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REVIEW

Cells and extracellular matrix interplay in cardiac valve disease: because age matters

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Abstract Cardiovascular aging is a physiological process affecting all components of the heart. Despite the interest and experimental effort lavished on aging of cardiac cells, increasing evidence is pointing at the pivotal role of extracellular matrix (ECM) in cardiac aging. Structural and molecular changes in ECM composition during aging are at the root of significant functional modifications at the level of cardiac valve apparatus. Indeed, calcification or myxomatous degeneration of cardiac valves and their functional impairment can all be explained in light of age-related ECM alterations and the reciprocal interplay between altered ECM and cellular elements populating the leaflet, namely valvular interstitial cells and valvular endothelial cells, is additionally affecting valve function with striking reflexes on the clinical scenario. The initial experimental findings on this argument are underlining the need for a more comprehensive understanding on the biological mechanisms underlying ECM aging and remodeling as potentially constituting a pharmacological therapeutic target or a basis to improve existing prosthetic devices and treatment options. Given the lack of systematic knowledge on this topic, this review will focus on the ECM changes that occur during aging and on their clinical translational relevance and implications in the bedside scenario.

Keywords Aging · Cardiac valves · Extracellular matrix · Valvular interstitial cells · Valvular endothelial cells

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Introduction

Cardiac valve degeneration represents the most common pathological condition for valve disease and the more frequent indication for surgery in the US and Europe [14]. Mitral valve (MV) and aortic valve (AV) are primarily interested by degeneration, causing severe valve dysfunction with important impact on the overall ventricle function over time [32]. MV is usually affected by a myxomatous degenerative phenomenon leading to severe regurgitation, while the process of calcification more often harnesses AV leaflets, causing different degrees of stenosis with subsequent repercussion on ventricular performance [14]. Aging is associated to a cascade of biological and molecular events that might translate and lead to structural deterioration and functional impairment of cardiac tissues [95]. Despite the interest and experimental effort lavished on aging of cardiac cells, increasing evidence is pointing at the pivotal role of extracellular matrix (ECM) in cardiac aging. ECM does not exert a role of mere support, but is a key factor in the tight interplay with the cellular compartment. Structural and molecular changes in ECM composition during aging are at the root of significant functional modifications at the level of cardiac valve apparatus [17]. Indeed, calcification or myxomatous degeneration of cardiac valves and their functional impairment can all be explained in light of age-related ECM alterations. Valvular interstitial cells (VICs) and valvular endothelial cells

(VECs) populating cardiac valves have been reported to be extremely sensitive to tissutal environment and physiological states and their activity is reciprocally influenced by changes in the surrounding matrix substrate [17, 137]. A bivalent mechanism is therefore established in which valve cells actively answer to mechanical load or biochemical signals through phenotype switches resulting in the production of different components of ECM [137]. At the same time, age-related or disease-related ECM changes are able in turn to deeply influence VICs and VECs behavior. Aged valve tissues undergo an ECM remodeling process resulting in fibrillar disarray with an imbalance in the ratio between collagen type I and type III and increased stiffness [159, 161]. Also, augmented concentrations of proteoglycans and glycosaminoglycans throughout the leaflets have also been reported and contribute to increased stiffness in the circumferential direction with subsequent impairment of the elastomechanical properties of the leaflet itself [158]. VICs have been shown to be at the base of ECM changes in elderly valve tissues contributing to myxomatous degeneration or calcification [101, 107]. However, ECM remodeling itself, stimulating the expression of different substrate matrix proteins and altering tissutal organization, influences both VICs and VECs biosynthetic activity eventually favoring leaflet tissutal modifications and therefore the risk of valvular disease [17]. Indeed, as valve ECM remodels during lifetime, the different biochemical composition of the supporting matrix or its simple structural deterioration triggers changes not only in valvular cells phenotype but also in their secretory activity. The age-related ECM remodeling, typical of older leaflets [161], impairs tissutal density and the barrage function of the fibrosa, determining an increased permeation of several VEC-secreted proteins throughout the valve layers. Accumulation of important mediators within the leaflet, as inflammatory or hemostatic proteins, might favor tissutal alteration and calcific nucleation [17].

Undoubtedly the two phenomena of physiological aging and pathological alteration are closely imbricated as they are reciprocal actors in the determinism of the disease. The mechanisms governing these intertwined pathways and the sequence of events leading to pathology is unclear even if it might be reliably speculated that the age-related alterations constitute a background for the development of pathological changes. In this context, the study of genetic factors predisposing to the onset of disease at later stages in life is very important. However, as in a multifactorial disease, the presence of external factors superimposed to a particular genetic background play a fundamental role and is able to trigger the actual "expression" and translation into disease of an underlying pathological potential. Similarly, ECM changes might be considered as an underlying framework on which pathologic changes can establish either affecting the biological activity of VICs and VECs or by means of a reciprocal interaction with them. In support of this, mechanical properties of the ECM and in particular increased degree of its stiffness, as in aged valve, have been shown to modulate and influence the differentiation of VICs to pathological phenotypes in response to biochemical cues [185]. Additionally, the fact that some of the characteristics found in aged valves reflect in different extents the changes observed in diseased valve further support this hypothesis. In a study by Kupari et al., increased circulating levels of collagen type III and I telopeptides were found in aortic stenosis patients [93]. This finding might be associated to the findings of increased collagen type III and I and telopeptides in aged valves as described in details below.

These initial findings clearly suggest the emerging need for a more accurate understanding of the changes occurring in valvular ECM during aging, which have been unjustly underestimated by the current literature. Considering the burden of age-related valve disease on an aging population [116], understanding the interplay between valve cells and ECM and their response to different physiological states appears fundamental to develop noninvasive therapeutic strategies [17] or to optimize existing treatment options. In light of the scarce availability of a comprehensive picture on this topic, this review will focus on changes of ECM components in the aged valves trying to unveil their clinical translational relevance and potential implications in the bedside scenario.

The clinical burden

As a result of the rise in life expectancy and the consequent progression and incidence of age-related valve disease, the need for surgical repair or replacement of degenerated valves is enormously increasing, determining a consistent burden on both surgical activity and general healthcare management [14]. Indeed, the amount of elderly patients requiring surgery is increasing and this subpopulation carries a significant intraoperative and postoperative risk due to their several comorbidities or simply poor general health conditions [31]. Heavily calcified valves, fragile cardiac and aortic tissue, chronic lung and renal disease profoundly affect both the surgical technical aspects and the postoperative care and retrieve from the operation [102]. Cardiac surgery in the elderly is associated with increased mortality, surgical complications, hospitalization time (length of stay, LOS), and usually demands for more complex procedures or combined operations of valve replacement and myocardial revascularization [31].

In mitral valve, aging mainly translates in myxomatous degeneration that determines redundancy and thickening of

the leaflets impairing coaptation and mostly causing regurgitation [137]. However, MV annulus calcification or leaflet retraction related to the changes in valve structure is also frequent, especially when associated to previous rheumatic disease. Abnormal age-related activation of myofibroblasts, deeply affecting the dynamic balance between the synthesis and degradation of connective tissue, is known to be at the root of these structural alterations [137]. Additionally, the aging process determines an intrinsic weakening of the whole heart skeleton, and this reflects in the general tendency to dilation of the mitral annulus under hemodynamic load and intraventricular telediastolic pressure [57]. This requires annulus reinforcement with prosthetic rings [62], but considering the natural elasticity and dynamic compliance of the heart, a great effort to simulate the mechanical properties of the skeletal part of the heart is mandatory in this context [35]. Different materials have been used in an attempt to mimic annulus properties, taking into account the redistribution of forces bearing on the leaflet and the annulus following reconstructive plasty of the MV [144].

