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Unfolding the Story of Proteoglycan Accumulation in Thoracic Aortic Aneurysm and Dissection

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Proteoglycans are important components of the extracellular matrix in vascular tissue and exert critical roles in maintaining aortic structure and function.[1] In the normal vasculature (Figure 1A), proteoglycans such as versican interact with hyaluronan to form large aggregates that enhance water retention and create reversible, compressive structures that are necessary for smoothing out pressure waves and abrogating the deformation of blood vessels.[2–5] Normal proteoglycan aggregates also pressurize the intralamellar spaces and promote the connection between smooth muscle cells (SMCs) and elastic laminae in the arterial wall, thereby facilitating mechanosensing.[5] In addition, proteoglycans maintain aortic hemostasis by regulating elastic fiber assembly[6,7] and SMC proliferation.[3,8] However, the abnormal accumulation of proteoglycans (Figure 2B) can be destructive to aortic structure and function. Recent studies using computational simulations have suggested that an abnormal increase in proteoglycan aggregate mass in the aorta can increase intralamellar

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swelling pressure, disrupt cell-matrix interactions, and delaminate the microstructure of the aortic wall, leading to compromised aortic mechanosensing and structural integrity.[5,9,10] Thus, an imbalance between proteoglycan production and turnover may disturb the integrity of aortic structure and function.[1,5,9,10]

In normal vascular tissue, the abundance of proteoglycans is low, but it is increased significantly in vascular disease.[1] Abnormal proteoglycan accumulation has been detected frequently in the degenerative aortic media of thoracic aortic aneurysms and dissections (TAAD), as described in mucoid extracellular matrix accumulation[11] and the outdated concept of "cystic medial necrosis."[12] The acidic glycosaminoglycans in proteoglycans give alcian blue staining its characteristic bluish-green color. Accumulation of glycosaminoglycans is observed often in areas with severe elastic fiber fragmentation and SMC loss. Recently, using liquid chromatography–tandem mass spectrometry analysis, Cikach et al[13] and Yin et al[14] have identified different proteoglycans and glycopeptides in ascending aortic tissues from TAAD patients, including those with Marfan syndrome. Large aggregates of aggrecan and versican were detected in ascending aortas from TAAD patients and mice with Marfan syndrome,[13,14] particularly in dissected and ruptured aortas.[13] These findings have suggested that the accumulation of aggregated proteoglycans, particularly aggrecan and versican, in the aortic media contributes to aortic degeneration in TAAD. Thus, it is critically important to understand the regulation of proteoglycan production, degradation, and turnover and the interaction between proteoglycan metabolism and other components of the aortic wall.

Proteoglycans are cleaved by matrix metalloproteases and a disintegrin-like and metalloprotease domain with thrombospondin-type motifs (ADAMTSs). In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Dupuis et al[15] describe the importance of ADAMTS5-mediated aggrecan cleavage for normal aortic wall development. The authors showed that *Adamts5*-deficient (*Adamts5*$^{-/-}$) mice displayed anomalies in the ascending aorta, such as increased aortic thickness, aortic stenosis, altered outflow tract rotation and aortic geometry, SMC loss, and blood cells in the aortic wall. These aortic abnormalities were associated with aggrecan accumulation. The authors further identified the site of ADAMTS5-mediated aggrecan cleavage that was important for aggrecan turnover and aortic development. Using antibodies that recognized neo-epitopes of aggrecan generated by ADAMTS-mediated cleavage (Figure 2C), they observed that cleaved aggrecan fragments with FFGVG, SSELE, FREEE, and GLGS neo-epitopes accumulated in aortas of *Adamts5*$^{-/-}$ mice. In contrast, they observed a reduced abundance of cleaved aggrecan fragments with the TEGE neo-epitope in the interglobular domain at the N-terminus core. These findings indicate that ADAMTS5 may be involved in aggrecan cleavage at the $TEGE_{373}{\downarrow}_{374}ALGSV$ site. Furthermore, the authors demonstrated that mice carrying a non-cleavable mutation at this site ($TEGE_{373}{\downarrow}_{374}NVYS$) displayed mild aortic abnormalities, suggesting that cleavage at this site may be important for aggrecan turnover and normal aortic wall development. These findings are in line with those of several recent studies that support an emerging role of ADAMTS5 as a key enzyme involved in proteoglycan turnover and aortic protection in mice.[16,17] Indeed, mice lacking the catalytic domain of ADAMTS5 (*Adamts5*$^{\Delta cat}$) showed aggrecan accumulation and thoracic aortic dilatation,[16] as well as exacerbated ascending aortic enlargement in mice with angiotensin II–induced aortic

dilatation.[17] Further analyses revealed that the accumulation of an ADAMTS-specific versican cleavage product (versikine) was decreased and that the abundance of versican was increased in *Adamts5*$^{\Delta cat}$ mice.[17] These findings suggest that the ADAMTS5-mediated cleavage and turnover of aggrecan and versican are critical for aortic morphogenesis during development and the maintenance of aortic integrity throughout adulthood. Others have reported the increased production of proteoglycans (particularly aggrecan and versican) in the aortic wall of TAAD tissues.[13] Together, these findings have raised the possibility that aggrecan and versican accumulation in ascending TAAD may result from increased production and reduced turnover of these proteoglycans.[13–17]

