1-24-2017

Transforming Growth Factor-β in Thoracic Aortic Aneurysms: Good, Bad, or Irrelevant?

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Digital Object Identifier (DOI)
https://doi.org/10.1161/JAHA.116.005221

Notes/Citation Information
Published in *Journal of the American Heart Association*, v. 6, issue 1, e005221, p. 1-3.

American Heart Association | American Stroke Association

Transforming Growth Factor-β in Thoracic Aortic Aneurysms: Good, Bad, or Irrelevant?

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Marfan syndrome is a multisystem disorder, but its most devastating manifestations are aortic aneurysms and dissection. This syndrome results from mutations in *FBN1*, which encodes fibrillin-1, a microfibrillar protein that decorates the surface of elastin fibers.[1,2] Over 100 known mutations in *FBN1* cause Marfan syndrome, resulting in a wide variance of clinical presentations in affected individuals.[3] While the genetic etiology of this disease is known, the mechanism explaining how these mutations promote a focal defect in the aorta has not been defined. One proposed hypothesis is through the intermediary role of a cytokine, transforming growth factor β (TGF-β).[4] A publication in this issue of *JAHA* addresses the complex roles of TGF-β in development of experimental aortopathies.[5]

TGF-β is secreted in a latent form as a complex that includes the cytokine, a latency-associated peptide, and 1 of 3 members of the latent TGF-β binding protein (LTBP) family. This complex retains TGF-β in an inactive form by binding to extracellular matrix elements, including fibrillin-1. It has been proposed that fibrillin-1 mutants associated with Marfan syndrome reduce the binding of this complex to facilitate release of bioactive TGF-β. TGF-β has 3 isoforms that are sequentially numbered. While increased total TGF-β1 protein has been detected in tissue surgically removed from individuals with aortopathies, there was no detected increase of bioactive TGF-β1, as defined by the presence of the 25 kDa form. TGF-β2 and 3 were detected in minimal amounts.[6] Although increased bioactive TGF-β has not been detected in Marfan syndrome–induced aortopathy, evidence for increased activity of this cytokine has been inferred from increased presence of mediators of intracellular signaling, primarily the phosphorylated form of SMAD2 (pSmad2). Indirect evidence supporting the role of excessive TGF-β in promoting aortopathies is that down-regulation of LTBP3 attenuated disease and was associated with decreased abundance of pSmad2.[7] More directly, a seminal publication demonstrated reduced dilation of the ascending aorta in fibrillin-1 haploinsufficient mice following administration of an antibody that neutralized activity of all TGF-β isoforms.[8] Neutralization of TGF-β also reduced aortic size in hypercholesterolemic mice with CXCL10 deficiency during chronic angiotensin II infusion.[9] Profound TGF-β neutralization using the mouse monoclonal antibody, 1D11, also improved survival in fibrillin-1 hypomorphic mice.[10]

While these early studies present a case for inhibition of TGF-β being a therapeutic strategy, evolving literature has painted a more confusing landscape for the role of TGF-β in aortopathies.[11,12] This includes studies in which manipulation of TGF-β activity provided diametrically opposing data: increased TGF-β is protective against aortopathies. These studies have either administered TGF-β neutralizing antibodies or genetically manipulated TGF-β and its receptors to attenuate physiological function.

Several studies have determined the effect of neutralizing TGF-β antibodies on experimental aortopathies with variable results. Some studies have demonstrated that administration of TGF-β antibodies had no effect on AngII-induced aortic dilation during profound neutralization.[13] Conversely, profound TGF-β neutralization has been demonstrated to increase aortic rupture rates and aneurysmal expansion in both fibrillin-1 hypomorphic mice and those chronically infused with AngII.[10,13,14] These studies reported increased incidence of aortic dissection and rupture in both abdominal and thoracic regions.

The role of TGF-β deficiency has also been studied, but the low postnatal viability of mice deficient in its different isoforms is a barrier to defining the effect on aortic diseases. One study has demonstrated augmented aortic root aneurysms in both TGF-β2 heterozygous deficient and fibrillin-1 haploinsufficient mice.[15] Although these mice demonstrated

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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J Am Heart Assoc. 2017;6:e005221. DOI: 10.1161/JAHA.116.005221.

decreased TGF-β2, the data were interpreted as being attributable to increased activity of TGF-β1. This needs to be tested directly and can now be defined with the availability of TGF-β1 floxed mice.