As far as degenerative AV disease is concerned, stenosis represents the most frequent cause of dysfunction in the elderly with a 2–7 % prevalence, which is in any case destined to rise because of the increasing life expectancy. AV stenosis is characterized by a silent chronic course with a steeper trend in clinical evolution and prognosis after the onset of symptoms [116]. Medical therapy is limited to the relief of symptoms, but is not able to have an impact on actual survival, while the surgical option remains the most effective treatment. However, despite this strategy carries low operative risk, improved survival, and optimal freedom from reoperation in younger patients, the 30-day mortality in patients aged over 80 years increases up to 15 %, with a significant increase in operative and postoperative complications [177]. In the recent years, new minimally invasive strategies are emerging to guarantee the treatment of AV stenosis in high-risk surgical patients (STS Score >10, logistic EuroSCORE >20). Transcatheter aortic valve implantation (TAVI), developed in 1992 by Andersen et al. [10], and clinically introduced in 2002 by Cribier [44], constitutes an option in this subpopulation, allowing avoidance of surgical complications essentially related to open chest operative technique, aortic cross clamping and cardioplegic arrest [102]. Differently from the MV, the major biological obstacle in this framework is represented by the heavy calcium deposition at the level of the connectival network of the leaflets and of the aortic annulus, which is thought to be due to a shift toward an osteoblastic phenotype of the cells within the aortic structure [87, 113].

The biological counterpart

In the physiology of the cardiac valve apparatus, the combined activity of myofibroblasts and connectival degrading enzymes normally warrant a strict regulation of matrix homeostasis within the valve and ensure an adequate functional architecture of the valve itself [148]. In this extent, the highly preserved stratified framework exhibited by the valve internal structure is crucial to meet the biomechanical needs related to the loading forces sustained by the valve over a lifetime. A continuous turnover of ECM is at the biological basis of the valve structure conservation, and is due to the activity of myofibroblast-like cells populating the leaflets, namely valvular interstitial cells (VICs). Also valvular endothelial cells (VECs) have been reported to exert a fundamental action in the maintenance of valve homeostasis, being responsible of hemostatic regulation [30] and displaying close interactions with VICs and valvular ECM [29]. VECs have been shown to actively respond to changes in the surrounding microenvironment via several mechanotransduction pathways [56] and their dysfunction has been claimed to be involved in the initiation of valvular disease [13, 46, 56, 152]. It is known that ECM components are responsible for the accommodation of the hemodynamic repetitive changes occurring throughout the cardiac cycle and that VICs and VECs mediate its continuous remodeling warranting valve durability [136, 137]. Early studies by Schoen et al. demonstrated specific topographical differences in ECM component within the valve [148], which will be discussed separately.

Aortic valve

In the physiological normal AV, the fibrosa (or arterial side) of the leaflet mainly contains type I collagen allowing for maximal coaptation during closure and preventing cusp prolapse, while the ventricularis is characterized by higher content in elastin, which provides better expandability during diastole and elastic recoil during systole. Shock and shear absorption is guaranteed by the hydrophilic proteoglycans present in the spongiosa [148]. Therefore, ECM spatial distribution and composition affect valve function, and alterations at the molecular level of such a delicate balance are able to determine the dysfunction and the morbidity that surgeons encounter at the operative table. Initial studies on explanted degenerated valves from adults demonstrated thickening phenomena with disorganization of collagen fibers, enhanced VICs density and calcifications [124]. An interesting study from Hinton et al. [75] on valvulogenesis, in which a comparison between characteristics of animal valves during embryogenesis and human

pediatric diseased aortic valves was performed, showed that the developmental program underlying leaflet remodeling during life includes a precise spatiotemporal coordination of ECM organization and a progressive VIC compartmentalization. Also, increases in collagen content, diameter of collagen fibrils and level of collagen cross-linking were reported in adult compared to fetal porcine heart valves [90]. These balanced mechanisms are disrupted in a pathological valve, underpinning the importance of the historarchitectural organization of ECM in valve pathobiology. Loss of collagen, misorientation and fragmentation of elastin fibers, and VIC disarray (with formation of cellular clusters alternated to cell-free areas in the interstitium) were demonstrated in pathological valves [75].

Valve aging shares several of the features described on pathological degenerated valves. The majority of the changes found in physiologically aged valves seems to be prodromal to the establishment of more defined pathological alterations. Conversely, another number of age-related alterations overlap with the ones described in pathological valves with the boundaries between physiological aging and pathology being very indefinite and the actual translation of these changes in a clinical evident pathological condition being associated to the superimposition of different other factors (genetic predisposition, environment, life-style, controllable risk factors as smoking physical inactivity hypercholesterolemia, uncontrolled hypertension or diabetes, etc.).

More specifically, aging is associated to changes in type I and type III collagen as found in diseases calcified valves [59]. Beside their relative content in AV, their distribution and degree of cross-linking acquire a relevant significance in valve aging. Indeed, after their secretion as propeptides, immature forms of collagen undergo cross-linking mainly at the lysine (Lys) and hydroxylysine (Hyl) residues in the telopeptide regions at both ends of the molecule. Telopeptides further bind with particular helical regions of another molecule to form a divalent immature cross-link, which undergoes a progressive maturation process towards a higher valence of cross-linking. The entity of cross-linking and proportion of telopeptides characterize many of the mechanical properties of the AV.

In physiological aged valves, a change in the nature of C-terminal telopeptide of collagen type I was reported, supporting the idea of a change in the maturation of cross-links together with an altered collagen turnover [59]. This has been explained by an age-related reduction in the activity of the LH isoenzyme, which is responsible for the hydroxylation of telopeptidyl lysine. At the same time, an overall decrease in the N–terminal telopeptide of type III collagen has been reported in aged valves [59]. Mechanisms underlying these changes are thought to be associated to increased activity of matrix metalloproteinases (MMP-1, MMP-2, MMP-3, MMP-9), their tissue inhibitor-1 and tissue inhibitor, which are mediating an ongoing ECM remodeling process [63, 93, 147].

Additionally, collagen-bound advanced glycation end products (AGEs), arising from non-enzymatic glycation of serum and vascular proteins, have been shown to play a role in the increase of aortic stiffness during aging [135]. AGEs accumulation within the ECM has been shown to induce collagen cross-linking in animals [141]. Additionally, AGEs are recognized by several receptor molecules, with the receptor of AGEs (RAGE) being the most extensively investigated. Its activation leads to a pro-inflammatory state and AGE–RAGE interaction has been shown to be involved in aortic valve changes due to metabolic stress from high fat intake in animal models [77]. These data found a counterpart in the observation that patient with calcific aortic valve stenosis have decreased levels of soluble RAGE, a natural inhibitor of the AGE–RAGE system with anti-atherogenic properties [20]. Interestingly, in animal studies Pioglitazone-mediated RAGEs downregulation attenuated the progression of AV calcification by reducing inflammatory cell infiltration and oxidative stress [100].

All these initial alterations of the main components of ECM, together with abnormal activity of VICs, define a framework that further becomes a theater for the establishment of leaflet calcification normally observed at surgery. However, other ECM components are altered during AV aging. Jian et al. [80] demonstrated increased levels of tenascin-C (a glycoprotein normally expressed in developmental bone tissue and atherosclerotic plaques) in aortic degenerated calcific valves. This protein participates in the physiological mineralization being associated to bone morphogenetic protein (BMP) pathways [103], and is normally expressed in low levels in aged valves in the subendothelial space. Tenascin-C expression might be induced by a number of cytokines as BMP, TGF-beta and TNF-alpha [85], and also by mechanical stress in fibroblasts [145]. Some studies suggested that the accumulation of tenascin-C, induced by several factors as cytokines, or collagen damage, might be prodromal to the progression of aortic valve disease to calcific stenosis. Accumulated tenascin-C in the deeper layers of the valve would up-regulate the expression of MMP-2 and the positive feedback by MMP-2 would induce further accumulation of tenascin-C. This would lead to cellular aggregation and increase in alkaline phosphatase (ALP) expression with subsequent deposition of calcium phosphates [80]. The induction of tenascin-C synthesis in fibroblasts through mechanical stress or collagen damage [145], together with the observation of increased level of this protein in pressure-overloaded valve in elderly hypertensive patients

[146], remark the idea that age-related structural changes of the ECM components can constitute a trigger for pathological alterations to occur and further explain the overlap in the biochemical and mechanical features of aged and diseased valves.

