Transforming Growth Factor- β Signaling in Thoracic Aortic Aneurysm Development: A Paradox in Pathogenesis

Jeffrey A. Jones^{a, b} Francis G. Spinale^{a, b} John S. Ikonomidis^a

^aDepartment of Surgery, Division of Cardiothoracic Surgery Research, Medical University of South Carolina, and

^bRalph H. Johnson Veterans Affairs Medical Center, Charleston, S.C., USA

Key Words

TGF- β • Aneurysm • Signal transduction • Extracellular matrix • Remodeling

Abstract

Thoracic aortic aneurysms (TAAs) are potentially devastating, and due to their asymptomatic behavior, pose a serious health risk characterized by the lack of medical treatment options and high rates of surgical morbidity and mortality. Independent of the inciting stimuli (biochemical/mechanical), TAA development proceeds by a multifactorial process influenced by both cellular and extracellular mechanisms, resulting in alterations of the structure and composition of the vascular extracellular matrix (ECM). While the role of enhanced ECM proteolysis in TAA formation remains undisputed, little attention has been focused on the upstream signaling events that drive the remodeling process. Recent evidence highlighting the dysregulation of transforming growth factor- β (TGF- β) signaling in ascending TAAs from Marfan syndrome patients has stimulated an interest in this intracellular signaling pathway. However, paradoxical discoveries have implicated both enhanced TGF- β signaling and loss of function TGF- β receptor mutations, in aneurysm formation; obfuscating a clear functional role for TGF- β in aneurysm development. In an effort to elucidate this sub-

ject, TGF- β signaling and its role in vascular remodeling and pathology will be reviewed, with the aim of identifying potential mechanisms of how TGF- β signaling may contribute to the formation and progression of TAA.

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Introduction

Thoracic aortic aneurysms (TAAs) develop as a result of maladaptive remodeling of the vascular extracellular matrix (ECM). These malignant alterations cause weakening of the aortic ultrastructure and lead to an increased propensity for dilatation, dissection, and rupture [1, 2]. ECM remodeling occurs by a highly regulated process involving both intracellular and extracellular mechanisms that function to balance matrix deposition and matrix degradation in order to maintain the structural integrity of the vascular wall [3, 4]. In the aneurysmal aorta, this balance is disrupted in favor of enhanced proteolysis, which results in the pathological remodeling of the vascular ECM [2, 5]. While the majority of previous studies have focused on the dysregulation of extracellular

This work was supported by NIH/NHLBI R01 HL075488-04.

protease systems in aneurysm formation, little attention has been focused on the small-molecule mediators that drive multiple signaling pathways upstream and regulate the remodeling process. One upstream signaling protein known to alter the structure and composition of the ECM, and known to play an important role in vascular remodeling is transforming growth factor- β (TGF- β) [6, 7]. TGF- β is a member of a superfamily of ligands and receptors that include the TGF- β , bone morphogenetic proteins, and the activins/inhibins. These soluble peptide growth factors are produced by multiple cell types and participate in a wide array of cellular responses including: proliferation, angiogenesis, differentiation, apoptosis, inflammation, and wound healing [8–10]. While TGF- β is probably best known for its role in matrix deposition (e.g. collagen synthesis) related to fibrotic disease [7], TGF- β has also been shown to regulate alternate pathways that can lead to matrix degradation [11–14]. Recent studies demonstrating altered TGF- β signaling in aneurysm formation have sparked an interest in how a well-known profibrotic growth factor can participate in a pathological process that is characterized by extensive matrix degradation. Thus, the goal of the present article is to review both the classical and alternative pathways of TGF- β signaling, its role in regulating ECM remodeling, and to propose mechanisms based on how this complex signaling pathway may be involved in TAA development.

Thoracic Aortic Aneurysms

Each year 15,000 lives are claimed by aortic aneurysmal disease, making it the 13th leading cause of death in people 55 years of age or older in the United States (National Center for Health Statistics, 2000). A TAA is defined as a localized dilatation of the supradiaphragmatic aorta exceeding 1.5 times its original diameter [15]. Anatomically, ascending TAAs are the most common, accounting for 40% of those diagnosed, while aneurysms of the descending thoracic aorta account for 35%, with the remaining composed of aneurysms of the aortic arch (15%) and the thoracoabdominal regions (10%) [16]. TAAs occur most frequently in Caucasians, and they afflict men two to four times more frequently than women. The mean age at diagnosis is 60–70 years of age [17]. The risk factors for developing aneurysms are similar to those for heart disease (atherosclerosis, hypertension, smoking, advanced age, and family history); however, the lack of aneurysm-specific symptoms often renders them un-

noticed until the aorta ruptures, resulting in significant morbidity and mortality [3, 16, 18–24]. While the most common etiology of TAA development is related to idiopathic aortic degeneration in patients with tricuspid aortic valves, other types of aortic aneurysm syndromes are associated with specific genetic conditions that carry a predisposition for TAA formation; these include possessing a congenital bicuspid aortic valve, Marfan syndrome, Loeys-Dietz syndrome, Ehlers-Danlos syndrome, and familial TAAs and dissections (TAADs) [3, 25]. When aortic dilatation is discovered, independent of the aneurysm etiology, aortic diameter is serially monitored over time using noninvasive imaging techniques. A high risk surgical procedure is often the only treatment option. There are currently no noninvasive interventional treatments available for these patients. Surgery is considered when: (1) the patient begins to experience specific symptoms; (2) the rate of aortic dilatation is determined to be greater than average (1.0 cm/years); or (3) the aortic diameter reaches a critical size (5.0–5.5 cm (ascending) or 6–6.5 cm (descending)) [26]; modified based on diagnosis, family history, or body surface area [27]. Thus, only when the risk of aortic rupture outweighs the risk of the surgical repair, is the patient treated. Although recent advancements, such as endovascular stent grafting and improvements in perioperative care have lessened the significant morbidity associated with open surgical repair, stenting may not permanently arrest aneurysm development, and continued disease progression may result in failure of the stent graft. Therefore, further diagnostic and therapeutic advancement is critical and especially relevant for those patients who have not yet reached surgical criteria.

Vascular Remodeling and Aneurysm Formation

Vascular remodeling collectively refers to the architectural alterations that occur in a vessel wall in response to hemodynamic changes or various forms of vascular injury. This adaptive process is induced to maintain the vessel lumen diameter and consistent blood flow under normal physiological conditions. Both clinical and basic research studies have characterized aneurysmal disease histologically based on the alterations that occur within the vascular ECM, primarily the pathological remodeling of collagen and elastin, the key structural proteins within the aortic vascular wall. This remodeling process is now understood to be driven by enhanced production of extracellular proteases and is accompanied by the loss

of vascular smooth muscle cells (SMCs), together leading to pronounced medial atrophy [2, 5, 28].

