The Pathogenic Transforming Growth Factor-β Overdrive Hypothesis in Aortic Aneurysms and Dissections

A Mirage?

Ziad Mallat, Hafid Ait-Oufella, Alain Tedgui

For >10 years ago, an unexpected role for the transforming growth factor-β cytokine pathway has been put forward in driving thoracic aortic aneurysms and dissections. Here, we reassess the evidence for a detrimental transforming growth factor-β overdrive in thoracic aortic aneurysms and dissections. In our view, most of the available mechanistic data argue against this theory.

Syndromic thoracic aortic aneurysms and dissections (TAADs) develop in patients with connective tissue disorders because of genetic mutations that affect structural components of the extracellular matrix and the cell contractile machinery. Early pathogenic hypotheses attributed the aortopathy to structural failure of the aortic tissue. Over 14 years ago, Neptune et al.,1 Habashi et al.,2 and Lindsay and Dietz3 proposed a novel hypothesis to explain how fibrillin-1 (FBN1) mutations in Marfan syndrome (MFS) lead to pulmonary emphysema and aortic aneurysm and pointed to increased transforming growth factor-β (TGFβ) activation as the culprit mechanism. This constituted a major paradigm shift, and a new hope emerged that the life-threatening manifestations of MFS might be prevented by a simple medical treatment, losartan, shown to prevent the disease in mice through its TGFβ-antagonizing properties.4

In 2010, we serendipitously discovered that TGFβ neutralization in mice treated with AngII (angiotensin II) unexpectedly induced fatal aortic dissections.5 Despite differences in the mouse models, the critical vasculoprotective role of TGFβ in our experiments highly contrasted with the reported pathogenic role of TGFβ in MFS and Loeys–Dietz syndrome (LDS), leading us to question the validity of the previous assumptions. Moreover, recent clinical testing of the concept in MFS patients failed to show any benefit of losartan over placebo or β-blockade.6 Thus, the time has come for a reassessment of the scientific evidence that supports a causal role for increased TGFβ signaling in TAADs.

What Is the Evidence for Increased TGFβ Signaling in TAADs?

Marfan Syndrome

The paradigm stipulates that FBN1 mutations are responsible for increased TGFβ signaling through increased bioavailability of TGFβ.

FBN1 contains 8-cysteine domains similar to those found in LTBP (latent TGFβ binding proteins) and directly interacts with LTBP1. An in vitro study showed that a recombinant FBN1 fragment (PF10) can interact with N-terminal FBN1 (which contains the hybrid domain required for binding to LTBP1) and inhibits its association with LTBP1.5 In cell layer extracellular matrix, PF10 releases endogenous TGFβ1, which stimulates SMAD2 phosphorylation (P-SMAD2). Because FBN1 mutations may increase proteolytic susceptibility of microfibrils, the above-described mechanism was proposed to account for increased TGFβ activity in MFS. However, those studies used engineered FBN1 fragments, which might not be relevant to FBN1 fragments generated in vivo. In fact, tissue-purified microfibrils did not increase P-SMAD2.6 Moreover, direct disruption of FBN1/LTBP interaction through deletion of the hybrid 1 region of FBN1 did not induce any MFS phenotype.7

The strongest evidence for increased TGFβ activity in MFS seems to be (1) the demonstration of increased TGFβ signaling in the lungs of MFS mice using a GFP reporter under the control of TGFβ-responsive promoter elements1 and (2) the detection of a TGFβ signature (increased TGFβ ligands, P-SMAD2/3, and TGFβ-responsive genes) in MFS tissues.8 Yet, no evidence is available that the TGFβ reporter activity can be abrogated by an anti-TGFβ antibody. TGFβ-responsive promoter elements are SMAD3/SMAD4-binding sequences and may well respond to TGFβ-independent SMAD activation. This is likely given that increased activation of SMAD2/3 can occur independently of TGFβ in MFS tissues.9 Furthermore, the increased aortic TGFβ signature tends to occur in advanced stages of disease development, suggesting that it is a compensatory, rather than a primary, detrimental process. Indeed, aortic TGFβ signaling is unaltered in young Fbn1c1039G/+ MFS mice, despite the presence of early signs of aortic disease.9