Recently, there have been several aortic studies in mice where the major TGF-β receptors (TGF-βR) have been either deleted or modified to decrease function. These mutations have primarily focused on TGF-βR1 and R2 that are obligate heterodimers for TGF-β signaling. Development of adult mice with deletion of either of the receptors has been hampered by embryonic lethality. Interestingly, embryonic lethality is attributed to maldevelopment of thoracic aortic smooth muscle cells leading to death secondary to aortic rupture.[16,17] Currently, there have been 2 major approaches. One was to develop functional mutants that attenuate TGF-β signaling.[18] Mice developed with this approach have reduced ability to promote TGF-β signaling and have markedly enhanced expansion of the aorta. A point of controversy in the interpretation of these data is that while these mutations decreased TGF-β signaling in cultured cells, they did not influence the abundance of pSmad2 in aortas from mice expressing these mutants. In fact, immunohistochemical staining of aortic tissue detected increased pSmad2. Another approach has been to delete TGF-βR2 postnatally in young adult mice, which has uniformly resulted in an increase of aortopathies.[19–21]

In the current issue of *JAHA*, Dichek and colleagues provide further confirmation that deletion of TGF-βR2 increases incidence and severity of aortopathies.[5] This is a meticulously executed study in which the rigorous experimental design is documented in detail. The experimental design incorporates many of the issues detailed recently in the National Institutes of Health requirements for rigor and reproducibility (https://grants.nih.gov/reproducibility/index.htm), including randomization and blinding of analysis. Also, the extensively described statistical approach provides confidence in the robustness of the conclusions.

This new publication is an extension of a previous study in which TGF-βR2 was deleted in smooth muscle cells of wild-type mice.[21] In this new study, Dichek and colleagues performed smooth muscle cell–specific postnatal depletion of TGF-βR2 in mice that were also haploinsufficient for fibrillin-1. This study included an extensive examination of proteins involved in intracellular signaling when the TGF-β ligand binds its cell surface receptor. Uniquely, this study determined the abundance of these signaling proteins prior to the appearance of overt pathology in fibrillin-1 haploinsufficient mice. Using this approach, Wei et al[5] demonstrated that smooth muscle cell deletion of TGF-βR2 led to the expected reductions in pSmad2 abundance. These data contradict other studies of thoracic aortic aneurysm in fibrillin-1 haploinsufficient mice that have demonstrated increased pSmad2.[22] However, these studies determined the abundance of pSmad2 in aortic tissue extracted from 12-month-old mice with extensive aortic disease. Use of diseased tissue makes it impossible to determine whether changes in protein abundance are a cause or consequence of the diseases. Hence, the approach used by Wei et al[5] lends great credibility to the concept that TGF-β signaling is reduced in the formative stage of aortopathies in fibrillin-1 haploinsufficient mice.

In a previous study from Dichek's laboratory,[21] deletion of TGF-βR2 led to aortic pathologies of thickened media and expanded aortic lumen. This pathology extended beyond the ascending aorta and into the descending and suprarenal aorta. To provide insight into the effect of smooth muscle cell–specific TGF-βR2 in Marfan aortopathies, these receptors were deleted in mice that were halpoinsufficient for fibrillin-1. As with deleting TGF-βR2 in mice with normal expression of fibrillin-1, deletion of this receptor in fibrillin-1 haplosufficient mice also augmented aortic pathology. Overall, this study provides further evidence that challenges the dogma stating that TGF-β overactivity is the cause of Marfan-associated aortic disease.

Collectively, the profound aortopathies found in TGF-β and TGF-βR manipulated mice demonstrate the critical need for further studies on the role of this cytokine and its receptors. The wide range of disparate opinions on whether TGF-β is harmful or helpful in aortopathies needs to be resolved by careful experimental design and objective interpretation. For example, use of pSmad2 as a surrogate marker of TGF-β signaling has caveats such as its lack of exclusivity. The approach of immunostaining pSmad2 also requires careful application of controls to demonstrate that tissue staining is specific. Furthermore, as described by Wei et al,[5] rigorous and reproducible ultrasonic measurement of aortic diameter is needed for meaningful interpretation into mechanisms.

Within the context of the current literature, the publication of Wei et al[5] provides further evidence that TGF-β is predominantly a protective cytokine against development of thoracic aortic disease. The effects of TGF-β on thoracic aortic aneurysms has been mechanistically linked to angiotensin II.[11] This link is based on evidence of angiotensin II both augmenting TGF-β secretion and stimulating the same intracellular signaling pathways. However, unlike the variable effects of TGF-β neutralization on experimental thoracic aortic disease, there is impressive consistency of angiotensin receptor antagonism in attenuating aortic dilation and rupture across a wide spectrum of mouse models.[8,10,18,19] Unfortunately, clinical studies on patients with Marfan syndrome have not mimicked the consistency in mouse models. However, lack of clear efficacy may be due to the use of losartan, which is a suboptimal angiotensin receptor antagonist because of its short half-life and surmountable antagonism. Given the profound unmet medical needs of patients with thoracic

aortic disease, it is imperative to continue efforts to define the mechanism and efficacy of drugs that influence these pleiotropic molecules.

Sources of Funding

Research in the authors' laboratory is supported by funding from the NIH (HL107319 and HL133723).

Disclosures

None.

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Key Words: Editorials • aneurysm • cytokine • transforming growth factor-β pathway aneurysm • vascular biology

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J Am Heart Assoc. 2017;6:e005221; originally published January 24, 2017;
doi: 10.1161/JAHA.116.005221
The *Journal of the American Heart Association* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Online ISSN: 2047-9980