As an additional confirmation, osteopontin and BMP were found to be up-regulated in calcified valves together with EGR-1, a transcription factor inducing both tenascin-C and osteopontin [67, 111].

Gene expressions of BMP-2 and ALP were significantly accelerated in AVICs from aged rats [150]. In parallel, ALP activity was found significantly increased in calcified valves [80]. Along with these findings, activity of matrix metalloproteinase type 2 (MMP-2), a gelatinase, was found enhanced in calcified valves [80]. In concert with the previous data, Fondard et al. identified changes in expression of MMP-3, MMP-9 and of their inhibitor, i.e. TIMP-1, in pathological valves [63]. Interestingly, in a study comparing tricuspid aortic valves from elderly patients with and without calcific aortic stenosis, both MMP-2 expression and its gelatinase activity was significantly higher in calcific stenotic valves, suggesting that that MMP-2 is present as a latent pro-enzyme in normal aged valves and turns activated in pathological valves [88]. Also, this observation confirms the idea of age as an underlying background for the establishment of pathology and the finding of the induction of MMP expression through mechanical shear stress, as in stiffened or aged tissues [15], further confirms this hypothesis.

Another interesting ECM component involved in AV degeneration is fibulin-4, a protein known to maintain the structure of the extracellular matrix. Reduction in its expression has been shown to lead to valve thickening and disarrangement of elastic fibers in an animal model [71]. Although the precise mechanism as not been elucidated yet, Hanada et al. associated this protein to perturbation of the TGF-beta signaling [71].

As mentioned above, ECM has been shown to be responsive to shear stress alterations, as testified by changes in the expression and activity of the proteases MMP-2, MMP-9, cathepsin L and cathepsin S, and an abnormal mechanical stimulation was shown to trigger calcification [166]. Altered ECM becomes a pabulum for the infiltration of inflammatory cells or for the phenotype switch of VICs into secreting myofibroblasts, with enhanced ECM deposition. This process is led by an increase in the expression of toll-like receptor (TLR)-2 and TLR-4 and in pro-inflammatory and pro-osteogenic responses [184]. The modulation of the nuclear factor-κB activity [186], of the osteoprotegerin (OPG)/receptor activator of nuclear factor kappa B (RANK)/RANK ligand (RANKL) pathway [83], and of associated signal transduction pathways [53] is thought to subtend the pro-osteogenic drive. Age-related VECs alterations determine lipid penetration and accumulation in areas of damage or inflammatory injury, and permit lipoproteins (LDL) implicated in atherogenesis to subsequently undergo oxidative modifications [121]. These ox-LDLs are cytotoxic and capable of enhancing inflammatory response, through TGF-β1, TNF-α, interleukin (IL)-1β pathways, eventually leading to both fibrotic and calcific processes, with an increase in valve stiffness [53]. The renin–angiotensin system is thought to modify this fibrotic process, since angiotensin-converting enzyme (ACE) and angiotensin-II are up-regulated and their effect on valve myofibroblasts has been shown [119].

In a genomic study, Bossè et al. identified many of the actors involved in calcific degeneration of AV stressing the role of inflammation and matrix remodeling in this context and eventually revealed phenomena of endochondral metaplasia in AV leading to calcification [24]. With this regard, Cappelli et al. have recently reported enhanced activity of gamma-glutamyltransferase (GGT), an enzyme linked to calcium metabolism and atherogenesis, inside the lipid component of valves leaflets by osteoclastic-like resident cells [34]. The findings of this study enlighten a potential link between atherosclerosis-related phenomena and the calcification of AV. Several study reported the association between atherosclerosis and aortic valve stenosis demonstrating as these two conditions share similar mechanisms and clinical risk factors, as age, male gender, hypertension, hyperlipidemia, smoking and diabetes [2, 8, 131]. The majority of these predisposing factors are strongly associated with inflammation and, interestingly, calcification in response to an inflammatory stimulus seems to be a proper feature of aortic valve, as it has not been described in other cardiac valves. In fact, only the aortic VICs exhibited an osteogenic in response to a pro-inflammatory stimulation via TLR-4 resulting in BMP-2 production [179]. The association between the calcification process and inflammation in aortic valve disease has been testified by the observation of inflammatory reaction as an early feature in histologic specimens from aortic stenosis patients [4, 124]. As above-mentioned, lipid accumulation, infiltration of macrophages and lymphocytes represent the substrate activating the calcification cascade [65, 120, 138]. A recent study using positron emission tomography proved that early aortic valve inflammation, prior to hemodynamic impairment, drives the pathophysiologic processes of valve calcification in humans [1], even if remains difficult to distinguish between the cause and the consequence.

Calcification of the aortic valve per se is associated with disruption of the basal membrane, infiltration of inflammatory cells and lipids deposition [58, 110], and cytokines and vesicles released from macrophages may initiate the calcification of the ECM. Conversely, calcium may promote inflammation which in turn would enhance further

calcification perpetuating a self-sustained "vicious cycle" [115, 172]. Furthermore, TNFα-mediated oxidative stress causes loss of endothelial protective function of VEC and chronic inflammation sustains fibrogenic and osteogenic activation [60]. The importance of inflammation pathways in the genesis of aortic valve disease has been extensively investigated and confirmed by many studies in recent years. Oxidized lipids are found in calcifying aortic valves, and could promote osteoblastic differentiation of valvular fibroblasts and macrophages via activation of LRP-5/Wnt and Runx2/Cbfa-1 pathways, eventually leading to calcification [118]. Also, the interaction between sphingosine 1-phosphate receptors and TLR-4 signaling leads to a cooperative up-regulation of inflammatory, angiogenic, and osteogenic pathways [61]. TLR-3, NF-κB and ERK1/2 pathways in VICs are also implicated in the pro-osteogenic effects [187]. Interestingly, the presence of numerous proinflammatory cytokines such as TNF-2, BMP molecules, and interleukins such as IL-1β/2/6 has been shown in degenerated diseased aortic valves [104]. Anti-inflammatory effects of Interleukin-1 receptor antagonist are reduced in stenotic valves in respect to normal valves; TGF-β, another anti-inflammatory mediator, is also ubiquitous in diseased valves, especially bound to matrix, and acts as a promoter of ECM fibrosis [97]. In established aortic stenosis, histopathology of the valves shows numerous new blood vessels associated with the expression of angiogenic factors such as vascular endothelial growth factor (VEGF) and endothelial nitric oxide synthase (NOS) [155], however, the origin of endothelial cells seen in this setting is poorly understood. In the early stages of calcific lesion formation, neoangiogenetic capillary sprouts exhibited the endothelial cell markers CD31, CD34 and von-Willebrand factor (vWF) as well as carcinoembryonic antigen cell adhesion molecule-1 (CEACAM1), Tie-2 and angiogenesis inhibitor endostatin indicating angiogenesis as a pathogenic factor in aortic stenosis [36]. In other studies the co-expression in VICs of smooth muscle alpha-actin (α-SMA) and VEGF receptor-2 suggested a mesenchymal-to-endothelial transition in early stage valve lesion with formation of new capillaries with inter-endothelial junctions and partial basement membrane-like structure, reinforcing the role of angiogenesis in the calcification process and valve remodeling [99, 129]. A recent study demonstrated a stretch-mediated activation of inflammatory pathways evaluated using miRNA analysis [128] but the multiple connections between inflammation, hemodynamics and aortic valve pathophysiology are far from being completely elucidated.