While the inciting pathological stimulus leading to aneurysm formation remains undefined, the development of experimental animal models that recapitulate many characteristics of human aneurysms has allowed hypothesis-directed investigation of specific proteolytic mechanisms driving aberrant vascular remodeling and aneurysm development [29–36]. Initial studies focused on the role of the matrix metalloproteinases (MMPs) in mediating ECM degradation during aneurysm formation [2, 3, 5, 35, 37–41]. The MMPs are a family of 27 unique extracellular proteases that are capable of degrading all aortic ECM constituents. MMP abundance and activity within the vascular wall is regulated by several mechanisms including transcriptional regulation, post-translational activation and release, and the regulated production of endogenous tissue inhibitors (TIMPs). Within the normal aorta, a balance between these mechanisms is maintained in order to tightly control matrix degradation and matrix deposition. Within the aneurysmal aorta, however, this balance is disrupted by an overproduction of MMPs or an underproduction of TIMPs, favoring an enhanced proteolytic state and driving matrix degradation [37, 42, 43]. Thus, while the physiological remodeling process within the aortic vascular wall operates to maintain normal aortic function, pathological dysregulation can result in excessive degradation of critical ECM components, leading to loss of mechanical strength and integrity. This results in aortic dilatation, dissection, or rupture.

While the role of enhanced ECM proteolysis in TAA formation remains undisputed, the fundamental mechanisms regulating the balance between matrix deposition and degradation remain largely undiscovered. With the majority of previous studies focusing on the role of specific end effectors of aneurysm development, such as the MMPs, little information exists regarding the upstream signaling pathways that manage the pathological remodeling process. Hence, interest is shifting toward a mechanistic understanding of aneurysm formation and progression with a focus on identifying critical upstream regulators.

TGF- β Signaling

TGF- β is a soluble peptide growth factor that has been implicated in numerous divergent cellular processes including proliferation, angiogenesis, differentiation, apop-

toxis, and wound healing [9, 44–48], and is well described as a modifier of the structure and composition of the ECM. Best known for its role in stimulating collagen production and deposition, dysregulated TGF- β signaling has been implicated in pathological fibrosis of the heart, lung, and liver [7, 49–51].

Classical TGF- β Signaling Pathway

In the classical TGF- β signaling pathway, upon release from sites of extracellular sequestration, TGF- β (TGF- β 1, TGF- β 2, TGF- β 3) dimerizes (forming predominantly homodimers) and binds to a heteromeric receptor complex consisting of two type I receptors and two type II receptors, both of which possess serine/threonine kinase activity (fig. 1) [52]. Upon ligand binding, the type II receptor recruits a type I receptor and activates it through a transphosphorylation event [53]. The activated type I receptor then phosphorylates a receptor-Smad (R-Smad), a class of intracellular signaling intermediates named for their homologues in *Caenorhabditis elegans* (*sma* genes; *SMALL*, regulators of body size) and *Drosophila* [*mad* genes; mothers against decapentaplegic (*dpp*)]. The R-Smad then interacts with a co-Smad (Smad4), forming a complex that is shuttled into the nucleus where, upon interaction with transcriptional coregulators (activators or repressors), it forms a competent transcription complex capable of inducing or repressing numerous genes [45, 48, 54, 55]. Accordingly, the classical TGF- β signaling pathway involving the Smad-mediated signaling cascade has been characterized by signals that induce ECM deposition (e.g. collagen, elastin) [7, 56, 57] while also repressing ECM degradation (e.g. TIMP-1, TIMP-3) [58, 59].

Regulation of TGF- β Signaling

TGF- β was originally named for its ability to *stimulate* anchorage-independent growth in fibroblasts by inducing cellular transformation [60, 61]. Not long afterwards, TGF- β was also shown to function as a potent growth *suppressor* [62]. Attempts to dissect the TGF- β pathway, separating its mitogenic responses from its growth suppression responses, initiated years of divergent results emphasizing the pleiotropic nature of TGF- β signaling. Since that time, the family of TGF- β signaling receptors and intermediates has dramatically expanded, and now defines TGF- β as one member of a superfamily of signaling components consisting of approximately 30 different ligands, 7 different type I receptors, 5 different type II receptors, and 8 different Smad proteins [45, 63].

Fig. 1. Classical TGF- β signaling pathway. The classical profibrotic TGF- β signaling pathway is initiated upon binding of ligand to a homodimer of the type II TGF- β receptor (TGF- β RII) (1). The type II receptor is autophosphorylated (2); it then recruits and transphosphorylates a type I receptor (TGF- β RI) (3). The activated type I receptor in turn phosphorylates and activates a receptor-Smad (R-Smad) (4). The R-Smad then binds the common co-Smad (5) and translocates to the nucleus (6). Once in the nucleus, it binds transcriptional cofactors and forms an activated transcriptional complex capable of inducing transcription of profibrotic genes (7).

TGF- β signaling is regulated at multiple levels including mechanisms such as the extracellular regulation of ligand availability [64, 65], regulation at the transcriptional level by co-activators, co-repressors, and transcriptional terminators [66], and by multiple feedback and cross-talk mechanisms that terminate or re-direct the intracellular signal [10, 46, 67]. For example, the extracellular TGF- β scavenging proteoglycan decorin binds TGF- β in the ECM and limits its ability to interact with TGF- β receptors. In a study by Coucke et al. [68], loss-of-

function mutations were identified in the *SLC2A10* gene that encodes the facilitative glucose transporter, GLUT10. Patients deficient in this transporter develop arterial tortuosity syndrome (ATS), characterized by a twisting/con tortion of the large and medium-sized arteries (including the aorta), as well as aneurysms. Interestingly, the decorin gene is regulated in part by a glucose response element in its promoter. SMCs isolated from patients with ATS demonstrated severely reduced expression of decorin versus control cells [68]. In this example, the loss of decorin translated to an increase in the abundance of TGF- β available for signaling within the ECM, which may be an underlying cause for ATS development. In another example, TGF- β stimulation results in the induction of the inhibitory Smads (I-Smads; Smad6 and Smad7), which function to both mediate TGF- β signaling cross-talk with other signaling pathways and attenuate the TGF- β signaling response [69]. Smad6 has been described to bind directly to the type-I TGF- β receptor (TGF- β RI), thus preventing subsequent R-Smad phosphorylation [70]. This has been shown to interfere with the R-Smad:co-Smad complex formation [71], attenuating nuclear translocation and the subsequent transcriptional activation mediated by the R-Smads. On the other hand, Smad7 interacts with the heteromeric TGF- β receptor complex, and recruits the E3 ubiquitin-ligases Smurf1 and Smurf2 (Smad ubiquitination regulatory factor-1 and -2), targeting the receptors for degradation, thus terminating the signaling response [67, 72, 73]. The R-Smads and the co-Smad have also been shown to be regulated by TGF- β -induced Smurf activity [48, 74, 75]. Accordingly, these factors all work together to modify and direct the response to signals through this complex pathway (table 1).

