

## The Emerging Role of the ADAMTS Family in Vascular Diseases

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**M**embers of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family are involved in the degradation of extracellular matrix (ECM) proteins during development, morphogenesis, tissue repair, and remodeling. They differ from other proteases like MMPs (matrix metalloproteinases) with respect to function, binding and anchoring properties, substrate recognition, and half-life. Thus far, the ADAMTS family has attracted less attention in vascular research than MMPs (Figure 1). Recent findings, however, point towards an important role of ADAMTS family members in atherosclerosis, restenosis, and aneurysm formation.

### ADAMTS Proteases and Hyaluronan-Binding Proteoglycans

ADAMTSs comprise a family of 19 secreted, extracellular enzymes with a common multidomain structure. Their organization consists of an N-terminal prodomain, a catalytic domain and a disintegrin-like domain, linked to an additional C-terminal region referred to as the ancillary domain. The latter is key for regulating the activity of ADAMTSs and the specification of their substrate-binding preferences. The importance of both domains in vascular biology is illustrated in context of angiogenesis where the catalytic and the noncatalytic domains revealed antiangiogenic properties.<sup>1</sup> Parallel evolution of ADAMTSs with hyaluronan and hyaluronan-binding proteoglycans alludes to their central role in the regulation of these proteoglycan substrates. Hyaluronan-binding proteoglycans are major components of the skeletal, nervous, and cardiovascular system. Their proteolysis is regulated by a subset of ADAMTSs called proteoglycanases (ADAMTS-1, -4, -5, -8, -9, -15, and -20). ADAMTS-1, -4, and -5 are the main enzymes responsible for cleaving hyaluronan-binding proteoglycans in the vasculature and will be the focus of this viewpoint (Figure 2). A shared feature of these large aggregating hyaluronan-binding proteoglycans is their tridomain structure containing a C-type lectin domain at the C-terminus, an N-terminal hyaluronan-binding domain, and a central domain harboring

the numerous glycosaminoglycan side chains. Aggregates of hyaluronan and hyaluronan-binding proteoglycans carry an enormous fixed negative charge, thus attracting counter-ions and water into the tissue. For this reason, they are responsible for vascular viscosity. They are also essential binding partners for various cell-associated and ECM molecules, including collagens, elastin, fibronectin, CD44, and integrins. Hence, although elastin and collagens are important for nonlinear elasticity of the vasculature, aggrecan and versican, the main hyaluronan-binding proteoglycans in the vasculature, give additional support and simultaneously dampen blood flow and pressure oscillations ensuring peripheral organ perfusion at steady flow and pressure because of their viscous properties.<sup>2</sup>

### ADAMTSs and Versican in Atherosclerosis

Besides granting viscous properties to the vasculature, glycosaminoglycans bound to hyaluronan-binding proteoglycans also interact with lipoproteins, in particular LDL (low-density lipoprotein). These lipid-retaining properties of the vascular ECM are key for the development of atherosclerosis, making ADAMTSs potential targets to reverse the retention of lipoproteins in the vasculature. Jönsson-Rylander et al<sup>3</sup> suggested that ADAMTS-1 may promote atherogenesis by cleaving ECM proteins, such as versican and inducing vascular smooth muscle cell migration. Ren et al<sup>4</sup> demonstrated that loss of ADAMTS-4 attenuates atherosclerosis in apoE (apolipoprotein E)-deficient mice on high-fat diet and results in a more stable plaque phenotype with reduced macrophage and lipid content. Our group first implicated ADAMTS-5 in the regulation of vascular proteoglycan turnover and lipoprotein retention in murine atherosclerosis.<sup>5</sup> Loss of ADAMTS-5 activity in aortas of young and old apoE-deficient mice on chow-fed diet was associated with an accumulation of versican and biglycan, the major lipoprotein-binding proteoglycans in atherosclerosis. Notably, ADAMTS-5 activity resulted in the release of LDL from the vascular ECM. This potent activity of ADAMTS-5 on the LDL-binding proteoglycan fraction of the ECM warrants further investigation.<sup>5</sup>

### ADAMTSs and Aggrecan in Vascular Injury

Versican is the main hyaluronan-binding proteoglycan in both veins and arteries; however, aggrecan is induced on vascular injury: using proteomics in mice fed with a stable isotope diet we observed a pronounced upregulation of aggrecan on grafting veins to the arterial circulation.<sup>6</sup> Furthermore, in a porcine model of stent-induced vascular injury, we noted an inverse association between cellularity and proteoglycan content in coronary arteries: aggrecan and versican accumulated in vessels treated with a drug-eluting stent as compared to a bare metal