Mutations in the TGFβ Signaling Pathway

LDS-associated mutations (TGFBRI, TGFBRII, TGFB2, TGFB3, SMAD3, and SMAD4) are expected to disrupt TGFβ...
signaling. However, this explanation has been challenged based on a tissue signature suggestive of increased TGFβ signaling. In fact, there is no evidence that this signature can be abrogated by neutralization of TGFβ. As in MFS, the TGFβ signature is detected only at late disease stages and is absent in aortas of young LDS animals. Moreover, vascular smooth muscle cells (VSMCs) from aortas of LDS mice displayed reduced, not increased, signaling in response to TGFβ.

**Does Increased TGFβ Activity Promote TAADs?**

**Marfan Syndrome**

New data suggest that the original finding of reduced aortic aneurysm in Fbn1<sup>C1039G/+</sup> MFS mice after TGFβ neutralization may not be reproducible. Cook et al. found that treatment of Fbn1<sup>C1039G/+</sup> mice with 1D11 anti-TGFβ antibodies was associated with an appreciable trend toward disrupting (rather than preserving) aortic tissue architecture. 1D11 treatment also dramatically exacerbated the aortopathy in the severe Fbn1<sup>mgR/mgR</sup> model, when initiated at postnatal day 16. Intriguingly, the authors suggested an improvement in survival when 1D11 treatment efficacy correlated with reduced TGFβ signaling after deletion of TGFβ in all VSMC subsets. However, treatment of Tgfbr2<sup>−/−</sup> mice failed to rescue the aortic phenotype of mice with deletion of TGFβ in all aortic VSMC subsets. Moreover, why would abrogation of TGFβ signaling in all aortic VSMCs prevent the development of aortic disease? It should rather promote TAAD as described after deletion of Tgfbr2 signaling in all VSMC subsets.

**Does Lineage-Specific Variation in TGFβ Signaling Predispose to Aortopathy Through a Pathogenic TGFβ Overdrive?**

Two types of aortic SMCs are found in the ascending aorta: cardiac neural crest (CNC)–derived VSMCs and mesoderm second heart field (SHF)–derived VSMCs. CNC-derived VSMCs show increased sensitivity to TGFβ compared with SHF-derived VSMCs. Lindsay and Dietz built on these observations and developed a new hypothesis to embrace the paradox of high TGFβ signaling in TAADs. According to this hypothesis, SHF-derived VSMCs are more sensitive to an alteration of TGFβ signaling compared with CNC-derived VSMCs. Loss of TGFβ signaling in SHF-derived VSMCs would initiate compensatory events leading to increased expression/accumulation of TGFβ, which, in turn, could drive (excessive) signaling in CNC-derived VSMCs to induce aortopathy. Despite the apparent attractiveness of the hypothesis, there is no actual data to support it, and there are reasons to think that the hypothesis is not valid. In fact, the aortic phenotype of mice with selective deletion of Tgfbr2 in SHF-derived cells, cited in support of the hypothesis, is different from the aortic phenotype of mice with deletion of Tgfbr2 in all aortic VSMCs. Moreover, why would abrogation of TGFβ signaling in CNC-derived VSMCs on top of the already defective signaling in SHF-derived VSMCs (leading to abrogation of TGFβ signaling in all aortic root VSMCs) prevent the development of aortic disease? It should rather promote TAAD as described after deletion of Tgfbr2 signaling in all VSMC subsets.

**Other TAADs**

Elastogenesis is altered in both Fibulin-4– and Fibulin-5–deficient mice. However, only Fibulin-4–deficient mice develop aortic aneurysm. In contrast to Fibulin-5, Fibulin-4 plays an additional role in targeting the enzyme lysyl oxidase (LOX) to microfibrils. Given the reported role of LOX in TGFβ inactivation, it has been argued that reduced LOX activity may be responsible for aortic aneurysm in Fibulin-4–deficient mice through increased TGFβ activation. However, neutralization of TGFβ signaling does not prevent the aortic disease of LOX-deficient embryos; it instead induces numerous hemorrhages. Thus, the aortic phenotype of LOX-deficient or Fibulin-4–deficient mice cannot be attributed to increased TGFβ signaling.