Smad-Independent and Alternative TGF- β Signaling

A growing body of evidence now supports the hypothesis that TGF- β signaling can proceed by alternative mechanisms that bypass key mediators in the classical pathway (fig. 2) [47, 76–78]. For example, these responses include: (1) signals propagated directly by type II receptors without type I receptor involvement [79, 80]; (2) type I receptor signals in the absence of Smad activity [78, 81–84]; (3) R-Smad signaling to parallel pathways in the absence of co-Smad involvement [85–87], and (4) activation of R-Smads by other signaling mediators in response to TGF- β , but not as a result of direct interaction with TGF- β receptors [88, 89]. Thus, in addition to the classical signaling pathway, many of the downstream effects of TGF- β may be mediated through alternative pathways

Table 1. TGF- β signaling pathway components and mediators

Abbreviation	Name	Description/function
<i>TGF-β pathway components</i>		
TGF- β	transforming growth factor beta	multifunctional growth factor that regulates ECM structure and composition; TGF- β -1, -2, and -3
LAP	latency associated peptide	TGF- β is synthesized as a prepropeptide that is cleaved into mature growth factor and an LAP
SLC	small latent complex	mature TGF- β and the LAP self-associated to form the SLC
LTBP	latent TGF- β binding protein	binding proteins that sequester the TGF- β SLC in the ECM
LLC	large latent complex	LTBP binds the SLC to form the LLC
TGF- β RI	TGF- β receptor 1	<i>TGFBRI</i> gene; type I TGF- β receptor with serine/threonine kinase activity; binds TGF- β RII and TGF- β
TGF- β RII	TGF- β receptor 2	<i>TGFBRII</i> gene; type II TGF- β receptor with serine/threonine kinase activity; binds TGF- β RI and TGF- β
TGF- β RIII	TGF- β receptor 3	type III TGF- β receptor; accessory receptor that binds TGF- β and facilitates interactions with type I and II receptors
ALK-1	activin receptor-like kinase-1	type I TGF- β receptor that binds TGF- β and activin
Smad	homologue to <i>C. elegans</i> SMA genes (SMALL, body size regulators) and drosophila MAD (mothers against decapentaplegic) genes	TGF- β signaling intermediates that translate receptor signals into transcriptional responses
R-Smads	receptor-Smads	phosphorylated by direct interaction with TGF- β receptor complex (Smad1, 2, 3, 5, 8)
Phospho-Smad	phosphorylated Smad	phosphorylated R-Smad, phosphorylation regulates interactions with co-Smad and transcriptional co-regulators.
Co-Smad	co-regulatory Smad	Smad4, binds phosphorylated R-Smad and translocates to the nucleus to regulate transcription
I-Smad	inhibitory Smad	Smad6, Smad7; assist in the regulation/termination of Smad-mediated signaling
SARA	Smad anchor for receptor activation	facilitates segregation of TGF- β receptor complexes into the clathrin-mediated endocytic pathway for recycling, and facilitates interactions with Smad2
<i>TGF-β-induced signaling mediators</i>		
COL1A1	type 1 collagen α_1 -chain	type I and type III collagen; major aortic fibrillar collagens induced by TGF- β
COL1A2	type 1 collagen α_2 -chain	
COL3A1	type 3 collagen α_1 -chain	
CTGF	connective tissue growth factor	TGF- β -induced growth factor that induces fibroblast proliferation, migration, and ECM protein production
PAI-1	plasminogen activator inhibitor-1	inhibits the activation of uPA and tPA
uPA	urokinase-type plasminogen activator	plasminogen activators that are known to activate several MMP species
tPA	tissue-type plasminogen activator	
Smurf	Smad ubiquitination regulatory factor	E3 ubiquitin ligase induced by TGF- β ; regulates TGF- β signaling by targeting TGF- β receptors, R-Smads, and co-Smads for proteasomal degradation
ERK1/2	extracellular regulated kinase 1/2	other signaling intermediates that are activated by TGF- β , primarily through alternate and non-Smad-mediated signaling pathways
p38MAPK	p38 mitogen-activated protein kinase	
PI3K	phosphatidylinositol-3-kinase	
PKC	protein kinase C	

that do not involve Smad-mediated transcriptional activity.

Through the appreciation of these combinatorial receptor interactions, multiple regulatory mechanisms,

and alternative signaling pathways, we are slowly gaining an understanding of the regulatory events that are responsible for the complexity and diversity of TGF- β signaling outcomes.

Fig. 2. Alternative TGF- β signaling mechanisms. Signaling directly mediated by the type II receptor (TGF- β RII) without type I receptor (TGF- β RI) function (1), type I receptor signaling independent of Smad function (2), R-Smad signaling independent of co-Smad interaction (3), and R-Smad activation in response to TGF- β but in the absence of direct TGF- β receptor interaction (4). Adapted from reviews by Derynck and Zhang [76] and Moustakas and Heldin [47].

TGF- β Effects on the ECM

Just as TGF- β was identified as a bifunctional regulator of cell growth, TGF- β signaling has been attributed to many other opposing and disparate cellular functions. As such, TGF- β has been implicated not only in matrix deposition, but also in matrix degradation, positioning it as a critical mediator of the structure and composition of the ECM.