Glycosaminoglycans (GAGs) content of AV also changes with age. In a study on porcine valves at different ages, Stephens et al. reported an aging-related increase in the 4-sulfation to 6-sulfation ratio of GAGs, that reversed the compressive tissue phenotype observed in younger AV into a tensile one [158]. Since GAGs are well represented in the central spongiosa layer, it has been speculated that this layer allows to reduce the shearing which results from the differences in motion kinetics of the fibrosa and ventricularis during leaflet deformations [149, 180]. GAGs are hypothesized to contribute substantially to the behavior of the bulk tissue at lower physiological force levels by means of mechanical interactions with the collagen, whereas at higher tissue stress levels, tissue response is dominated by the fully loaded and straightened collagen fibers [171]. The specific arrangement of GAGs within the leaflet, with increased content of water-binding GAGs localized in regions that experience more compression, achieves an important significance in controlling tissue behavior over the cardiac cycle [69, 70]. In an experimental setting, the removal of GAGs from the aortic valve had actually no measureable effect on extensional mechanical properties including peak stretch, hysteresis, or creep. However, in the low force range, hysteresis was markedly reduced after GAG removal. Therefore, GAGs do not play a direct role in modulating the time-dependent tensile properties of valvular tissues, but they are strongly connected with fiber–fiber and fiber–matrix interactions at low forces levels. In this context, GAGs are thought to be important in providing a damping mechanism to reduce leaflet flutter when the leaflet is not under high tensile stress [54]. Considering the role of GAGs, the changes occurring with aging result in increased tensile loading on the aortic leaflet, and may partially explain the age-related decrease in aortic compliance [45].

Additionally, a recent study from Balaoing et al. underlined the hemostatic imbalance in old aortic pig valve due to age-related changes in the activity of VECs [17]. Interestingly, these Authors found an increased secretory activity of prothrombotic proteins such as vWF, tissue factor pathway inhibitor (TFPI) and tissue plasminogen activator (tPA) in older valves. This aberrant secretory activity, coupled with the age-related changes in collagen type I and elastin organization, justified the sequestration of the soluble hemostatic protein deeply within the ventricularis part of the AV leaflet, as the barrier function exerted by the aligned collagen fibrils of healthy valves is lost in older valves. Authors demonstrated in vitro that the presence of a large burden of vWF induced a pro-osteogenic activity in the interstitial cells leading to calcific nodule formation [17]. The importance of vWF is supported by the fact that inhibition of the metalloproteinase ADAM-17, the physiologic inactivator of vWF, during mouse development results in perinatal lethality and heart valve abnormalities. Thus, ADAM-17 is required in VECs for proper cell regulation and ECM homeostasis in semilunar valves [183]. The age-related imbalance in hemostatic protein

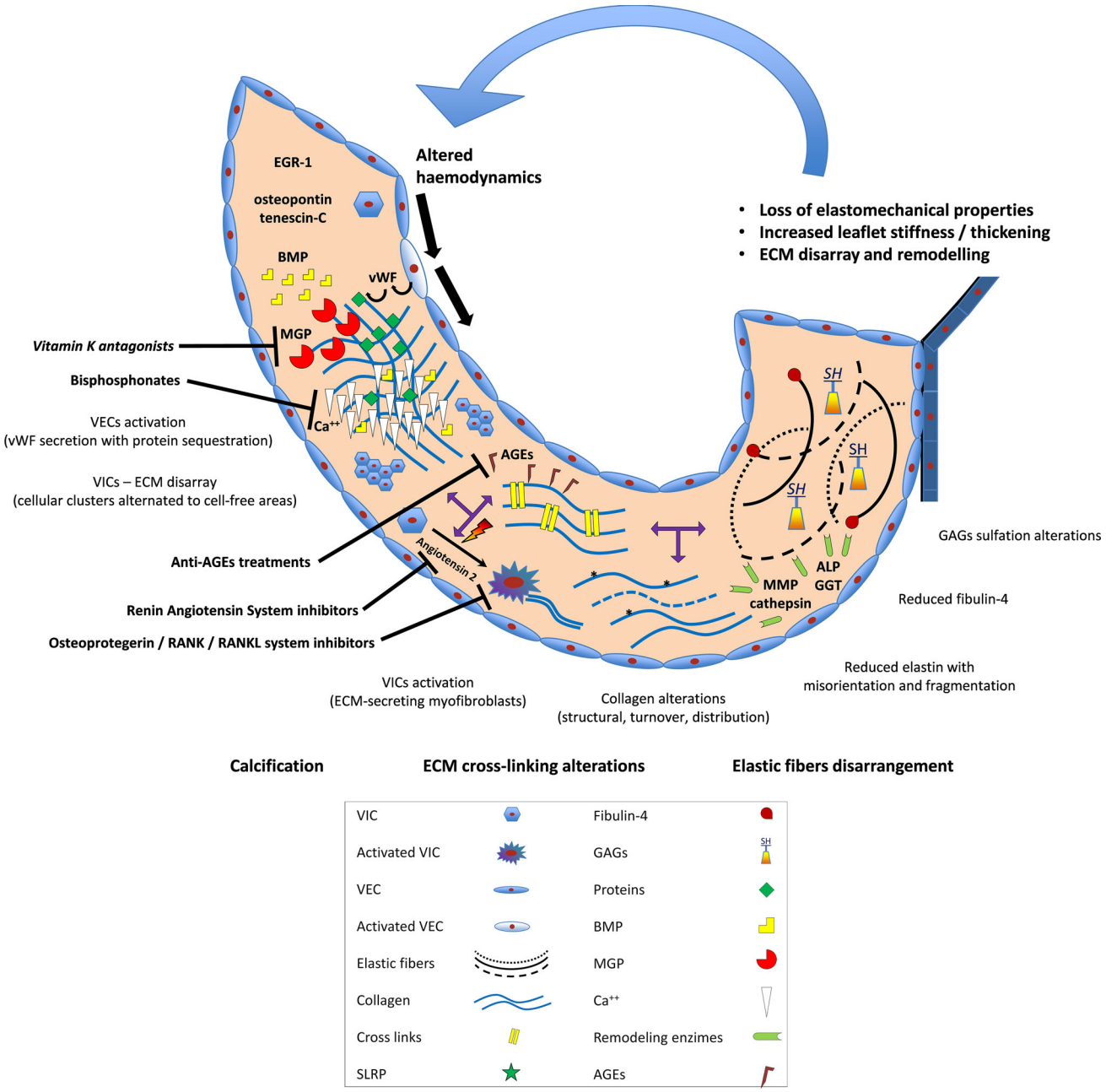

Fig. 1 Schematic diagram showing ECM changes in aortic valve leaflets during aging and potential therapeutic approaches to counteract aging processes

regulation, leading to prothrombotic mediators accumulation within the leaflet and accompanied by the mentioned ECM disarray, might favor the development and progression of aortic calcific disease [17]. In this context, the matrix Gla protein (MGP), a vitamin K-dependent inhibitor of calcification, has been reported to play an important role in the pathogenesis of valve calcific disease. γ-carboxylation of its glutamic acid residual (via vitamin K-dependent γ-carboxylase) activates the protein which in turn binds BMP-2 exerting an inhibitory activity and therefore protecting from vascular and valvular calcification. Undercarboxylated MGP circulating levels have been associated to increased mortality in aortic valve stenosis [176] and Sweatt and colleagues demonstrated reduced levels of carboxylated MGP in valve tissues of old rats establishing a pathological link between aging, ECM valve remodeling and calcific aortic disease [168]. MGP is currently under investigation as an inhibitor of vascular calcification, and lack of this "anti-calcification" protein contributes to the calcification in the aortic valve [178].