The elegant study by Dupuis et al[15] leads to several additional questions. First, it is not clear whether induction of proteoglycan production results from aortic inflammation or the adaptive response to aortic injury. Further work is needed to determine the factors and pathways that stimulate proteoglycan production and to characterize the dynamic roles of proteoglycan metabolism in aortic inflammation, injury, repair, and remodeling. Second, the comprehensive regulation of proteoglycan cleavage is not well understood. Proteoglycans are important for the homeostasis of aortic structure and function. Unwanted proteoglycan cleavage not only causes the disruption of normal proteoglycan function but also generates cleaved products that can further promote an inflammatory response. In contrast, insufficient proteoglycan cleavage and turnover cause abnormal proteoglycan accumulation. Therefore, the precise control of proteoglycan cleavage is critical. It is not clear whether site-specific cleavage is specifically responsible for degrading functional proteoglycans or clearing dysfunctional proteoglycan fragments. The possibility also remains that cleavage at the same site, through more complex regulation, has diverse effects on proteoglycan processing, degradation, and clearance. Finally, it is likely that different ADAMTS family members such as ADAMTS1,[18] ADAMTS5,[15–17] and ADAMTS4[19] are differentially involved in proteoglycan metabolism. Their specific proteoglycan substrates and their regulation of the specific cleavage sites on each proteoglycan remain to be determined. The upstream stressors and pathways that regulate ADAMTS expression and activity and subsequent proteoglycan dysfunction and/or turnover also remain to be identified. With the increasing use of advanced technologies such as proteomics (glycoproteomics, proteoglycanomics) and conditional knockout mice, our understanding of the dynamic regulation of proteoglycan metabolism will continue to unfold.

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Abbreviations

ADAMTS	disintegrin-like and metalloprotease domain with thrombospondin-type motif
SMC	smooth muscle cell
TAAD	thoracic aortic aneurysms and dissections

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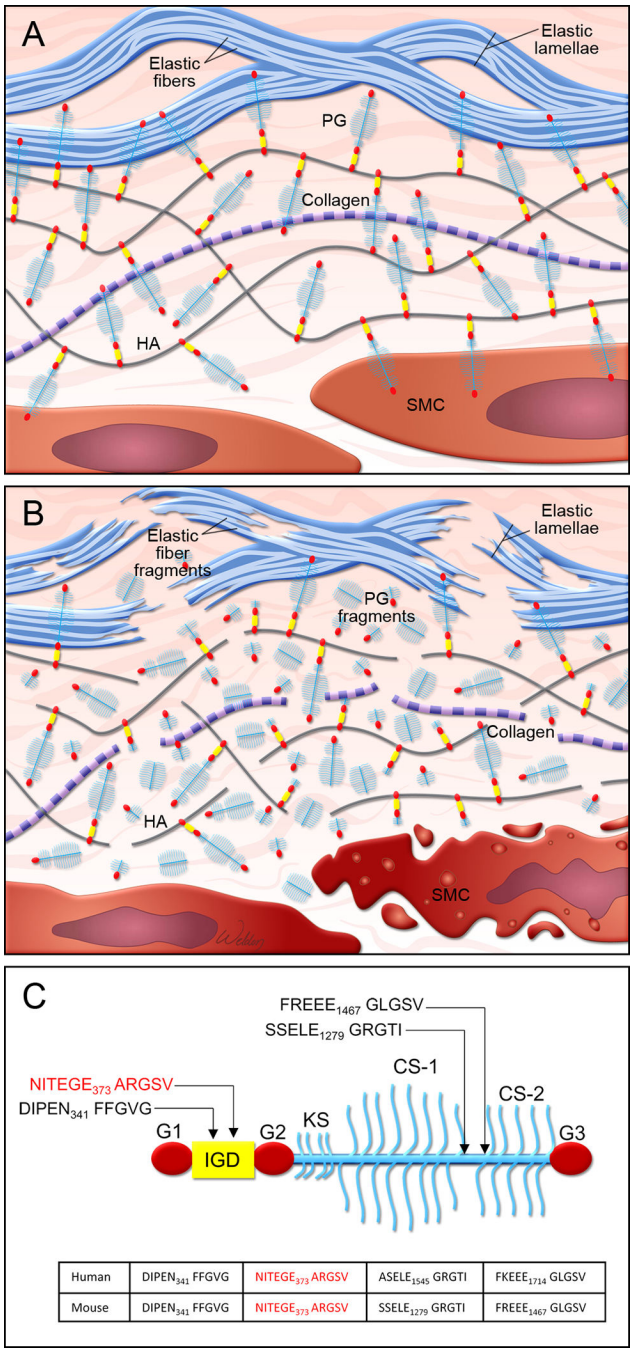

Figure. Proteoglycan metabolism in aortic wall.

A, Illustration of normal aortic wall with intact proteoglycans (PG), such as aggrecans, attached to hyaluronan (HA) and healthy smooth muscle cells (SMC). **B**, Illustration of degenerative aortic wall with accumulated proteoglycan (eg, aggrecan) fragments, elastic fiber fragmentation, and SMC death. **C**, Schematic structure of an aggrecan core protein that contains globular domains (G1, G2, and G3) and an interglobular domain (IGD). Glycosaminoglycan keratan sulfate (KS) and chondroitin sulfate (CS1 and CS2) chains are

attached to the core protein. The location of cleavage sites along the core protein is indicated.