Early studies examining the biological effects of TGF- β on both primary and cultured cells demonstrated that TGF- β treatment could enhance the production of type I and type III collagen [90–93]. Subsequent studies revealed that collagen production was a result of enhanced

Fig. 3. Mechanisms for TGF- β -induced matrix degradation. TGF- β stimulates matrix degradation primarily through non-Smad-mediated pathways that may also involve Smad activation, and results in the increased expression of extracellular protease genes. PLAU/PLAT = Urokinase-type plasminogen activator/tissue-type plasminogen activator.

collagen gene expression [94–96]. These studies defined a role for TGF- β in normal fibrogenesis. It was then postulated that the TGF- β signaling pathway may serve as a significant therapeutic target for the treatment of patho-

logical fibrosis. Indeed, in a study by Smith et al. [97], delivery of a recombinant soluble type II TGF- β receptor prevented adventitial fibrosis and collagen deposition in a rat carotid balloon-injury model. In similar fashion, cells exposed to the proteoglycan decorin, which binds and sequesters TGF- β , dose-dependently inhibited collagen synthesis in isolated scar-derived fibroblasts [98]. As the TGF- β signaling pathway has become more defined, a clear role for TGF- β in collagen production and pathological fibrosis has now been well established [99, 100].

In addition to stimulating the production of matrix proteins, TGF- β has also been shown to affect matrix deposition through other modalities. For example, other profibrotic genes are induced in response to TGF- β , including connective tissue growth factor (*CTGF*) which acts on fibroblasts to induce proliferation, migration, adhesion, and ECM protein production [101]. Furthermore, tropoelastin mRNA stability was increased in fibroblasts treated with TGF- β [56]. This response was shown to be dependent on Smad-mediated signaling, but also required protein kinase C and p38 mitogen activated protein kinase (p38MAPK) activity [57]. As well, TGF- β has been shown to actively inhibit matrix degradation by at least two independent mechanisms. First, it was demonstrated that TGF- β could induce endogenous protease inhibitors. The expression of plasminogen activator inhibitor-1 (PAI-1), an endogenous inhibitor of the plasminogen activators (urokinase-type plasminogen activator and tissue-type plasminogen activator), which are well known upstream activators of the MMPs [102], was found to be induced in response to TGF- β treatment [12, 103]. Similarly, TGF- β was shown to induce several TIMP species which function as direct inhibitors of MMP activity. In fibrosarcoma cells, Kwak et al. [58] demonstrated TGF- β -mediated induction of TIMP-1 through an extracellular regulated kinase 1 and 2 (ERK1/2)-dependent pathway. Likewise, work by Garca-Alvarez et al. [59] established that TGF- β 1 could induce *Tim*p-3 gene expression and protein production in primary lung fibroblasts. Second, several of the MMP species (-1, -7, and -13) have promoter binding regions (termed TGF- β inhibitory elements) for transcription factors that mediate the direct repression of *MMP* gene expression [104, 105]. Additionally, Kerr et al. [106] also demonstrated TGF- β -induced repression of *MMP*-3 mediated in part by *c-fos*-containing protein complexes. Furthermore, Edwards et al. [107] demonstrated that the co-treatment of fibroblasts with TGF- β 1 could mediate the repression of growth-factor-induced collagenase expression while synergistically en-

Table 2. Outcomes of classical TGF- β signaling affecting matrix deposition

Mediator	Outcome (genes involved)	Ref.
<i>Effects on matrix synthesis</i>		
Collagen	enhanced collagen expression, synthesis, and deposition (<i>COL1A1</i> , <i>COL1A2</i> , <i>COL3A1</i>)	90–93
CTGF	increased connective tissue growth factor expression (<i>CTGF</i>)	101
Elastin	tropoelastin mRNA half-life is stabilized, increasing elastin expression (<i>ELN</i>)	56, 57
<i>Effects on matrix degradation</i>		
PAI-1	increased PAI-1 expression; inhibits activation of uPA and tPA, activators of MMPs (<i>PLAU</i> and <i>PLAT</i> , respectively)	12, 103
TIMP	induction of TIMP-1, -3 expression, direct inhibitors of MMP activity (<i>TIMP-1</i> , <i>TIMP-3</i>)	58, 59
MMPs	direct repression of MMP expression mediated by TGF- β inhibitory elements (TIE) within the MMP promoter (<i>MMP</i> -1, -7, -13); <i>MMP</i> -3 repression by <i>c-fos</i> complexes	104–106

hancing *TIMP* expression. Hence, TGF- β can drive matrix deposition by inhibiting matrix-degrading enzymes (either directly or by blocking activation) as well as by repressing their transcription. In terms of the functional consequences of TGF- β -mediated inhibition of matrix degradation, it was suggested that overexpression of TGF- β may therefore be able to stabilize the degenerative ECM remodeling observed during aneurysm development. Indeed, in a study by Dai et al. [108], virus-mediated overexpression of TGF- β 1 in rat abdominal aortic aneurysms increased endogenous TGF- β 1 levels, stabilized aortic dilatation, and attenuated vascular degeneration.

Taken together, these studies suggest that the multiple outcomes of TGF- β signaling, specifically the production of matrix proteins along with the repression or inhibition of matrix degrading enzymes, work in concert to regulate matrix deposition (table 2).

In stark contrast to these results, TGF- β signaling has also been implicated in pathways directly leading to enhanced ECM degradation and the production of various MMP species. In human skin fibroblast cultures, TGF- β treatment induced the production of plasminogen activator [12]. In breast cancer cells, TGF- β treatment rapidly activated p38MAPK, leading to the production and release of MMP-2 and MMP-9 [11]. In rat osteoblast cultures, TGF- β was shown to stimulate *Mmp*-13 expres-

sion, which was dependent on Smad2, p38MAPK, and ERK1/2 signaling [14]. Furthermore, Safina et al. [13] revealed that breast cancer cell invasion and tumor angiogenesis were dependent on signaling through the type I TGF- β receptor, and resulted in MMP-9 production. Interruption of the TGF- β signaling pathway by overexpressing a kinase-deficient type I receptor attenuated both angiogenesis and MMP-9 expression. Thus, these data suggest that non-Smad-mediated TGF- β signaling pathways dependent on TGF- β RI, and often in combination with Smad-mediated signaling, can stimulate the proteolytic destruction of the vascular ECM (fig. 3).

TGF- β and Vascular Pathology

It is becoming apparent, as the above data illustrate, that TGF- β signaling can regulate the production of critical vascular matrix proteins as well as matrix-degrading enzymes, and suggests that perturbations in the TGF- β signaling pathway may be detrimental to normal vascular function and architecture. Accordingly, alterations in normal TGF- β signaling have been implicated in the pathophysiology of several vascular disorders including atherosclerosis [3, 109–112], primary pulmonary hypertension [113–116], and a host of aortic aneurysm syndromes which are placed in context below.

