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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 Dietz[3] 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.[2]

In 2010, we serendipitously discovered that TGFβ 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 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.[5] 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 LTBPs (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.[6] 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 elements[1] and (2) the detection of a TGFβ signature (increased TGFβ ligands, P-SMAD2/3, and TGFβ-responsive genes) in MFS tissues.[2] 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.[8] 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 *Fbn1*$^{C1039G/+}$ MFS mice, despite the presence of early signs of aortic disease.[9]

Mutations in the TGFβ Signaling Pathway

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

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signaling.[3] However, this explanation has been challenged based on a tissue signature suggestive of increased TGFβ signaling.[10] 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.[10] Moreover, vascular smooth muscle cells (VSMCs) from aortas of LDS mice displayed reduced, not increased, signaling in response to TGFβ.[10]

Does Increased TGFβ Activity Promote TAADs?

Marfan Syndrome

New data suggest that the original finding of reduced aortic aneurysm in *Fbn1*$^{C1039G/+}$ MFS mice after TGFβ neutralization[2] may not be reproducible. Cook et al[11] found that treatment of *Fbn1*$^{C1039G/+}$ mice with 1D11 anti-TGFβ 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 TGFβ 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 TGFβ or phosphorylated SMADs in *Ltbp3*$^{-/-}$ mice.[13]

Genetic manipulations of TGFβ signaling support a protective role of TGFβ 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 TGFβ-dependent noncanonical pathway.[14] However, no data were presented to show that blockade of TGFβ abolishes the aortopathy of *Fbn1*$^{C1039G/+}$ *Smad4*$^{+/-}$ mice. In contrast, further reduction of TGFβ2 using *Tgfb2*$^{+/-}$ mice[15] or deletion of *Tgfbr2* in VSMCs[9,16] substantially aggravated the aortopathy of *Fbn1*$^{C1039G/+}$ mice.

Mutations in the TGFβ Signaling Pathway

If TGFβ signaling is pathogenic in LDS, TGFβ neutralization should prevent the disease. However, treatment of *Tgfbr2*$^{G357W/+}$ 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 TGFβ1 expression and P-SMAD2. However, losartan is not a selective TGFβ antagonist, and its protective effects in LDS (or MFS) mice cannot be used as a proof of the pathogenic role of TGFβ signaling in those settings. Actually, other studies strongly suggest that direct blockade of residual TGFβ signaling in LDS would be detrimental. Deletion of *Tgfbr2* selectively in VSMCs (*Myh11*^{CreERT2} *Tgfbr2*$^{fl/fl}$) induces severe TAAD.[16] Although the model is not a true LDS model, the phenotype is consistent with that of the *Tgfbr2*$^{G357W/+}$ strain.[10] Interestingly, TGFβ neutralization in *Myh11*^{CreERT2} *Tgfbr2*$^{fl/fl}$ 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 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.[3] However, neutralization of TGFβ 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 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 signaling.[18] Patient fibroblasts seem to display an increased TGFβ signature.[18] However, the increased P-SMAD2/3 and P-ERK were seen in vitro in the absence of TGFβ, and intriguingly, responses to TGFβ2 stimulation (increase from baseline) were similar between the patient and control fibroblasts.[18] Moreover, *SKI* knockdown does not necessarily impair TGFβ-dependent transcriptional responses,[19] 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.

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[3] 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.[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 *Tgfbr2* in SHF-derived cells,[20] 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.[16]

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]

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Disclosures

None.

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