MMP17/MT4-MMP and Thoracic Aortic Aneurysms
OPNing New Potential for Effective Treatment

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In recent years, many advances have been made in understanding the pathophysiology of thoracic aortic aneurysms and dissections (TAAD). Mutations in several genes leading to aneurysm formation have been identified thus far, and include extracellular matrix (ECM) genes (FBN1, COL3A1, EFEMP2, MFAP5), molecules involved in transforming growth factor-β (TGFBR1, TGFBR2, SMAD3, TGFβ2, TGFβ3), and genes involved in regulation of the smooth muscle cell (SMC) contractile apparatus (MYH11, ACTA2, MYLK, PRKG1). However, for the majority of TAAD patients, a causative genetic mutation has not yet been identified. A whole-exome sequencing approach to identifying new genes predisposing to TAAD has provided a means of rapidly identifying additional genetic alterations.

Studies using mouse models have recapitulated some key molecular pathways disrupted by mutations in currently identified TAAD genes, including angiotensin II and transforming growth factor-β-mediated signaling pathways, vascular SMC phenotype, and pathways regulating SMC contractile function. In addition, multiple studies have highlighted the importance of the connections formed between the concentric layers of elastic fibers and SMCs in the aortic wall for transducing mechanical signals and maintaining vascular contractility and homeostasis.

Despite advances in our understanding of the mechanisms underlying the development of aortic aneurysms, current pharmacological treatment of TAAD is limited. β-adrenergic receptor blockers are currently recommended for reducing aortic wall stress and potentially slowing the rate of aneurysm growth. Angiotensin II receptor blocker losartan showed great potential in preventing aortic aneurysms in a mouse model of Marfan syndrome: however, a larger scale clinical trial in Marfan patients showed losartan and the β-adrenergic receptor blocker atenolol to be equally effective. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers were used in a cohort of patients with abdominal aortic aneurysms (AAAs) and resulted in reduced mortality rates but no reduction in the rate of surgeries to repair AAAs.

One of the hallmark characteristics of pathology for both thoracic and abdominal aneurysms is matrix degradation, and matrix metalloproteinases (MMPs) are well-established culprits in promoting this degradation, regardless of the underlying mutation. The MMP family of proteins has 23 members including 5 membrane-type (MT)-MMPs, with many substrates identified, including both ECM and matrix cellular proteins. MMP2 and MMP9 are the most well-studied elastin degrading MMPs in both thoracic and abdominal aneurysms. MMP14 and MMP19 were also reported to be increased in TAA. Their role in matrix degradation has led to the idea that blocking of MMPs should either slow or prevent aortic disease progression. Treatment with doxycycline improved the aneurysm phenotype in a fibrillin-1 hypomorphic mouse model of Marfan syndrome (mGr/mGr), and survival time was increased by breeding onto an Mmp2-null background. Although initially promising in mouse models, the use of doxycycline to treat AAA has had mixed results. Interestingly, in mice, deletion of Mmp2 improved the aortic phenotype in CaCl2-induced AAA; however, this resulted in increased susceptibility to TAA after angiotensin II infusion.

Taken together, these studies highlight the complex role of MMPs in aneurysm formation. The diversity of MMP substrates and their effects on the aortic wall adds an additional layer of complexity to the role(s) of MMPs in TAAD. However, for some MMPs, including MMP17/MT4-MMP, relatively few substrates have been confirmed, and substrate identification is an ongoing process. Osteopontin is a matrixcellular protein highly expressed in the aortic wall and is shown to be targeted for cleavage by MMP-2, -3, -7, -9, and -12. Osteopontin promotes atherosclerosis through its functions in SMC survival, adhesion, and migration, promotes inflammation of carotid plaques in hypertensive patients, and mitigates vascular calcification. When considering the importance of transduction of mechanical signals in the aortic wall, osteopontin is of particular interest because it contains an arginine-glycine-aspartate (RGD) sequence as well as a cryptic integrin–binding site revealed by proteolytic cleavage by thrombin. The N-terminal domain of...
osteopontin can also act on MMPs by enhancing the activity of pro-MMP9. Furthermore, osteopontin has been found to be increased in some instances of TAA. However, much still remains unknown about specific functions of osteopontin in the aortic wall, and its role in aneurysm formation, including the degree to which it is cleaved in the aortic wall.

In this issue of Circulation Research, Martin-Alonso et al reported a missense mutation in MMP17 gene (p.Arg373His) as the possible causal mutation for acute ascending aortic aneurysms using whole-exome sequencing. The R373H mutation was predicted to cause a conformational change in the C-terminal region that hinders the binding of the glycosylphosphatidylinositol (GPI)-tail to the endoplasmic reticulum and form an energy unstable heterodimer with wild-type MMP17. Indeed, the authors showed compromised expression of R373H protein in transfected cells, suggesting a functional homozygous loss, which is surprising considering the well-known functions of MMPs as matrix degrading enzymes and the increased expression reported in aortic aneurysms in mice and humans.

The authors then turned into a mouse model (Mmp17−/−) to investigate the impact of loss of MMP17 in the aortic development and aneurysm formation. Although these mice showed a marginal increase in aortic lumen diameter, no spontaneous aneurysm or dissection was observed. However, when challenged with chronic infusion of angiotensin II or carotid artery ligation, the mutants exhibited higher incidence of TAA (but not AAA) and developed more prominent neointimas, respectively, suggesting an altered response to pathological stimuli and dysfunctional SMCs. Ultrastructural analyses revealed various morphological abnormalities in the Mmp17−/− aorta, including radially oriented SMCs with round cell shape, disconnection of SMCs from elastin lamelli, an increase in collagen fibers, altered distribution of SMC marker proteins, and a transient increase in proliferating SMCs, affecting the maturation of SMCs (Figure).