Shprintzen–Goldberg syndrome is caused by mutations in SKI and shares features with MFS and LDS. Most of the mutations are missense mutations within the R-SMADs, suggesting a role for altered TGFβ activity. Patient fibroblasts seem to display an increased TGFβ signature. However, the increased P-SMAD2/3 and P-ERK were seen in vitro in the absence of TGFβ, and intriguingly, responses to TGFβ stimulation (increase from baseline) were similar between the patient and control fibroblasts. Moreover, SKI knockout does not necessarily impair TGFβ-dependent transcriptional responses, and there is currently no evidence that abrogation of TGFβ signaling rescues the phenotype of Shprintzen–Goldberg syndrome. Furthermore, SKI interacts with and regulates many other TGFβ-dependent or TGFβ-independent pathways.

**Mutations in the TGFβ Signaling Pathway**

If TGFβ signaling is pathogenic in LDS, TGFβ neutralization should prevent the disease. However, treatment of Tgfb2<sup>2G577W/+</sup> mice with 1D11 antibody failed to rescue the aortic phenotype. As in MFS, the disease was prevented by losartan, and treatment efficacy correlated with reduced TGFβ1 expression and P-SMAD2. However, losartan is not a selective TGFβ antagonist, and its protective effects in LDS would be detrimental. Deletion of Tgfb2 selectively in VSMCs (Myh11<sup>CretERT</sup> Tgfb2<sup>2G577W/+</sup>) induces severe TAAD. Although the model is not a true LDS model, the phenotype is consistent with that of the Tgfb2<sup>2G577W/+</sup> strain. Interestingly, TGFβ neutralization in Myh11<sup>CretERT</sup> Tgfb2<sup>2G577W/+</sup> mice aggravated the disease and induced fatal aortic ruptures.
Conclusions

Fourteen years after the pathogenic TGFβ hypothesis, there is still insufficient evidence that MFS, LDS, or other TAADs are mediated by an overdrive of TGFβ signaling. In fact, most of the available data indicate that TGFβ is vasculoprotective in those settings.

We think that the concept was based on 2 disputable interpretations. The first one has considered increased expression of P-SMAD2/3 and TGFβ-responsive gene products in diseased aortas as a pathognomonic signature of increased TGFβ signaling and a primary mechanism in disease pathogenesis, with little attention to any other plausible interpretation. The second one attributed the beneficial effect of losartan in mouse models of MFS and LDS to its TGFβ-antagonizing properties, not considering a large body of evidence that showed induction or aggravation of aortic aneurysm and dissection after direct inhibition of TGFβ activity or signaling.

We think that strategies aimed at inhibition of TGFβ-dependent signaling are unlikely to provide any benefit to patients with TAADs and may even aggravate their disease. The time has come to abandon the unproven hypothesis of detrimental TGFβ overdrive in TAADs and explore new concepts and horizons.21,22

Sources of Funding

This study was supported by British Heart Foundation, European Research Council, and Institut National de la Sante et de la Recherche Medicale.

Disclosures

None.

References

2. Habashi JP, Judge DP, Holm TM, et al. Losartan in mouse models of MFS and LDS to its effect of losartan in mouse models of MFS and LDS to its antag-

Key Words: aneurysm • aorta • aortic dissection • connective tissue • extracellular matrix • losartan
The Pathogenic Transforming Growth Factor-β Overdrive Hypothesis in Aortic Aneurysms and Dissections: A Mirage?
Ziad Mallat, Hafid Ait-Oufella and Alain Tedgui

*Circ Res.* 2017;120:1718-1720
doi: 10.1161/CIRCRESAHA.116.310371

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/120/11/1718
Free via Open Access

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/