Hereditary Hemorrhagic Telangiectasia

In addition to the type I and type II TGF- β receptors, the type III auxiliary receptors (endoglin, betaglycan; TGF- β RIII) bind TGF- β and function to sequester it in the ECM, thereby regulating ligand availability and its interaction with the type I and II receptors [117]. Recently, TGF- β RIII was shown to directly influence TGF- β -mediated growth inhibition by enhancing both Smad3- and p38MAPK-dependent signaling [118]. Interestingly, mutations in endoglin were directly linked to the development of hereditary hemorrhagic telangiectasia (HHT1, OMIM #187300), a disorder that results in vascular dysplasia and arteriovenous malformations. Similarly, mutations in the *ACVRL1* [activin A receptor, type II-like 1 or activin receptor-like kinase-1 (*ALK-1*)] gene, a type I TGF- β receptor that binds TGF- β /activin and has enhanced expression in highly vascularized tissues and endothelial cells [119, 120], has been linked to the development of HHT2 (OMIM #600376) [119]. Moreover, the targeted deletion of *Acvrl1* (the *Alk-1* gene in mice) has been associated with enhanced production of angiogenic factors and plasminogen activators; both of which serve to stimulate

vascular remodeling through the induction and activation of extracellular proteases [121]. Interestingly, in the *Acvrl1* deficient mice, dilatation of the yolk-sac vasculature was observed at day E9.5, prior to embryonic lethality at day E11.5 [121]. Thus it was postulated that ALK-1 signaling negatively regulates angiogenesis and vascular remodeling. It is interesting to speculate that interruption of ALK-1-mediated signaling may induce angiogenic factors (i.e. specific MMPs) that contribute to the degradation of the vascular ECM and aortic dilatation. Indeed enhanced angiogenesis has been implicated in the development of abdominal aortic aneurysms [122–124].

Marfan Syndrome

Marfan syndrome (MFS; OMIM #154700) is an inherited connective tissue disorder characterized by cardiovascular, skeletal, and ocular abnormalities that display an autosomal dominant inheritance pattern with variable penetrance [125]. The primary gene defect lies on chromosome 15q21.1 within the coding sequence for the fibrillin-1 gene (*FBN1*); a principal component of the 10- to 12-nm microfibrils that form the scaffold for elastin assembly within the ECM [125, 126]. The primary cause of morbidity and mortality in MFS patients relates to the common development of cardiovascular complications including annulo-aortic ectasia and dilatation of the aortic root, thus leading to the formation of ascending aneurysms and dissections [127, 128]. These lesions develop secondary to pathological remodeling events that occur within the medial and adventitial vascular ECM consisting of SMC loss, elastin breakdown, and accumulation of cyst-like structures containing mucopolysaccharide [125].

Fibrillin-1 (and the closely related fibrillin-2) is a 350-kDa glycoprotein comprised of tandem repeats of an epidermal growth factor (EGF)-like motif; most of which contain a calcium-binding sequence (cbEGF) [129]. Fibrillin monomers self-assemble into macroaggregates that form the basic structure on which mature elastin fibers are synthesized from tropoelastin subunits. The cbEGF modules function to sequester extracellular calcium to protect against ECM proteolysis, mediate interactions between fibrillin monomers and other cellular components such as integrin α v β 3, and stabilize the structure of the microfibrils to favor lateral packing [130, 131]. Thus, mutations in fibrillin-1 within the aorta result in weakened and disordered elastic fibers, as well as disruption of the microfibril network connecting the elastic lamellae to the adjacent interstitial cells [132, 133].

In addition to directing elastogenesis and providing structural integrity to the elastic lamellae, fibrillin-rich

microfibrils have also been shown to sequester TGF- β within the ECM. TGF- β is synthesized as a prepropolypeptide that is cleaved in a post-Golgi compartment to yield the mature growth factor and a latency-associated peptide (LAP). Homodimers of mature TGF- β and LAP form a tight biologically inactive complex termed the 'small latent complex' (SLC) [134]. The SLC is covalently bound to a latency-associated TGF- β binding protein (LTBP) through disulfide bonds that form between cysteine residues in the LAP and a cysteine-rich motif in LTBP [131, 135, 136]. This large latent complex is secreted from the cell and functions to target TGF- β to the ECM, and specifically to fibrillin microfibrils, which have been shown to directly bind LTBP [137, 138]. TGF- β can be made available for signaling upon release from the LAP through a number of activation mechanisms including: proteolysis of latent complexes, thrombospondin-1 competition with SLC for LTBP binding, integrin binding to embedded RGD peptide sequences within the LTBP (the central peptide sequence that binds $\alpha v \beta 3$ integrin), or exposure to acidic/oxidative stress [135, 136, 139]. Thus, it was suggested that abnormalities in FBN-1 function such as those observed in MFS may lead to impaired sequestration of latent TGF- β complexes, thereby making more available for activation, and potentially leading to enhanced TGF- β signaling [125]. This hypothesis was confirmed by two key studies. First, Neptune et al. [140] demonstrated that lung abnormalities, evident in the immediate postnatal period in mice deficient in fibrillin-1 (*Fbn1*^{mg Δ /mg Δ}), were related to enhanced TGF- β activation and signaling, and that the perinatal administration of a TGF- β -neutralizing antibody could rescue the defect in alveolar septation. Second, in mice carrying a hypomorphic allele of the Fbn-1 gene (*Fbn1*C1039G/+), effectively recapitulating a mouse model of Marfan syndrome, Habashi et al. [141] observed that treatment with a TGF- β -neutralizing antibody or the angiotensin II type I receptor antagonist Losartan was sufficient to attenuate spontaneous aortic root dilatation, elastic fiber fragmentation and Smad2 activation. Together, these studies suggest that sequestration of TGF- β in the ECM is critical to its regulated activation, and mutations that functionally impair its sequestration likely contribute to the pathogenesis of MFS, and in particular the pathogenesis of ascending TAA.

