ADAMTS7 in cardiovascular disease: From bedside to bench and back again?

Alicia G. Arroyo, MD, PhD[1], Vicente Andrés, PhD[2]

[1] Laboratory of Matrix Metalloproteinases in Angiogenesis and Inflammation, and [2] Laboratory of Molecular and Genetic Cardiovascular Pathophysiology, Vascular Biology Program, Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain

Correspondence to:

V. Andrés, CNIC, Melchor Fernández Almagro 3, 28029 Madrid (Spain)

Phone: +34-91 453 12 00 (Ext. 1502)

Fax: +34-91 453 12 65

E-mail: vandres@cnic.es

Metzincins, a family of zinc metalloproteinases able to process all the extracellular matrix (ECM) components, include the matrix metalloprotease (MMP), a disintegrin and metalloproteinase (ADAM), and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) subfamilies. Metzincins are important regulators of tissue remodeling, particularly vascular remodeling during atherosclerosis development.[1] In the atherosclerotic artery wall these enzymes cause profound alterations to the ECM, and these alterations instigate changes in the behavior of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs).[2] Recent genome-wide association (GWA) studies have identified *ADAMTS7* as a novel locus associated with human coronary atherosclerosis.[3, 4] However, a causal link between this secreted zinc metalloprotease and atherosclerosis has yet to be established. In this issue of *Circulation*, Bauer and colleagues[5] directly address this hypothesis by generating whole-body knock-out mice for *Adamts7* for the investigation of atherosclerosis development. The authors crossed *Adamts7*-null mice with the atherosusceptible *apoE*-KO and *Ldlr*-KO mouse models and found that deletion of *Adamts7* significantly reduced atherosclerotic lesion formation in the aortas and aortic roots of both hyperlipidemic strains. The atheroprotective effect of *Adamts7* deletion occurred without significant changes in plasma lipid levels or plaque composition, and was associated with impeded migration of *Adamts7*-null VSMCs in response to TNFα. The early and transient upregulation of Adamts7 in the plaques of atheroprone mice suggests that Adamts7 makes an important contribution at early stages of the disease. However, mouse models are of limited use for the analysis of late atherosclerotic and thrombotic events, and ADAMTS7 is expressed at all stages in human plaques. Therefore further analysis is clearly required in order to understand the significance of these observations. Consistent with the association of *ADAMTS7* with atherosclerosis but not with myocardial infarction, Bauer et al[5] detected a tendency of plaques in *Adamts7*-null mice to develop a larger fibrous cap, a finding that should be fully explored to determine whether targeting ADAMTS7 in patients might result not only in decreased atherosclerosis but also in more stable plaques. Studies are also needed to investigate whether atherosclerosis-associated *ADAMTS7* genetic variants associate with restenosis or arterial calcification, as recently suggested.[6] Overall, the studies presented by Bauer at al[5] provide the first firm evidence

that mouse Adamts7 plays a proatherogenic role, likely through the promotion of VSMC migration.

The long-term success of percutaneous coronary intervention is limited by restenosis, a pathological process characterized by excessive neointimal thickening caused by the inflammatory response associated with mechanical injury to the vessel wall.[7] Like native atherosclerosis, restenosis involves activation of zinc metalloproteinases that alter the ECM.[2] The studies by Bauer and colleagues[5] and Kessler and colleagues[8] (a second study also published in this issue of *Circulation*) both report that genetic deletion of *Adamts7* reduces neointimal thickening after wire injury to the femoral and carotid arteries. These results are consistent with previous studies showing that Adamts7 increases neointima formation in balloon-injured rat arteries by stimulating VSMC migration through the degradation of cartilage oligomeric matrix protein (COMP, also called thrombospondin-5).[9] Kessler and colleagues[8] shed further light on the role of Adamts7 in vascular remodeling by focusing on vessel re-endothelialization, which is inversely related to neointima formation. They found that Adamts7 inhibits EC proliferation and migration in vitro and that reendothelialization is strongly augmented in the injured vessels in *Adamts7*-null mice.[8] Surprisingly, COMP expression did not affect EC proliferation/migration in vitro, and *Comp* deficiency had no effect on reendothelialization in injured arteries, suggesting that Adamts7 retards endothelium repair via COMP-independent mechanisms.[8] Using label-free LC MS/MS secretome analysis, coimmunoprecipitation strategies and mammalian two-hybrid analysis, Kessler and coworkers[8] found that Adamts7 can bind directly to thrombospondin-1 (TSP-1) and degrade it in vitro. In agreement with earlier mouse studies showing the beneficial effects of TSP-1 inactivation on reendothelialization and neointima formation,[10] the inhibitory effect of Adamts7 overexpression on EC proliferation and migration was blunted in *Tsp-1* silenced endothelial cells in vitro, and Adamts7-dependent inhibition of reendothelialization was circumvented in *Tsp-1*-null mice.[8] The study by Kessler et al[8] thus suggests that ADAMTS7 exerts complementary functions in neointima formation by selective and cell-type-dependent substrate processing: COMP cleavage mediating augmented VSMC migration whereas TSP-1 degradation mediates impaired EC recovery (Figure 1). However, the in vivo relevance of Adamts7-mediated processing in EC responses during neointima formation remains