During mouse embryogenesis, SMC progenitors from various embryonic origins migrate and surround the endothelial tube in a location-specific manner beginning at around E10.5 and gradually add layers of SMCs to form the aortic wall. The majority of vascular ECM proteins, including fibrillar collagens (I, III, and VI), elastin, and fibrillin-1, are upregulated at around E14.5 following the recruitment of SMC progenitors and continue to be expressed until the early postnatal period. These ECM proteins provide structural support with tensile strength and elasticity and form connections with SMCs. Interestingly, Mmp17 expression was detected in periarterial progenitors at E10.5 to E11.5 and peaked at E14.0, suggesting a potential role for MMP17 in the early construction of the aortic wall and SMC maturation. This led to 2 important questions. First, what is the molecular mechanism of MMP17 function in these processes? Second, is the proteolytic activity required for the MMP17 function, and if so, what are the biologically relevant substrate(s) for MMP17?

The authors addressed these questions first by showing that lentivirus-mediated rescue of the aortic phenotype in MMP-17−/− mice was dependent on the catalytic activity of MMP-17. What is surprising, however, is that although MMP-17 exerts its function during embryogenesis, immediate postnatal restoration of MMP17 activity was sufficient to achieve full rescue of the Mmp17−/− aorta. Second, the authors used SILAC (stable isotope labeling of amino acids in culture) to compare protein expression between wild-type and Mmp17−/− cells and identified osteopontin as a putative substrate among others for MMP17-mediated cleavage. The authors determined that the cleavage site was between Asp210 and Leu211 in human osteopontin, which was consistent with a previously determined MMP cleavage site, and demonstrated the presence of liberated N-terminal osteopontin fragments (N-OPN) in the developing aorta, which was reduced in the Mmp17−/− aorta and led to decreased amounts of phospho-c-Jun N-terminal kinase (p-JNK). Reintroduction of N-OPN, but not full length osteopontin, restored p-JNK levels and reduced phospho-H3-positive proliferating cells in the aortic wall and improved SMC morphology in vitro, establishing a cleavage-specific function of osteopontin in vivo.

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**Figure.** Loss of matrix metalloproteinase (MMP17) affects maturation of vascular smooth muscle cell (VSMC) and integrity of the aortic wall. Osteopontin (OPN, blue) is a biologically relevant substrate for MMP17 during aortic development. Cleaved N-terminal OPN (N-OPN), but not full-length OPN, phosphorylates c-Jun N-terminal kinase (JNK) in SMCs and facilitates SMC maturation, as well as forms connections to elastic lamina. Loss of MMP17 results in increased susceptibility to angiotensin II (AngII)-induced thoracic aortic aneurysms (TAA).
Martin-Alonso et al provided compelling evidence for the biological function of N-OPN generated by MMP17 in the aortic development. However, the results from this study also raise several important questions for follow-up. First, osteopontin was shown to be cleaved by multiple proteases, including MMP3, MMP7, thrombin, plasmin, and cathepsin D at multiple sites containing the RGD sequence. How is the specificity of osteopontin cleavage by MMP17 determined during embryogenesis? Is MMP17 the only relevant protease expressed at the right time and right location? Second, what is the signaling pathway for N-OPN and its relationship with full-length osteopontin? The RGD site in osteopontin has been shown to bind subsets of integrin heterodimers, including αvβ3, αvβ1, α5β1, and α8β1. The differential biochemical activities for N-OPN and full-length osteopontin have begun to be addressed in various disease settings. Experiments using purified proteins in cell culture system should allow for comparison of the differential effects on migration and proliferation of vascular SMCs. Third, the intracellular signaling pathway mediated by N-OPN was shown to involve p-JNK; however, p-JNK positive cells constitute a subset of cells in the developing aorta. The nature of these p-JNK positive cells, including whether they represent specific progenitor cell population or how they contribute to the maturation of entire aortic wall, warrants further investigation.

Fourth, the authors clearly showed that MMP17 was required for the development of contractile filaments, correct positioning and orientation of SMCs within the elastic lamella, and formation of connections to elastic fibers. This structural continuity between SMC and elastic lamina is essential to prevent development of thoracic aortic aneurysms. In addition, ECM exhibits profound effects on SMC phenotypes and induces differential responses to stiffness-induced mechanotransduction. The current study further demonstrated that remodeling of ECM and liberation of cleaved fragment by MMP17 during embryogenesis plays a critical role in establishing and maintenance of the vessel integrity, although the long-term rescue effects of N-OPN and whether Mmp17−/− aortas with N-OPN overexpression prevents from angiotensin II-induced TAA need to be addressed in future studies. Finally, the identification of a specific MMP17-cleaved osteopontin fragment raises the question of whether other matricellular or ECM protein fragments cleaved by MMPs also have important roles in the aortic wall. The identification of MMP17 substrates other than osteopontin may facilitate our understanding of aortic remodeling in pathological conditions accompanied by increased inflammatory responses. Further consideration of the balance between degradation of ECM and generation of ECM fragments is required to develop effective therapeutic strategies using MMP inhibitors for aortic aneurysms.

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References


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