MFS Type 2

The diagnosis of MFS has been based on a defined set of clinical criteria [142–144]. Interestingly, approximately 10% of patients classified as having MFS failed to show

a defect in the *FBN1* gene, suggesting that a second genetic locus is linked to MFS [145–147]. Originally reported in a French family by Boileau et al. [148], this syndrome was clinically very similar to classic MFS but with no ocular involvement. Subsequent linkage analysis by Collod et al. [145] mapped the defect to a locus on chromosome 3p25-24.2 and designated the syndrome 'Marfan syndrome type 2' (MFS2; OMIM #154705). When a Japanese individual with MFS was found to have a chromosome break at 3p24.1, within the gene encoding the type II TGF- β receptor (*TGFBR2*), Mizuguchi et al. [149] hypothesized that the *TGFBR2* gene may be linked with the MFS2 locus. Subsequent analysis revealed a mutation (1524G to A) which resulted in a synonymous amino acid substitution (Q508Q), but disrupted an RNA splice site, leading to early termination and truncation of the TGF- β RII protein [149]. Three other mutations in two unrelated families were also identified, all of which fell within the kinase domain of TGF- β RII and led to the loss of functional TGF- β signaling. Thus, the MFS phenotype can be caused by mutations in both *FBN1* and *TGFBR2*, and in both cases, the alterations in the TGF- β signaling may contribute to the underlying cause of aneurysm formation. While enhanced TGF- β signaling was associated with dilatation of the ascending aorta in a mouse model of MFS [141], the loss of TGF- β signaling appeared to be linked to aneurysm formation in MFS2 [149].

Familial TAAs and Dissections

In addition to the classified aneurysm syndromes that are directly associated with specific gene defects, such as MFS, as described above, an expanding collection of studies have identified TAAs and dissections that are not clearly associated with an identifiable syndrome. Of these individuals, approximately 20% display a genetic predisposition that was inherited in an autosomal dominant manner with decreased penetrance and variable expression [150–152]. These nonsyndromic cases have collectively been referred to as familial TAAs and dissections (TAADs). Currently, six causal genetic loci have been identified and linked to TAAD: 11q23.3-q24 (AAT1; OMIM #607086), 5q13-q14 (AAT2; OMIM #607087), 3p24-25 (AAT3; OMIM #608967), 16p13.13-p13.12 (AAT4; OMIM #132900), 9q33-q34 (AAT5; OMIM #610380), and 10q22-q24 (ACTA2; OMIM #102620). Candidate genes mapping to these intervals have revealed several interesting mutations in genes encoding the smooth muscle myosin heavy chain (β) (*Myh11*; AAT4) [153], α -smooth muscle actin ($\alpha 2$; *ACTA2*) [154], and in

both TGF- β receptors (*TGFBR1*, *AAT5* and *TGFBR2*, *AAT3*) [155–157].

In a study by Pannu et al. [158], four unrelated families were found to have mutations in the *TGFBR2* gene, but tested negative for any signs of MFS. In all four cases, structural analysis revealed mutations of the arginine residue at position 460; a key residue that was found within the highly conserved serine/threonine kinase domain and was predicted to disrupt receptor function [158]. In similar fashion, Matyas et al. [156] screened 70 individuals that displayed phenotypes related to MFS, but tested negative for mutations in *FBN-1*. Of these individuals, 9 were identified to have *TGFBR1* sequence variants that segregated within family groups and with TAAD disease, suggesting a causative link. Thus taken together, the loss of signaling through TGF- β receptors I and II may therefore be associated with the development of familial TAAD.

Loeys-Dietz Syndrome

Work by Loeys et al. [159] as well as other laboratories [160] has recently described another condition that presented with symptoms similar to MFS, but included a greater cardiovascular risk. This disorder was designated Loeys-Dietz syndrome (LDS, OMIM #609192), and was characterized by the enhanced development of aortic aneurysms and dissections that occur at a younger age and smaller aortic diameter [159]. Heterozygous mutations were identified in both *TGFBR1* and *TGFBR2* genes, thus it was initially suggested that the loss of TGF- β signaling may be the underlying cause of LDS. Interestingly, aortic tissues derived from affected patients showed elevated protein levels of collagen and CTGF; both of which are well-described indicators of active TGF- β signaling [159]. Even more compelling, the tissues displayed enhanced nuclear translocation of phospho-Smad2, suggesting that there was an enhancement, not a repression, of TGF- β signaling in these aortic specimens. Upon culturing fibroblasts from affected individuals it was hypothesized that, although the mutant TGF- β receptors were unable to transmit signals, the wild-type receptor (also expressed in a heterozygous individual) retained its acute responsiveness to TGF- β , and in fact displayed elevated activity [159]. Thus, consistent with the observations from the MFS patient studies and the MFS mouse model, the authors concluded that an enhancement of TGF- β signaling drove aortic dilatation and aneurysm formation in patients with LDS.

Ehlers-Danlos Syndrome Type IV

Ehlers-Danlos syndrome type IV (EDS, OMIM #130050), also known as vascular type EDS, primarily affects the skin and large arteries and can lead to medial degenerative disease of the aorta resulting in acute dissection. The original cause was linked to a 3.3-kb DNA deletion in one allele of the type III procollagen gene (*COL3A1*), which results in a truncated procollagen monomer that has decreased thermal stability, cannot be proteolytically processed, and cannot be efficiently secreted [161]. The end effect on the large arteries (including the aorta) is a diminished collagen network, with a low intimal-medial thickness, increased wall stress, and a propensity for acute dissection and rupture [162]. Diagnosis of EDS type IV can be difficult since there is significant phenotypic overlap with patients presenting with LDS. Loeys et al. [151], while characterizing 52 LDS-affected families for mutations in *TGFBR1* and *TGFBR2* genes also assessed a cohort of EDS type IV patients that lacked the *COL3A1* gene mutations and the craniofacial features of the typical LDS patient. Interestingly, 12 EDS type IV probands were identified that possessed *TGFBR1* or *TGFBR2* mutations, suggesting a possible re-classification of this group as LDS type 2. Hence, in addition to *COL3A1* mutations, this predisposition to acute aortic dissection may also be driven by mutations in TGF- β receptors, like the LDS patients with TGF- β receptor mutations that display signs of elevated TGF- β signaling [159]. It is interesting to speculate whether EDS type IV patients with *COL3A1* mutations show enhanced TGF- β signaling in an effort to compensate for the loss of type III collagen within the aorta. In either case, within the aorta, the importance of proper regulation of the TGF- β signaling pathway and its downstream transcriptional targets is further emphasized.

Proposed Mechanisms of TGF- β -Mediated Aneurysm Formation

As put forward by the pathophysiological studies above, alterations in TGF- β signaling may therefore be an underlying factor contributing to the development of TAAs. While the majority of studies demonstrated that loss-of-function mutations within the kinase domain of the type I or type II TGF- β receptors were associated with TAA formation, the predominant emerging theory suggests that overstimulation of the TGF- β signaling is associated with enhanced proteolysis of the vascular ECM. Accordingly, based on additional in vitro and in vivo

studies, some potential mechanisms have emerged that may help to explain (1) how the loss of TGF- β RII kinase activity can result in enhanced TGF- β signaling, and (2) how stimulation of the TGF- β signaling pathway, generally believed to be profibrotic, can drive matrix degradation during aneurysm development.