undefined. EC-specific *Adamts7* deletion in conditional mouse models will help to confirm the EC-selective function of Adamts7 and reconcile the data about its expression and function in ECs in vivo (see below).

Both *Adamts7*-null mouse strains have a *LacZ* reporter gene in the gene-trapping cassette, allowing for X-gal staining as readout of active *Adamts7* expression, which was detected in heart tissue and pulmonary vasculature.[5, 8] This staining allowed analysis of the dynamics of Adamts7 expression in SM-α-actin-immunoreactive cells in response to mechanical vascular injury and hyperlipidemia, revealing an early, transient upregulation,[5] consistent with the action of ADAMTS7 as a positive regulator of neointimal thickening. Notably, previous in vitro findings showed upregulation of ADAMTS7 expression in VSMCs by inflammatory cytokines (TNFα, IL-1, PDGFB), but not by anti-inflammatory cytokines (TGFB) or oxidized LDL.[4] This would suggest that ADAMTS7 responds to inflammation rather than to hyperlipidemia, in line with the recognized role of ADAMTS7 in arthritis.[4] The study by Bauer et al[5] also provides insight into the cell distribution of Adamts7, which is mainly detected in the media and adventitia of mouse aortas but not in ECs. Further expression studies are needed to clarify the spatial and temporal pattern of ADAMTS7 expression in the cell types of the injured vessel wall—including direct immunohistochemical detection of *Adamts7* in rodent arteries using specific antibodies and quantification of expression levels (e.g. real-time PCR, western blot). In contrast to the Adamts7 expression pattern in the media and adventitia of mouse arteries, immunohistochemistry analysis in human coronary and carotid arteries revealed ADAMTS7 expression in only a proportion of VSMCs in atherosclerotic plaques, predominantly near the media-intima border and the fibrous cap.[5, 11] The absence of ADAMTS7 staining in CD68-labeled macrophages indicates the need for further work to expand the repertoire of molecular markers for selective cell subsets, in order to better define the populations expressing ADAMTS7 in the intima of human atherosclerotic plaques. It will be also important to analyze ADAMTS7 expression in human atherosclerotic and restenotic lesions at different stages of disease progression.

The study by Bauer et al[5] positions ADAMTS7 in primary aortic VSMCs in specialized membrane protrusions called podosomes, which are actively involved in matrix

degradation and cell invasiveness. In this location, ADAMTS7 might associate with adhesion receptors such as integrins or other proteases to exert coordinated functions in vascular remodeling.[12] Given the emerging idea that podosomes can sense matrix stiffness,[13] it is appealing to propose that ADAMTS7 modulates matrix tension in the vessel wall by processing COMP near podosomes to interfere with its binding to α7β1 integrin—a recognized mechanosensor at myotendinous junctions[14]—which could ultimately lead to pathologic vascular remodeling.[15]