Table 1 ECM changes and functional consequences in the aortic valve

Component	Mechanism leading to component alteration	Findings in aged valve	Findings in stenotic valve	Pathobiological effect
Collagen	Increased remodeling mediated by elevated activity of matrix metalloproteinases, their tissue inhibitors and cathepsins [3, 54, 62, 71, 72, 79, 84, 87, 146, 155, 156] Disorganized deposition of newly synthetized fibers [58] Reduction in the activity of the LH isoenzyme, responsible for the hydroxylation of telopeptidyl lysine [58]	Increased collagen content, diameter of collagen fibrils and cross-linking [89] Increased C-terminal telopeptide of collagen type I, and decreased N-terminal telopeptide of collagen type III, indicating a change in the maturation of cross-links together with an altered collagen turnover [58]	ECM disarray with formation of cellular clusters alternated to cell-free areas [74] Increased synthesis of collagen, with changes in distribution and degree of cross-linking [58] Basement membrane appears thin or absent [37]	Fibrosis [58] Leaflet thickening [58] Matricellular regulation of VICs leading to myofibroblast differentiation [37]
AGEs	AGE-RAGE interaction leads to a pro-inflammatory state [24, 25] RAGE activation results in increased inflammatory cell infiltration and oxidative stress [99] Decreasing circulating sRAGE, which reduces AGE-RAGE signaling cascade [19]	Increased AGE accumulation and RAGE expression [26, 27, 76]	RAGE activation-induced inflammation promotes aortic valve calcification [99]	Stiffness [26, 27, 76] Enhanced cross-linking, with structural and functional changes in collagen [140] AGE-RAGE interaction leads to increased proinflammatory cytokine synthesis [116, 117]
Tenascin-C	TGF-beta, BMP and TNF-alfa induce tenascin-C expression [80, 84, 111, 112, 173, 174] Tenascin-C expression can be induced mechanically in fibroblasts by collagen damage [144] Tenascin-C up-regulates the expression of MMP-2 with a positive feedback leading to further accumulation of tenascin-C [79]	Participates in normal mineralization [102]	Increase, co-localizes with MMP-2 [79] Enhanced accumulation around calcified nodules [79, 145] Increased in case of pressure overload (hypertension) [145]	Increased ALP activity and MMP expression in VIC, leading to deposition of calcium phosphates [79, 172] Modulates VICs signaling and increases ECM production [107, 108] calcification [79, 145]
BMP	Induced by various inflammatory factors (TNF-alfa, TGF-beta, and interleukins) [80, 81] ICAM-1 signaling activates the Notch-1/NFkB pathway which up-regulates BMP-2 expression, translating a pro-inflammatory signal into a pro-osteogenic response [180, 181] Increased expression by elevated stretch [14] Increased after TLR stimulation [183] Altered hemodynamics stimulates the expression of adhesion molecules and BMP [163]	Increased expression [149]	Increased expression, mostly in cell-rich areas associated with focal calcium deposits [83]	Increased ALP activity [149] Calcification through Smad and Wnt signaling [83, 149]

Table 1 continued

Component	Mechanism leading to component alteration	Findings in aged valve	Findings in stenotic valve	Pathobiological effect
Osteopontin	Differentiation of an osteoprogenitor subpopulation in VICs [38, 39] Egr-1 up-regulation is associated with increased tenascin-C and osteopontin [66]	Unknown	Increased, co-localizes with calcific deposits [110, 129]	Calcification [110, 129, 130] Affects cellular HMGB1 function in VICs, enhancing the calcification process [126, 127] Regulation of VECs activation [129, 130]
MMP	Induced by shear stress alterations [14–16, 153, 165] Induced by TNF-alfa [6]	Participates in physiologic remodeling [62, 87]	Enhanced [54, 55, 79, 84–86] Changes in expression of MMP-3, MMP-9 and TIMP-1 [62]	Calcification [62, 79, 84] Collagen remodeling and ECM disarray [54, 55, 62] Generation of proinflammatory collagen fragments [124, 125, 133, 134]
ALP	Induced by supra-physiological stretch [14, 15, 153, 154]	Enhanced activity [149]	Enhanced activity [79]	Calcification [104]
Fibulin-4	Induced by TGF-beta signaling [70]	Reduced [70]	Unknown	Leaflet thickening [70] Elastic fiber disarrangement [70]
GAGs	Mechanical stimulation (?)	Increase in the 4-sulfation to 6-sulfation ratio [157]	Present in regions of early calcific nodule formation [157] Differential distribution [161, 162]	Modification from compressive into a tensile tissue phenotype [157] Decrease in aortic compliance [157, 158] Localizes TGF-beta and TNF-alfa [37] Matricellular regulation of VICs via TLR-2/NFkB pathway, with enhanced inflammatory response [50, 51]
Elastin	Increased expression of MMPs and cathepsins [3, 62, 71–73, 87]	Reduced and fragmented [74]	Reduced and fragmented [74]	Increases ECM stiffness [97, 98] Osteogenic differentiation of VICs [97, 98] Elastin fragment induce ALP activity [3]

The protease calpain-1 has been recently considered as an antagonist of ECM adverse remodeling through inhibition of MMP-2 in rat aortic wall [82], but its importance in aortic leaflet remains to be clarified. ECM changes of the aortic leaflet occurring with aging are summarized in Fig. 1, and a comprehensive schema of all the reported changes together with the possible pathobiological mechanisms in both aged and diseases AV ECM is given in Table 1.

Mitral valve

Normal MV structure and function rely on a more complex valvular apparatus including annulus, leaflets and chordae tendinae. Leaflets are composed of four layers, atrialis, spongiosa, fibrosa, and ventricularis. Atrialis, spongiosa and ventricularis are characterized by loose collagen, while the fibrosa is constituted by dense circumferentially aligned collagen providing tensile strength to the valve [92].

Interspersed GAGs within the other layers determine the compressive strength of the leaflet, while the elastin-rich side of the atrialis layer at the level of the inflow side permits to bear considerable stretch and allows for elastic recoil to the original shape during the phases of the cardiac cycle [96].

A specific analysis of GAGs within the valve ECM revealed high concentration of hyaluronan (HA) and hydrated chondroitin/dermatan sulfate proteoglycans in the spongiosa [158], while regularly spaced elastic fibers and circumferentially oriented collagen fibers characterized the thinner ventricularis side. Small leucine-rich proteoglycans (SLRPs) are mostly present in the collagen-rich region, in which they interconnect and provide mechanical support to the fibers [96, 158]. Additionally, there are functional differences in the composition of the free edge of the anterior leaflet, historically named "rough zone", where the chordae tendineae attach to the valve, and the center of the leaflet referred as the "clear zone", where no chordae are attached. The latter, experiencing tensile stresses, has a thicker fibrosa layer and shows lower concentration of GAGs, in particular less unsulfated, 6-sulfated, and 4-sulfated glucuronate than the free zone. In the rough zone, designed for compressive load absorption, the spongiosa layer is more represented and contains an overall higher concentration of HA [158]. The posterior or mural leaflet is suited for compressive load bearing and shows similar characteristics to those of the rough zone of the anterior one [69]. Chordae tendineae exert an important action during systole phase and chordal replacement is quite frequently required during surgical repair for degenerative mitral disease, mainly due to age-related alteration in chordal native structural composition. Dense collagen fibers orientated according to the direction of load characterize the core of the chorda, while elastic properties are conferred by elastin present in the outer sheath [5]. SLRPs (such as decorin and biglycan) are found interspersed within the aligned collagen fibers of the chordae [69].