Enhanced Signaling by Mutant Receptors

In the study by Loeys et al. [159], LDS was associated with deficient TGF- β RII receptor kinase function, yet paradoxically displayed evidence of enhanced TGF- β signaling; including enhanced expression of PAI-1, collagen, and CTGF, as well as nuclear localization of phospho-Smad2. Thus, other aneurysm syndromes associated with *TGFBR2* mutations may also be a result of overstimulation of the TGF- β pathway. While it is difficult to reconcile how kinase-deficient TGF- β receptors could result in enhanced TGF- β signal transmission, there are several potential mechanisms that could account for these observations.

First, Denton and coworkers developed a transgenic mouse that expressed a fibroblast-restricted kinase-deficient TGF- β RII under control of the *COL1A2* promoter [163]. Based on in vitro results characterizing the overexpression of the mutant receptor, the authors had predicted that the transgene would have a dominant-negative effect in mice, thus resulting in the fibroblast-specific suppression of TGF- β signaling. Surprisingly, they observed the opposite: the mice developed dermal and pulmonary fibrosis, and isolated transgenic fibroblasts displayed hallmarks of enhanced TGF- β signaling. To explain these paradoxical results, the authors suggested that the dominant-negative TGF- β receptor may enhance TGF- β signaling in functional TGF- β receptor complexes by facilitating ligand interactions with the functional receptors, similar to the type III TGF- β receptors endoglin and betaglycan [164]. They also suggested that the presence of the mutant TGF- β RII may modify the orientation of wild-type receptors in a manner that would more easily facilitate signaling. Thus, the enhanced TGF- β signaling observed in aneurysm syndromes associated with heterozygous TGF- β receptor mutations may be a result of mutant receptors functioning as accessory receptors that facilitate ligand binding.

Secondly, kinase-deficient receptors may enhance TGF- β signaling by altering the cellular dynamics of receptor stability. It is well known that endocytosis of cell surface receptors is an important regulatory step in the transduction of intracellular signals. Di Guglielmo et al. [165] described internalization of TGF- β receptors by

both clathrin-mediated and caveolin-mediated endocytic pathways. Interestingly, segregation into the clathrin-mediated pathway sustained receptor recycling and propagated receptor signaling, whereas segregation into the caveolin-mediated pathway led to receptor degradation and termination of TGF- β signaling. This process is regulated by receptor interactions with accessory proteins that direct the fate of TGF- β signaling. For example, Di Guglielmo et al. [165] showed that the Smad anchor for receptor activation (SARA) interacted with TGF- β RII at the plasma membrane, facilitating segregation into the clathrin-dependent endocytic pathway, as well as facilitating the interaction of the receptor complex with Smad2. This resulted in signal propagation and eventual receptor recycling to the plasma membrane. Alternatively, it was shown that Smad7 interacted with the heteromeric TGF- β receptor complex (requiring both receptors for efficient interaction), facilitating segregation into the caveolin-mediated endocytic pathway, and recruiting the E3 ubiquitin ligases Smurf1 and Smurf2 [165, 166]. This resulted in the ubiquitin-mediated proteasomal degradation of TGF- β receptors, and thus the attenuation of TGF- β signaling. Hence, it is possible that the *TGFBR2* mutations observed in the above aneurysm syndromes may facilitate interactions with SARA or diminish association with Smad7, preventing segregation into the caveolin-mediated pathway, subsequently resulting in the prolonged activation of TGF- β signal transmission (fig. 4).

Lastly, it is also possible that TGF- β receptor complexes that incorporate kinase-deficient receptors, while unable to signal directly, retain the ability to form functional signaling platforms and mediate interactions between other critical intracellular signaling components (fig. 5). For example, Runyan and coworkers showed that stimulation of human mesangial cells with TGF- β resulted in the induction collagen gene expression (*COL1A2*) that was dependent upon phosphoinositide 3-kinase (PI3K) activity [88]. Using a pharmacological inhibitor of PI3K (LY294002) in combination with phosphorylation site mutants of Smad3, they demonstrated that PI3K catalyzed collagen gene expression by directly phosphorylating Smad3 at residues other than those within the TGF- β RI target site. Thus, the binding of TGF- β induced a receptor signaling complex which included at least PI3K and Smad3, and led to Smad3-mediated collagen gene expression that was *not* dependent on phosphorylation by TGF- β RI. Therefore, TGF- β RII mutants that retain the ability to bind TGF- β may induce signaling through alternate pathways that are independent of receptor kinase activity.

Fig. 4. Potential mechanism for enhancement TGF- β signaling in the presence of receptor mutations. The endocytosis and subsequent recycling or degradation of TGF- β receptors is an important mechanism for regulating TGF- β signaling. There are two independent endocytic pathways, regulated by accessory protein interactions that exist to regulate TGF- β signaling. The TGF- β RII mutations identified in several aneurysm syndromes may contribute to the enhanced TGF- β signaling by facilitating interactions with SARA, or by diminishing association with Smad7, favoring receptor recycling and resulting in prolonged activation of TGF- β signals. **A** Clathrin-mediated pathway leading to receptor recycling. **B** Caveolin-mediated pathway leading to proteasomal degradation of TGF- β receptors.

TGF- β -Induced Matrix Degradation

As detailed above, elevated TGF- β levels and/or evidence of functional TGF- β signaling have been associated with TAA development. Furthermore, it is becoming clear that TGF- β not only functions to induce matrix

Fig. 5. Potential mechanism for enhancement of TGF- β signaling in the presence of nonfunctional receptors. Ligand-induced receptor signaling complexes that are unable to transmit signals because of mutations within the receptor kinase domain (TGF- β RII/TGF- β RI/both), may still function by seeding active signaling platforms that localize other signaling components capable of inducing downstream signaling events that contribute to aneurysm development.

deposition, but has also been implicated in regulating matrix degradation, in part due to its ability to induce matrix-degrading enzymes such as the MMPs (fig. 3). In fact, the majority of studies demonstrating TGF- β -dependent protease production implicate activated TGF- β RI and MAP kinases ERK1/2 and p38 [11–14]. Hence, TGF- β -induced matrix degradation may be driven by non-Smad-mediated pathways, either alone or in combination with Smad-mediated signaling [45, 167, 168]. Whereas hypotheses explaining elevated TGF- β tissues levels have been proposed for some aneurysm syndromes [125], the mechanisms determining the overall (or temporal) cellular response to TGF- β (matrix deposition vs. matrix degradation) remain unclear. Accordingly, while all cell types within the aortic wall are known to respond to TGF- β , each cell type may respond differently. Thus, the combined aortic response will ultimately be deter-