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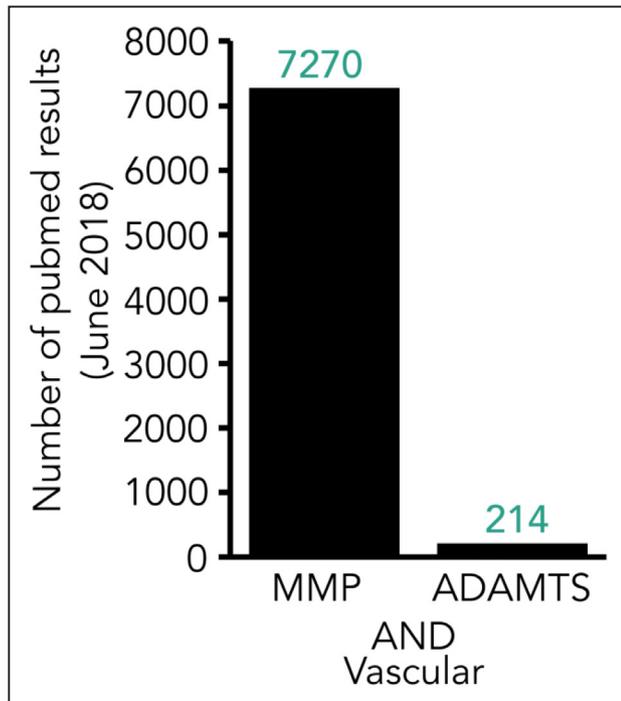
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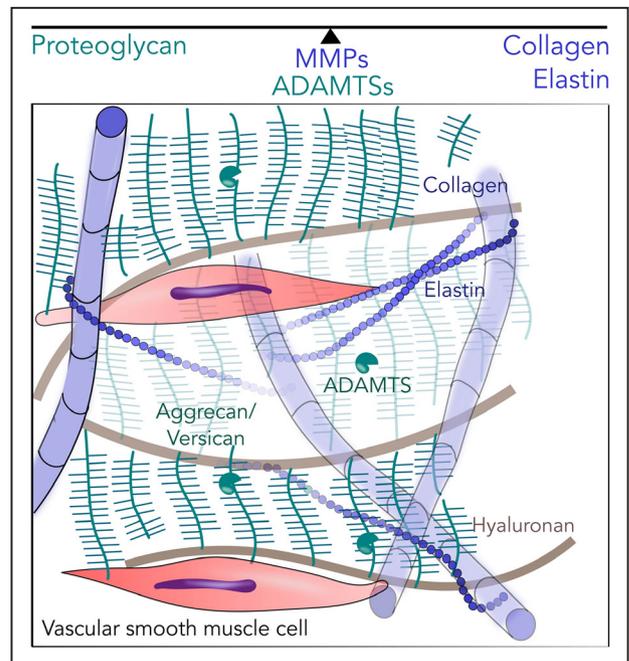


**Figure 1.** Current literature on MMPs (matrix metalloproteinases) and ADAMTSs (a disintegrin and metalloproteinase with thrombospondin motifs) in the vasculature. Number of entries returned in a Pubmed search for “MMP and vascular” or “ADAMTS and vascular”.

stent.<sup>6</sup> These changes were accompanied by reduced expression of Adamts-1 and -5, but an increase in Adamts-4.<sup>6</sup> The observation that aggrecan, the main large aggregating proteoglycan in cartilage, is higher in arteries than in veins, induced in coronary arteries after stenting, and by grafting veins from a low- to a high-pressure environment is intriguing. Larger arteries require increased viscosity compared with veins because of blood pressure propagation. Although the precise role of aggrecan in arterial viscoelasticity remains to be explored, a recent report has linked aggrecan degradation to vascular stiffness.<sup>7</sup> Notably, MMPs are less efficient in cleaving aggrecan compared with ADAMTSs (Figure 2).<sup>8</sup> Correspondingly, proteomics analysis revealed an accumulation of aggrecan in murine aortas lacking ADAMTS-5 activity.<sup>6</sup>

### ADAMTSs in Aneurysmal Disease

Interestingly, Oller et al<sup>9</sup> showed that haploinsufficiency of ADAMTS-1 in mice leads to thoracic aortic aneurysm formation. In contrast, we found that ADAMTS-5 deficient mice also display increased aortic dilatation in response to Ang II (angiotensin-II)-induced hypertension, despite a compensatory increase of ADAMTS-1.<sup>10</sup> On Ang II infusion, reduced versicanolysis resulted in an exacerbated build-up of versican in the aortic wall. This reduced versican cleavage was still observed despite higher ADAMTS-1 levels.<sup>10</sup> Aggrecan and versican accumulation is also evident in human thoracic aortic aneurysm together with a downregulation of *ADAMTS-5* expression.<sup>11</sup> Thus, the balance of ADAMTSs may affect the development of aneurysm formation by influencing the abundance of the proteoglycans involved in maintaining the viscoelastic properties of the vasculature. In addition,



