

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

A Mirage?

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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 (*FBNI*) mutations in Marfan syndrome (MFS) lead to pulmonary emphysema and aortic aneurysm and pointed to increased transforming growth factor- β (TGF β) 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 TGF β -antagonizing properties.²

In 2010, we serendipitously discovered that TGF β neutralization in mice treated with AngII (angiotensin II) unexpectedly induced fatal aortic dissections.⁴ Despite differences in the mouse models, the critical vasculoprotective role of TGF β in our experiments highly contrasted with the reported pathogenic role of TGF β 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.⁵ Thus, the time has come for a reassessment of the scientific evidence that supports a causal role for increased TGF β signaling in TAADs.

What Is the Evidence for Increased TGF β Signaling in TAADs?

Marfan Syndrome

The paradigm stipulates that *FBNI* mutations are responsible for increased TGF β signaling through increased bioavailability of TGF β .

FBNI contains 8-cysteine domains similar to those found in LTBP1s (latent TGF β binding proteins) and directly interacts with LTBP1. An in vitro study showed that a recombinant *FBNI* fragment (PF10) can interact with N-terminal *FBNI* (which contains the hybrid domain required for binding to LTBP1) and inhibits its association with LTBP1.⁶ In cell layer extracellular matrix, PF10 releases endogenous TGF β 1, which stimulates SMAD2 phosphorylation (P-SMAD2). Because *FBNI* mutations may increase proteolytic susceptibility of microfibrils, the above-described mechanism was proposed to account for increased TGF β activity in MFS. However, those studies used engineered *FBNI* fragments, which might not be relevant to *FBNI* fragments generated in vivo. In fact, tissue-purified microfibrils did not increase P-SMAD2.⁶ Moreover, direct disruption of *FBNI*/LTBP interaction through deletion of the hybrid 1 region of *FBNI* did not induce any MFS phenotype.⁷

The strongest evidence for increased TGF β activity in MFS seems to be (1) the demonstration of increased TGF β signaling in the lungs of MFS mice using a GFP reporter under the control of TGF β -responsive promoter elements¹ and (2) the detection of a TGF β signature (increased TGF β ligands, P-SMAD2/3, and TGF β -responsive genes) in MFS tissues.² Yet, no evidence is available that the TGF β reporter activity can be abrogated by an anti-TGF β antibody. TGF β -responsive promoter elements are SMAD3/SMAD4-binding sequences and may well respond to TGF β -independent SMAD activation. This is likely given that increased activation of SMAD2/3 can occur independently of TGF β in MFS tissues.⁸ Furthermore, the increased aortic TGF β signature tends to occur in advanced stages of disease development, suggesting that it is a compensatory, rather than a primary, detrimental process. Indeed, aortic TGF β signaling is unaltered in young *Fbn1*^{C1039G/+} MFS mice, despite the presence of early signs of aortic disease.⁹

Mutations in the TGF β Signaling Pathway

LDS-associated mutations (*TGFBR1*, *TGFBR2*, *TGFB2*, *TGFB3*, *SMAD3*, and *SMAD4*) are expected to disrupt TGF β

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signaling.³ However, this explanation has been challenged based on a tissue signature suggestive of increased TGF β signaling.¹⁰ In fact, there is no evidence that this signature can be abrogated by neutralization of TGF β . As in MFS, the TGF β signature is detected only at late disease stages and is absent in aortas of young LDS animals.¹⁰ Moreover, vascular smooth muscle cells (VSMCs) from aortas of LDS mice displayed reduced, not increased, signaling in response to TGF β .¹⁰

Does Increased TGF β Activity Promote TAADs?

Marfan Syndrome

New data suggest that the original finding of reduced aortic aneurysm in *Fbn1*^{C1039G/+} MFS mice after TGF β neutralization² may not be reproducible. Cook et al¹¹ found that treatment of *Fbn1*^{C1039G/+} mice with 1D11 anti-TGF β antibody 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*^{mgR/mgR} model, when initiated at postnatal day 16.¹¹ Intriguingly, the authors suggested an improvement in survival when 1D11 was initiated at day 45. However, the Kaplan–Meier curves indicate that the difference in survival was already present before the initiation of 1D11 injections, with survival curves being almost parallel after treatment initiation.