Changes in structural composition accompanying the aging process are at the basis of functional reflexes on the valve itself and often translate into clinical evident morbidity requiring surgical treatment. One of the main findings in degenerated aged MV regards the thickening of the collagen-rich fibrosa layer and the increase in collagen production and remodeling [159]. In 2001, Rabkin et al. [137] perceived that the imbalance between MMPs and cysteine endoproteases (cathepsins), mainly altering collagen features, could play a role in myxomatous degeneration of MV. These observations suggest that the abnormalities of collagen result primarily from excessive collagenolytic activity rather than decreased collagen synthesis. Therefore, the deranged mechanical properties of myxomatous valves result from defective ECM and altered layered architecture. Indeed, collagen derangement has been shown to determine striking defects in the mechanical properties of myxomatous leaflets, leading to loss of tensile strength and inherent weakness. The synergistic effect of intrinsic elastomechanical defects and aberrant stresses engendered by hemodynamic cyclic load on altered and enlarged leaflets contributes to both disease pathogenesis and occurrence of postoperative failure [18]. In this context, David et al. in a multivariate analysis, reported myxomatous changes of the leaflets as the only statistically significant variable predicting the risk for reoperation [47].

In support of the hypothesis of an aberrant ECM remodeling in degenerated valve, it has been demonstrated that VICs maintain an "activated" phenotype and immunohistological co-localization of these cells with enzymes deputed to ECM turnover has been shown [161]. VICs are known to be mechanosensitive, and their synthesis of ECM components influences valve properties. Analysis of markers of VIC activation, such as α-SMA, and collagen synthesis, as heat shock protein-47 (HSP47) and prolyl 4-hydroxylase (P4H), proved that there are age-specific and valve-region-specific changes in VIC phenotype depending on the leaflet regional matrix where the VICs reside [160]. Potential mechanisms underlying tissutal degeneration in MV have been elucidated. Activation of resident interstitial valvular cells seems to be crucial in matrix degeneration. Interstitial valvular cells were found in higher number and expressing increased proteolytic enzymes, such as MMPs, that mediate ECM degradation, in degenerated valves compared with normal valves. Additionally, they have been shown to activate a catalytic state as demonstrated by their immunoreactivity for cathepsins S and K and IL-1β. Co-localization of IL-1β co-with MMP-13 and cathepsin S might additionally indicate that VICs are activated by IL-1β in response to a variety

stimuli and modulate the ECM enzymatic breakdown of the connective tissue described in myxomatous valves [137]. Specifically, collagen degradation process is thought to be initiated by interstitial collagenase that operates the breakdown of type I collagen fibrillar network, leading to the generation of fragments which become further accessible to gelatinases, completing collagen catabolization [91]. Collagen type III is conversely more represented in the aged valve [159]. Elastin degradation is in turn performed by cathepsins, in particular cathepsin K, which also exhibits collagenolytic activity [165]. VICs activation and ECM degradation itself trigger the production of a cascade of extracellular and paracrine messengers, such as IL-1, boosting and perpetuating the lytic process also for other components of the leaflet, such as proteoglycans [49, 137]. Indeed, there is a reduction in unsulfated and 6-sulfated glucuronate, as well as a decrease in the ratio of chondroitin sulfate to dermatan sulfate. Conversely, decorin and biglycan have a greater representation in the valve and co-localize with collagen in attempt to lend support to the above-mentioned remodeling occurring throughout aging [158]. In a study on porcine valves, Stephens et al. reported that compressive regions of MV showed an aging-related decrease in the fraction of 4-sulfated GAGs (associated with tension), paralleled by an increase in the fraction of 6-sulfated GAGs (associated with compression), whereas the tensile regions of the MV showed the opposite pattern. The distribution of these changes is not uniform in the degenerated myxomatous MV: the rough zone of the leaflet on the ventricular side, at the attachment of the chordae, is more prone to develop disease together with the interchordal hoodings at the level of the apposition line of the leaflets (the so-called parachute change) [64]. Additionally, the middle section of the leaflets, especially the middle scallop, which is normally undergoing the strongest pressure, shows the greatest incidence of changes [64]. Additionally, the interaction and relative content of GAGs with other ECM components is important in defining the elastomechanical properties of the leaflets and the age-related alterations of ECM further demonstrate the significance of GAGs function in this context. In an animal study on mitral valve, old mice showed increased leaflet stiffness due to increased collagen and matrix fiber alignment. Interestingly in Marfan-like mice deficient in Fbn1 gene, the loss fibrillin, a crucial element in extracellular microfibrils organization and function, produced fiber misalignment and a relative increase in GAGs content, which eventually resulted in myxomatous degeneration [68]. Therefore, the loss of a leading ECM framework constituted by fibrillin, determines an excessive accumulation of GAGs leading to degenerative changes. This finding testifies the importance of an homeostatic balance between structural proteins and load absorption glycans also in non-Marfan conditions, as the deterioration or loss of important structural ECM protein occurs in aging too, as described above. Whether the deregulation of this balance is triggered by an age-related change in VICs secretory activity or by an intrinsic ECM age-related degradation needs to be clarified, but it might play a crucial role in the determinism of disease as shown in the genetic model.

Even if, with advancing age, the layers become microstructurally more delineated [161], the amount of elastin decreases, especially in subjects over the age of 50 [106]. Other important changes seen during aging regard lipids accumulation and calcification throughout the valve. These phenomena represent both a pathogenic moment for age-related degeneration of the valve and an obstacle during valve surgical repair.

Changes in the structural and functional composition of ECM translate in an alteration of the mechanical properties of the valve. Collagen modifications during aging, with particular reference to the augmented cross-linking, together with elastin fragmentation, determine an increase in the overall valve stiffness in both the radial and circumferential directions, leading to permanent tissue stretch with less crimped collagen fibers, analogously to what occurs in valves subjected to glutaraldehyde fixation [26]. Additionally, these changes in ECM composition might underlie another interesting clinical finding elucidated by the group of Levine et al. who demonstrated an adaptive enlargement of the leaflets of chronically insufficient MV in patient with dilated ventricles, therefore bearing longitudinal stress and tethering forces [21]. In support of this clinical evidence, further studies demonstrated that stress application to mitral leaflets are able to induce a switch in VICs phenotype, promoting the synthesis of different ECM patterns with consequent increase in leaflet stiffness [107]. Taken together, these data underpin the importance of age-related alterations of the ECM at the molecular level. ECM abnormalities can be thought as the true origin of the pathological changes responsible for tissutal degeneration and for mechanical/hemodynamic failure of the MV. A careful exploitation of the knowledge achieved in this context might in the future lead to the development of preventive or therapeutic strategies in cardiac disease. ECM changes occurring in mitral leaflets over time are summarized in Fig. 2, and a comprehensive schema of all the reported changes in MV ECM is given in Table 2.