ADAMTS7 posseses mucin-proteoglycan domains, and interacts with COMP through its four C-terminal TSP repeats.[4] Additional structural studies of the ADAMTS7/COMP complex will shed light on the potential value of targeting the ADAMTS7 catalytic site or selective exosite binding motifs to avoid adverse effects on related proteases such as ADAMTS12, which is also able to process COMP.[16] The identification by Kessler et al[8] of the matricellular protein TSP-1 as a novel ADAMTS7 substrate in ECs is important, but the selectivity of this processing and its role in vascular remodeling is as yet unclear since TSP1 can be cleaved by other metalloproteases, including ADAMTS1, ADAMTS13 and MT1-MMP.[17] It will also be important to identify the ADAMTS7 clevage sites in COMP and TSP-1 and define whether they are unique or shared with other metalloproteases, and whether cleavage can generate bioactive polypeptide fragments able to bind cell receptors that trigger EC and VSMC responses. The identification of specific ADAMTS7 cleavage sites would also permit direct in vivo investigation of the relevance of COMP and TSP-1 processing to atherosclerosis by generating cleavage-resistant knock-in mice, as previously achieved for collagen I processing.[18] Since ADAMTS7 is thought to be a nonredundant member of the ADAMTS family,[4] the search for other unique ADAMTS7 substrates and interacting proteins in the artery wall might also provide new opportunities for therapeutic intervention.

The studies by Bauer and colleagues[5] and Kessler and colleagues[8] conclusively demonstrate a pro-atherogenic role for mouse *Adamts7*. A key outstanding question is whether any of these laboratory findings in mouse models can be translated back to the clinic (Figure 2). Recent studies have begun to assess whether human *ADAMTS7* alleles associated with high risk of coronary atherosclerosis are linked to higher ADAMTS7

expression or activity in tissues and cells involved in disease development. For example, the rs3825807 G/G genotype in the *ADAMTS*7 locus, which is associated with lower atherosclerosis prevalence and severity, reduces not the expression of ADAMTS7 but its maturation and activity, resulting in reduced COMP cleavage and attenuated VSMC migration.[11] It will be of interest to assess whether the rs3825807 G/G genotype also affects TSP-1 degradation in ECs. Further research in this area could lead to personalized medicine based on the identification of *ADAMTS7* genetic variants in patients with atherosclerosis who could benefit from strategies targeting this proteolytic pathway. Quantification of COMP or TSP-1 fragments in plasma of these patients might also provide valuable information about the severity or progression of atherosclerotic disease, as shown for COMP in arthritis.[19] Despite the lack of success with inhibitors of the closely related MMP subfamily, the strong GWAS association of *ADAMTS7* with atherosclerosis, together with the solid knowledge being generated about the mechanisms of action of ADAMTS7-mediated vascular remodeling, may pave the way for the development of novel strategies to ameliorate atherosclerosis and restenosis. These therapeutic approaches might include targeting ADAMTS7 catalytic or C-terminal exosites, locking the ADAMTS7 propetide-catalytic domain conformation (as in the G/G rs3825807 variant), delivering substrates to restore homeostasis (e.g. via viral-based approaches), inhibiting signaling pathways triggered by ADAMTS7 substrate fragments, or decreasing ADAMTS7 expression by miR29-mimics.[20]

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CONFLICT OF INTEREST DISCLOSURES

None.