Reduced aortopathy in *Fbn1*^{mgR/mgR} mice under *Ltbp3*^{-/-} background has been attributed to normalization of TGF β activity.¹² However, this is a speculation given that the authors were unable to detect differences in activity or signaling using antibodies that recognize either active TGF β or phosphorylated SMADs in *Ltbp3*^{-/-} mice.¹³

Genetic manipulations of TGF β signaling support a protective role of TGF β in MFS. MFS aortopathy is aggravated in *Fbn1*^{C1039G/+} mice with disrupted canonical SMAD4 (*Smad4*^{+/-}).¹⁴ This was interpreted as resulting from a detrimental increase of TGF β -dependent noncanonical pathway.¹⁴ However, no data were presented to show that blockade of TGF β abolishes the aortopathy of *Fbn1*^{C1039G/+} *Smad4*^{+/-} mice. In contrast, further reduction of TGF β 2 using *Tgfb2*^{+/-} mice¹⁵ or deletion of *Tgfb2* in VSMCs^{9,16} substantially aggravated the aortopathy of *Fbn1*^{C1039G/+} mice.

Mutations in the TGF β Signaling Pathway

If TGF β signaling is pathogenic in LDS, TGF β neutralization should prevent the disease. However, treatment of *Tgfb2*^{G357W/+} mice with 1D11 antibody failed to rescue the aortic phenotype.¹⁰ As in MFS, the disease was prevented by losartan, and treatment efficacy correlated with reduced TGF β 1 expression and P-SMAD2. However, losartan is not a selective TGF β antagonist, and its protective effects in LDS (or MFS) mice cannot be used as a proof of the pathogenic role of TGF β signaling in those settings. Actually, other studies strongly suggest that direct blockade of residual TGF β signaling in LDS would be detrimental. Deletion of *Tgfb2* selectively in VSMCs (*Myh11*^{CreERT2} *Tgfb2*^{fl/fl}) induces severe TAAD.¹⁶ Although the model is not a true LDS model, the phenotype is consistent with that of the *Tgfb2*^{G357W/+} strain.¹⁰ Interestingly, TGF β neutralization in *Myh11*^{CreERT2} *Tgfb2*^{fl/fl} mice aggravated the disease and induced fatal aortic ruptures.¹⁶

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 TGF β inactivation, it has been argued that reduced LOX activity may be responsible for aortic aneurysm in Fibulin-4-deficient mice through increased TGF β activation.³ However, neutralization of TGF β signaling does not prevent the aortic disease of LOX-deficient embryos; it instead induces numerous hemorrhages.¹⁷ Thus, the aortic phenotype of LOX-deficient or Fibulin-4-deficient mice cannot be attributed to increased TGF β 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 TGF β signaling.¹⁸ Patient fibroblasts seem to display an increased TGF β signature.¹⁸ However, the increased P-SMAD2/3 and P-ERK were seen in vitro in the absence of TGF β , and intriguingly, responses to TGF β 2 stimulation (increase from baseline) were similar between the patient and control fibroblasts.¹⁸ Moreover, *SKI* knockdown does not necessarily impair TGF β -dependent transcriptional responses,¹⁹ and there is currently no evidence that abrogation of TGF β signaling rescues the phenotype of Shprintzen–Goldberg syndrome. Furthermore, *SKI* interacts with and regulates many other TGF β -dependent or TGF β -independent pathways.

Does Lineage-Specific Variation in TGF β Signaling Predispose to Aortopathy Through a Pathogenic TGF β 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 TGF β compared with SHF-derived VSMCs. Lindsay and Dietz³ built on these observations and developed a new hypothesis to embrace the paradox of high TGF β signaling in TAADs. According to this hypothesis, SHF-derived VSMCs are more sensitive to an alteration of TGF β signaling compared with CNC-derived VSMCs. Loss of TGF β signaling in SHF-derived VSMCs would initiate compensatory events leading to increased expression/accumulation of TGF β , which, in turn, could drive (excessive) signaling in CNC-derived VSMCs to induce aortopathy.³ 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 *Tgfb2* in SHF-derived cells,²⁰ cited in support of the hypothesis, is different from the aortic phenotype of mice with deletion of *Tgfb2* in all aortic VSMCs. Moreover, why would abrogation of TGF β signaling in CNC-derived VSMCs on top of the already defective signaling in SHF-derived VSMCs (leading to abrogation of TGF β signaling in all aortic root VSMCs) prevent the development of aortic disease? It should rather promote TAAD as described after deletion of *Tgfb2* signaling in all VSMC subsets.¹⁶

Conclusions

Fourteen years after the pathogenic TGF β hypothesis, there is still insufficient evidence that MFS, LDS, or other TAADs are mediated by an overdrive of TGF β signaling. In fact, most of the available data indicate that TGF β 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 TGF β -responsive gene products in diseased aortas as a pathognomonic signature of increased TGF β 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 TGF β -antagonizing properties, not considering a large body of evidence that showed induction or aggravation of aortic aneurysm and dissection after direct inhibition of TGF β activity or signaling.

We think that strategies aimed at inhibition of TGF β -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 TGF β overdrive in TAADs and explore new concepts and horizons.^{21,22}

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Disclosures

None.

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