The genetics contribution

The role of genetics in the context of aging and development of valvular disease is being increasingly recognized. Genetic variations may act as a silent substrate which can

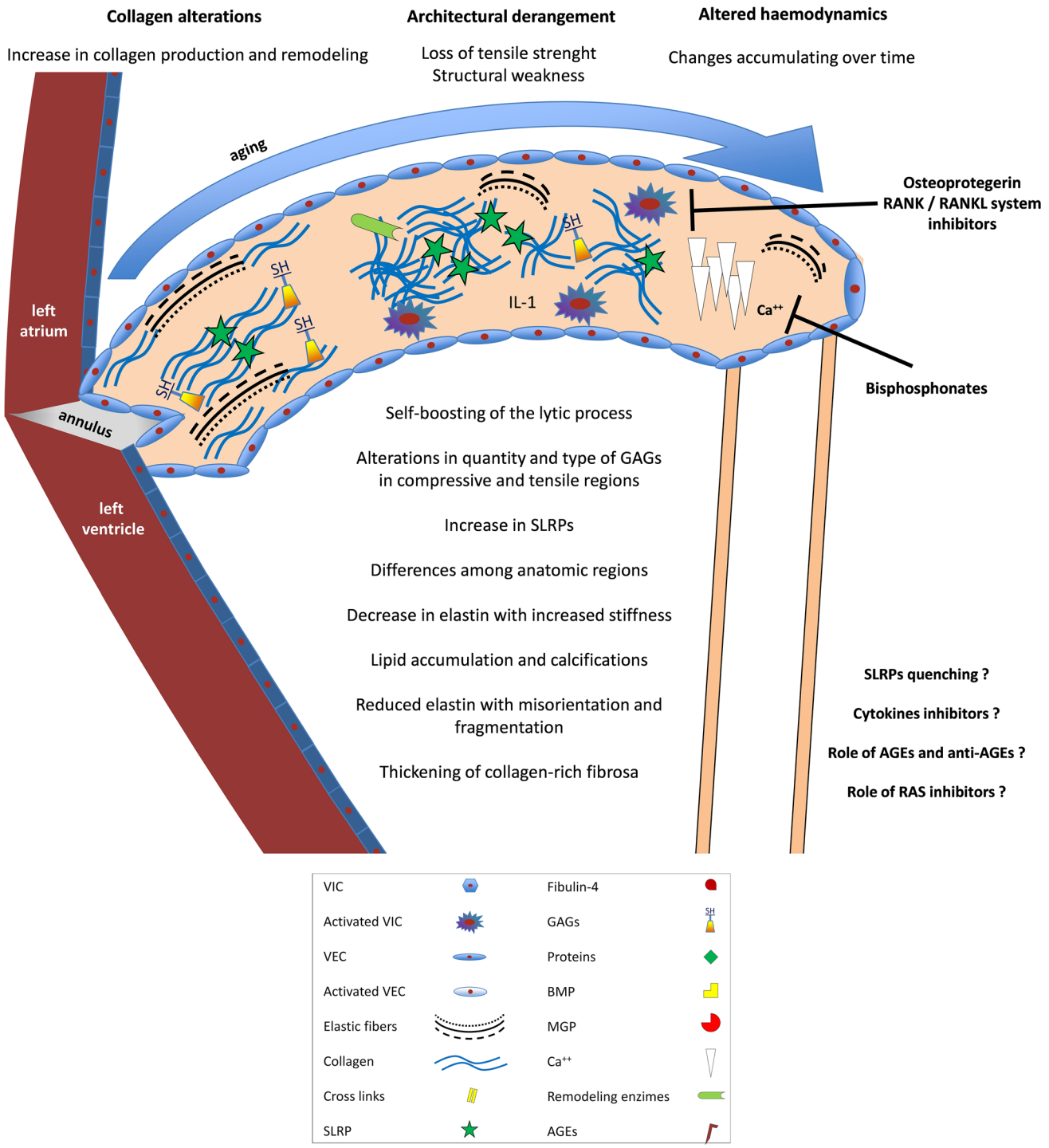

Fig. 2 Schematic diagram showing ECM changes in mitral valve leaflets and potential mechanisms underlying valve alterations during aging. Note the progression of changes in extracellular matrix composition and arrangement during aging (*blue arrow*). Potential therapeutic approaches to counteract age-related alterations and consequences are reported. Note that pharmacologic approaches followed by the *question mark* indicate only presumably effective treatments, as studies evaluating those compounds are still required

be triggered by environmental variables to initiate the process of valvular degeneration. The knowledge of the genetic determinants of valvular disease may foster the development of new therapies to prevent, rather than treat, these diseases [94]. Particular interest has been lavished in the study of valvular calcification, however, the exact role of specific genetic variations is still under investigation [22, 79, 133].

Table 2 ECM changes and functional consequences in the mitral valve

Component	Mechanism leading to component alteration	Findings in aged valve	Pathobiological effect
Collagen	Increased expression of proteolytic enzymes, as MMPs [136] Impaired MMPs and cathepsins activity [136] Mechanical stress results in VICs maintaining an "activated" phenotype with increase in proteolytic enzymes production [106, 160] Breakdown of type I collagen by collagenase [90]	Excessive collagenolytic activity [136] Increased collagen production and remodeling [158] Increased cross-linking [158] Increased diameter of collagen fibrils [89]	Leaflet thickening [150, 151, 158] Loss of tensile strength, weakness [136, 150, 151]
Elastin	Increased cathepsin K activation [164]	Reduced by degradation and fragmentation [105, 164]	Loss of elastomechanical properties [105, 164]
GAG	Mechanical stress	Reduction in 4-sulfated and increase in 6-sulfated GAGs in the compressive regions of the mitral valve; opposite pattern in the tensile regions of the leaflet [157]	More pronounced differences between tensile and compressive regions [157] Myxomatous degeneration [157]
SLRP	Unknown	Increased [157]	Altered mechanical support to collagen [157]

Wnt signaling has multiple functions at different stages of heart development, and its signaling sustained by Wnt receptor Lrp5 and stabilized β-catenin has been associated with adult calcific valve disease in humans [7, 32, 138]. The polymorphism rs13290979 of NOTCH gene was significantly associated with adult-onset of aortic stenosis [52], probably via enhanced BMP-2 production and osteogenic differentiation of the interstitial cells [12, 109]. In a study comparing the transcriptional profiles and cellular functions of human aortic VICs and mitral VICs, a higher expression of cardiac-specific transcription factor NKX2-5 and a lower expression of TBX5 were found in aortic VICs compared to mitral VICs [167]. NKX2-5 is an important transcriptional regulator during early embryonic development regulating the conduction system [140], while TBX5 plays a key role in cardiogenesis since its mutations result in Holt–Oram syndrome [19]. TBX5 expression in developing atrioventricular valves and ventricular trabeculae has been shown to account for the structural and functional defects in mitral and tricuspid valves [28, 33] and might represent an important actor in the aging process.

Lipoprotein(a) is known to be a risk factor for coronary artery disease, and several evidences have convincingly shown its causal role in ischemic heart disease due to lipid oxidation and an induction of a prothrombotic state [40, 89]. In a large cohort of patients, one single nucleotide polymorphism in the lipoprotein(a) locus (rs10455872) was associated with aortic valve calcification, and, in prospective analyses, was related to aortic stenosis and aortic valve replacement. Two single nucleotide polymorphisms (SNPs) (rs17659543 and rs13415097) located in vicinity of the proinflammatory gene IL1F9 achieved genomewide significance for mitral annular calcification [169].

Inflammation is a prominent feature of valve calcification and plays an important role in the development of valvular calcification [42]. Therefore, many genes involved in the process of inflammation have been considered to be candidate genes predisposing to valvular calcification. IL-10 is an anti-inflammatory cytokine that is secreted by various cells inhibiting TNF-α production by activated macrophages [175] and its production is genetically regulated [182]. Polymorphisms in the IL-10 gene (rs1800871, rs1800872, rs1801082, rs1800819, rs1800592 and rs1800872) have been found associated with valvular calcification in population studies [9, 66, 123].

Low-density lipoprotein cholesterol (LDL-C) and adult atherosclerosis risk factors are important in the determinism of aortic and mitral valve disease in epidemiologic studies [163, 170], and a large cohort study confirmed that genetic predisposition to elevated LDL-C levels is linked to development of aortic calcific disease and aortic stenosis in the adulthood [153]. Indeed, LDL-C play an important role in the early calcification and mineralization phase through the formation of cholesterol micro-crystals that act as pabulum for initial calcification. Additionally, oxidized-LDL enhances the osteogenic phenotype in valvular cells [76, 126].