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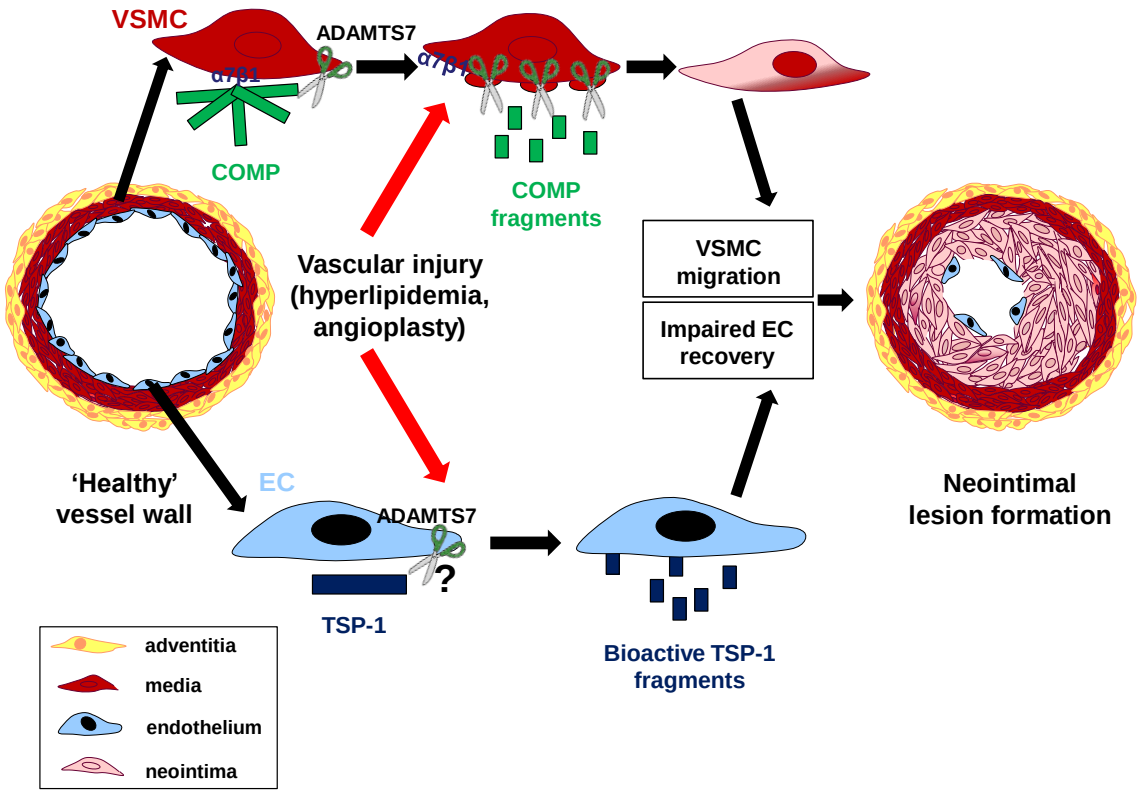

Figure 1. **ADAMTS7-mediated actions on vascular smooth muscle cells and endothelial cells promote neointima formation**. ADAMTS7 expression is upregulated in vascular smooth muscle cells (VSMCs) upon vascular injury, leading to processing of the α7β1 integrin ligand COMP (cartilage oligomeric matrix protein) and increased VSMC migration. Complementary actions of ADAMTS7 have been proposed in endothelial cells (ECs) via cleavage of thrombospondin-1 (TSP-1), resulting in bioactive TSP-1 fragments that would reduce EC migration and proliferation and thus impair EC recovery. However, more evidence is needed about ADAMTS7 expression and actions on ECs in vivo. The combined effect of Adamts7-mediated increased VSMC migration and impaired re-endothelialization ultimately leads to increased neointima formation.

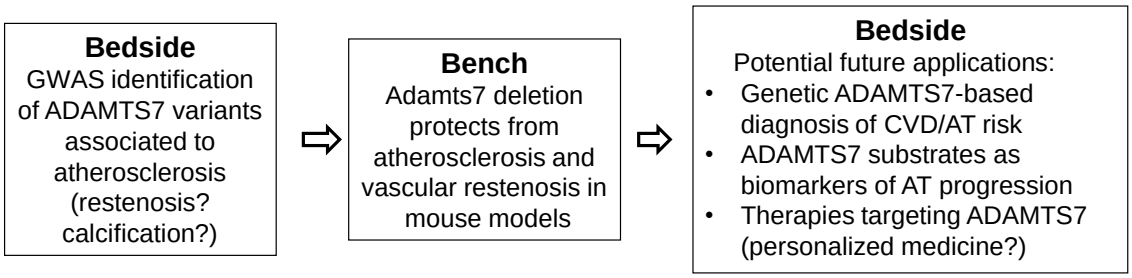

Figure 2. Proposed translation of research on ADAMTS7 in atherosclerosis and cardiovascular disease from bedside to bench and back again. Clinical and basic research on ADAMTS7 in cardiovascular disease (CVD) exemplifies successful translational research. The identification of ADAMTS7 variants associated with atherosclerosis (AT) by GWAS in patients prompted basic researchers to generate loss-of-function mouse models to directly test the pathogenic role of ADAMTS7 in atherosclerosis. Two independent studies in this issue of Circulation show that *Adamts7* deletion protects mice from atherosclerosis and restenosis.[5, 8] Potential applications of Adamts7-related laboratory findings in the clinic include genetic analysis of CVD risk, use of ADAMTS7 substrates as biomarkers for AT progression, and personalized medicine in selected patients with pathogenic ADAMTS7 variants.