**Figure 2.** ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) proteases regulate proteoglycan content. Members of the ADAMTS family degrade versican and aggrecan. These hyaluronan-binding proteoglycans are the main components responsible for vascular viscosity. Although extracellular matrix components like elastin and collagens are predominantly degraded by MMPs (matrix metalloproteinases), hyaluronan-binding proteoglycans, such as versican and aggrecan, are mainly regulated by members of the ADAMTS family. Decreased ADAMTS activity results in proteoglycan accumulation, which has been associated with lipid retention and atherosclerosis, as well as elastin breaks and aortic aneurysm formation. Thus, members of the ADAMTS family as key regulator of proteoglycan turnover should be studied more widely in the context of vascular diseases.

haploinsufficiency of ADAMTS-1 resulted in a lower systolic and diastolic blood pressure. Similarly, mice lacking the catalytic domain of ADAMTS-5 had lower blood pressure in response to Ang II-induced hypertension. Oller et al<sup>9</sup> convincingly demonstrate that haploinsufficiency of ADAMTS-1 mediates aortic dilation by upregulating inducible nitric oxide synthase yet the precise mechanism remained elusive. Although the proposed target, syndecan-4, has been implicated in nitric-oxide-mediated vasodilation<sup>9</sup> and can be cleaved by ADAMTS-1,<sup>12</sup> its accumulation was not shown in aortas of ADAMTS-1 haploinsufficient mice. Also, syndecan-4 transgenic animals have no pronounced vascular phenotype and normal blood pressure.<sup>13</sup> To further our understanding of the function of proteases, all ECM constituents should be taken into consideration. This is best achieved using proteomic techniques.

### Regulation of ADAMTS Activity by Cellular Uptake

LRP1 (low-density lipoprotein receptor-related protein 1) acts as a protease sink for ADAMTS-5 regulating its abundance, hence, affecting its proteolytic activity.<sup>14</sup> We have recently shown that silencing of *LRP1* in human aortic smooth muscle cells is associated with reduced gene expression of ADAMTS-5 and a concomitant increase in the extracellular

bioavailability of ADAMTS-1.<sup>10</sup> Similarly, on treatment with Ang II, aortas from ADAMTS-5 deficient mice displayed lower levels of LRP1 but increased abundance of ADAMTS-1. Thus, ADAMTS enzymes must not be studied individually, but the cross-talk of the different ADAMTS proteases and their receptors has to be taken into consideration. It is also plausible that hyperlipidemia may influence vascular ECM homeostasis not only by inducing the expression of proteases but also by impairing their cellular uptake. LRP1-mediated endocytosis of lipoproteins could impact the uptake of proteases from the extracellular space and shift the balance towards proteolysis in the vessel wall.

### Regulation of ADAMTS Activity and Substrate Recognition by Glycosylation

In addition to their abundance, ADAMTS activity depends on a number of other factors including temperature, pH, substrate availability, coenzymes, inhibitors, and posttranslational modifications. One of the major posttranslational modifications regulating ADAMTS activity is glycosylation. In fact, all ADAMTS enzymes, with the exception of ADAMTS-4, are N-glycosylated and, in addition, their ancillary domains are subjected to proteolytic processing affecting their secretion, localization, and activity. Thus, ADAMTS activity can be increased directly by higher enzyme expression, indirectly by influencing LRP1-mediated ADAMTS uptake, but also by posttranslational modifications affecting ADAMTS secretion and activity. Glycosylation alters both ADAMTS activity and the susceptibility of certain ECM substrates for ADAMTS-mediated cleavage. Indeed, chondroitin sulfate side chains on versican are mandatory for proteolysis by ADAMTS-5 and ADAMTS-1.<sup>15</sup> Understanding how glycosylation affects ADAMTS activity and substrate specificity may reveal a new therapeutic strategy.

### Concluding Remarks and Future Perspectives

The late evolutionary rise of proteoglycanases and their main substrate allowed for the development of a more complex viscoelastic vasculature. Although the importance of arterial elasticity (elastin, collagens) has been commonly accepted in vascular disease, arterial viscosity provided by hyaluronan-proteoglycan aggregates is less well studied in the vasculature. Proteoglycanases of the ADAMTS family regulate proteoglycan turnover in the vasculature. Therefore, they have potential as therapeutic targets in atherosclerosis and aneurysm formation. Here, specific attention should be given to glycosylation as a potential mechanism for influencing ADAMTS activity and substrate recognition. Although, at present, our knowledge on the extent of ECM glycosylation and the subsequent functional consequences is limited, novel mass spectrometry methods for the analysis of intact glycopeptides will pave the way in this area of research.

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