Clinical significance

This review underlined the relevance of ECM changes during aging process in heart valves demonstrating its influence on both their mechanical and functional

properties and in the development of aged-related disease.

Several findings in this review point at the idea of aging as an underlying background for the establishment of pathology and that age-related structural changes of the ECM components can constitute a trigger for pathological alterations to occur. This might further explain the overlap in the biochemical and mechanical features seen in aged and diseased valves. The factors responsible to determine a shift of this delicate balance towards the disease are numerous and not completely identified, but genetic predisposition, environment, life-style, controllable risk factors are all relevant. From these observations a number of clinical implications and insights might be drawn.

Characterization of ECM modifications associated to aging and to valve disease is crucial in the development of valve substitutes or living tissue engineered constructs. Indeed, in the recently affirming field of detergent-based tissue decellularization for tissue engineering purposes, the importance of protecting and preserving the molecular and structural arrangement of ECM, including both proteins GAGs and regulatory molecules of matrix homeostasis, has been acknowledged [11]. Detergents have been shown to be retained in the ECM affecting cell survival rate when recellularization is attempted and the role of specific biochemicals to enhance matrix production and thereby creating a protective environment for the seeded cells while nourishing them, has been advocated [11]. These examples, further remark the importance of ECM and its activity. Additionally, a deep understanding of the mechanism underlying age-related ECM changes is fundamental to optimize and ameliorate currently used prosthetic valves which are widely known to undergo deterioration and aging processes. A close interplay between ECM and cells populating the valve has also been unveiled, enlightening a reciprocal influence on the development and progression of valve disease. VICs and VECs actively contribute to valve remodeling being extremely sensitive to changes of the ECM and of the surrounding hemodynamic environment. Several examples of functional VICs biosynthetic reactions aimed at maintaining valvular tissue homeostasis or compensating functional loss in leaflet mechanical properties have been reported in in vitro studies [17, 92, 107] and in clinical scenarios [21]. In light of such an adaptive mechanism inherent in the biology of the valve itself, pharmacological or molecular means of augmentation and assistance of leaflet remodeling might be developed. It has been suggested that the local delivery of biological mediators able to enhance the endogenous VICs compensating activity might be attractive and enlighten new avenues also in the surgical treatment of valve disease [157].

However, biological changes occurring at the cellular and ECM levels in the aging valve are likely to have an impact also on the clinical management of valve disease. Several pharmacological attempts have been done to inhibit or slow down valvular calcification or degeneration especially in aortic stenosis. Those approaches are mostly based on the concept of an atherosclerosis-like pathogenesis for aortic valve disease, in which an initial valvular lesion due to mechanical or increased shear stress determines lipids deposition resulting in an early subendothelial plaque which further triggers the development of the classic fibrocalcific remodeling. This phenomenon is sustained partially by VICs dysfunction, but is caused mainly by the ECM disarrangement occurring during aging. Indeed, the mentioned disproportion in collagen content and characteristics together with the reduction in elastin and the reported connectival modifications, lead to progressive loss of leaflet and aortic compliance and increase in valvular stiffness rendering the endocardium more prone to pressure or shear-mediated damage [74]. Pharmacological approaches currently tested consist in lipid-based intervention and aim to reduce the biological cascade triggered by lipid deposition [48]. Statins have been firstly used for both their lipid lowering action and for their pleiotropic anti-inflammatory and antioxidant effect with the aim to biologically stabilize the lesions and avoid fibrocalcific progressions. However, the unsatisfactory results of large clinical trials as the SEAS or the SALTIRE put on hold the initial enthusiasms about these compounds [74]. Also, statins failed to demonstrate any impact on aortic valve disease in three prospective randomized trials [37, 43, 142]. Increased presence of angiotensin-converting enzyme (ACE), angiotensin-II, and angiotensin-II type 1 receptor in fibrocalcific valves, together with the proinflammatory effect of the biological pathway sustained by macrophage-associated ACE and mast cell chymase, prompted the clinical use of ACE inhibitors in aortic stenosis. Renin-aldosterone system blockade proved to slow calcium accumulation but, disappointingly, did not show any benefit on stenosis progression [118]. Additionally, bisphosphonates have been shown to have a role in the inhibition of calcium deposition in vascular and valvular tissues in preclinical models. This effect has been claimed to be due to an inhibitory action on the release of calcium phosphate particles from bone [132]. However, nitrogen-containing bisphosphonates exert statin-like actions as they inhibit the farnesyl-pyrophosphate synthase, an enzyme distal to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the cholesterol biosynthesis pathway [41]. Bisphosphonates might therefore share calcium regulating effects and pleiotropic anti-inflammatory actions resulting in appealing tools against valvular calcification. Pretreatment of bioprosthetic valve with bisphosphonate inhibited calcific degeneration [139], but, taking from the MESA study, there was an increase in the risk of

calcification in women younger than 65 years [58]. In recent years, monoclonal antibodies targeting specific signal transduction pathways have been used clinically. Since one of the key regulatory roles in leaflet calcification is played by the OPG/RANK/RANKL system, denosumab (an anti-RANKL monoclonal antibody) is a promising pharmacologic approach to prevent aortic valve calcification [53].

Pharmacologic treatment against advanced glycation end products (AGEs) and their receptors has been gradually introduced in preclinical settings and in clinical research, with encouraging results in diabetic patients with cardiovascular disease [114]. In rabbits, inflammation induced by RAGE activation promotes aortic valve calcification, but both inflammation and RAGE expression can be attenuated by pioglitazone, a thiazolidinedione normally used in clinical settings for diabetes [100]. A more profound understanding of AGE role in ECM remodeling might potentially stimulate further research on pharmacologic approaches in the next years.

Aging has been associated to a shift towards a prothrombotic balance in the secretion of hemostatic protein by VECs and the structural disarray typical of old valves contribute to sequester thrombotic mediators within the leaflet. This phenomenon on a side favors the calcific nucleation inside the leaflet, but on the other exposes to an increased risk of valve thrombosis [17]. Additionally, the reduced endothelial expression and release of the leaflet accumulated vWF might justify the clinically reported augmented sensibility of elderly patients to anticoagulant drugs and their reduced clotting ability [143]. Another important lesson from aging-related ECM biology applicable in the clinical scene regards the possible detrimental effect of vitamin K-antagonists in the management of elderly patients in need of anticoagulant treatment or bearing prosthetic valves. Inhibiting vitamin K activity through coumarinic agents blunts γ-carboxylation of MGP leading to a rescue of the BMP-2-mediated osteogenic activity with eventual initiation of the calcific processes. This process has been clinically described but is still poorly understood [78]. A recent study showed that an increase in the decarboxylated, and thus inactive, form of MGP was associated with an increase in vascular calcification [50]. Accurate knowledge on these mechanisms could on a side discourage the use of standard anticoagulant in these patients, and on the other side prompt further research on both alternative anticoagulation options and on prosthetic valve design to avoid the risk of long-term calcification.

However, this delicate balance of cellular activities finds its substrate in the valve ECM which, far to behave as a mere support, is dynamically affected by hemodynamic conditions and aging and exert an active role in the interplay with VIC and VEC providing fundamental biological signaling [184]. The evidence that structural disarray induced by fixation methods in prosthetic valve might lead to premature valve degeneration and failure, supports this idea [23, 122]. Miscomprehension of the significance of ECM role in this context might explain the failure of anti-calcification approaches uniquely directed to the cellular component of valve leaflet and might also explain the contradictory results of the SEAS trial.

However, conclusions of this review are remarking the importance of the ECM in valve aging and might trigger new experimental efforts aimed at controlling or modulating age-related changes of its components to avoid premature degeneration of both native and prosthetic biological valve.

Compliance with ethical standards

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