Fig. 6. Potential mechanism of TGF- β -induced changes in the cellular constituents of the vascular wall during aneurysm development. **A** Nonaneurysmal thoracic aorta showing normal homeostatic content of medial SMCs and adventitial fibroblasts. **B** Thoracic aorta during aneurysm formation. TGF- β -mediated SMC apoptosis leaves the adventitial fibroblast as the predominant endogenous cell type in the vascular wall. TGF- β can induce adventitial fibroblasts to differentiate into myofibroblasts, conferring SMC-like characteristics including α -smooth muscle actin expression, enhanced contractile properties, and the ability to migrate. Migrating myofibroblasts respond to TGF- β stimulation by inducing MMP production and secretion. Thus, the resulting myofibroblast population may dominate the vascular remodeling process leading to enhanced matrix degradation.

mined by the representative amounts of the constitutive cells present during aneurysm development.

There are three major endogenous cell types found within the aortic wall: (1) endothelial cells, found populating the intimal layer lining the lumen of the aorta; (2) SMCs, the predominant cell type found in the medial elastic layer, and (3) fibroblasts, found primarily in the adventitial layer. Importantly, it is well known that the cellular constituents within the aortic wall change during the course of aneurysm development. It has been well documented that aortic dilatation is accompanied by SMC apoptosis [169–172]. In fact, Fukui et al. [173] demonstrated in human abdominal aortic aneurysm specimens that high levels of TGF- β expression colocalized with SMC markers that stained positive for fragmented DNA (TUNEL assay). This suggested that SMC apoptosis during aneurysm formation might be mediated by TGF- β signaling pathways. Accordingly, as the aneurysm progresses, the SMC content within the aortic wall decreases, leaving fibroblasts as the predominant cell type to manage the vascular remodeling process. Thus, the degradative changes occurring within the aortic wall may be a result of the differential responses to TGF- β mediated by the changing proportions of cellular constituents.

In addition to changes in the cellular make-up of the aortic wall during aneurysm formation, the adventitial fibroblasts themselves may also undergo phenotypic changes in response to vascular injury/stress [174]. This

transition from fibroblast to myofibroblast confers SMC-like characteristics including increased expression of SMC markers (e.g. α -smooth muscle actin) and contractile properties. Interestingly, TGF- β may provide the signal to induce this fibroblast differentiation [175]. Shi et al. [176] demonstrated a direct correlation between autocrine TGF- β production and the expression of α -smooth muscle actin in adventitial fibroblasts. Likewise, Vaughan et al. [177] demonstrated that TGF- β promoted both morphological and functional differentiation of myofibroblasts, and demonstrated that the differentiated phenotype was dependent on the presence of TGF- β ; when TGF- β was removed, the morphological markers of myofibroblast differentiation resolved. Mechanistically, studies in lung and coronary fibroblasts demonstrated that TGF- β -induced myofibroblast differentiation was dependent on enhanced signaling through the classical TGF- β signaling pathway [178, 179]. Pharmacologic inhibitors of ERK1/2, p38MAPK, and TGF- β RI were used to differentiate the Smad-dependent responses from the alternative signaling pathways. This was confirmed by a study in which the overexpression of a mutant TGF- β RI (lacking critical amino acid residues to interact with the Smads) in mammary epithelial cells failed to induce myofibroblast differentiation in response to TGF- β , but maintained an ability to activate p38MAPK [78]. Interestingly, Shi et al. [180, 181] demonstrated that adventitial fibroblasts from coronary arteries displayed enhanced migratory prop-

erties characterized by increased MMP-2 and MMP-9 production. Moreover, they demonstrated a differential response from medial SMCs, in which medial explants were characterized by diminished migration and enhanced TIMP production.

Taken together, it is interesting to speculate that Smad-mediated TGF- β signaling may drive SMC apoptosis and catalyze the differentiation of fibroblasts into myofibroblasts during aneurysm development. It is then conceivable that upon acquisition of the new functional properties, the myofibroblasts may respond differently to TGF- β signals, promoting non-Smad-mediated pathways that enhance matrix-degrading enzyme production and cell migration (fig. 6). While myofibroblast transition has not been characterized in the aortic vascular wall during TAA formation, myofibroblasts have been identified in association with inflammatory aneurysms; a relatively rare subset of abdominal aortic aneurysms [182]. Accordingly, additional studies are needed to address the role of myofibroblast differentiation during TAA development, as well as the role of non-Smad-mediated signaling in myofibroblast function.

Significance

TAA disease is a potentially devastating disease process which often causes death by rupture in the absence of symptoms. There are currently no effective nonsurgical clinical treatment protocols available which will halt or reverse the aortic remodeling process once diagnosed. While it is clear that the pathological alterations in the structure and composition of the vascular ECM are associated with reduced aortic compliance and resiliency, and lead to aortic dysfunction, there remains a paucity of

information regarding the regulation of specific upstream signaling intermediates and pathways involved in the remodeling process during aneurysm development. As highlighted here, mutations in key TGF- β signaling pathway components are invariably associated with vascular pathology, and have been directly implicated in the development of ascending TAAs. While the majority of aneurysm syndromes are associated with inactivating mutations in the kinase domain of TGF- β receptors (primarily TGF- β RII), direct evidence has implicated enhanced TGF- β signaling during TAA formation. As each cell type within the aortic wall is capable of responding differently to TGF- β , it is conceivable that the homeostatic balance between matrix deposition and degradation is maintained through differential cellular responses. This may be driven by TGF- β -mediated effects on the cellular composition of the aortic wall. For example, TGF- β may induce apoptosis in the SMC population while at the same time inducing adventitial fibroblasts to transform into myofibroblasts. Thus, newly acquired cellular functions (including the production of MMPs) may work in concert with alterations in the relative number of constitutive cells, to change the overall tissue response to TGF- β , shifting the balance toward matrix degradation and aneurysm development.

These studies demonstrating enhanced signaling in the presence of mutant receptors not only speak to the complexities of TGF- β signaling, but also emphasize the importance of continued study of TGF- β in TAA development. Thus, it is likely that by enhancing our understanding of the complex events that mediate malignant vascular remodeling and aortic dilatation, we may also uncover interventional strategies to interrupt aneurysm formation.

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