

Review

Transforming Growth Factor β Signaling Pathway as a Potential Drug Target in Treating Aortic Diseases

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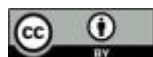
Abstract: The transforming growth factor β (TGF- β) signaling pathway is crucial for preserving the structural homeostasis of the aorta and promoting aortic development. This pathway encompasses both SMAD-dependent canonical pathway and SMAD-independent non-canonical signaling pathway. Heritable thoracic aortic aneurysms and dissection are highly correlated with genetic alterations in TGF- β canonical signaling-related genes. However, depending on the stage of the disease, the TGF- β signaling pathway can have either inhibitory or aggravation effects, making its roles in aortic disease complex and occasionally contradictory. This review aims to elucidate the biological mechanisms underlying the TGF- β signaling pathway in the most common aortic diseases, namely acute aortic syndromes and aortic aneurysms, and to evaluate the potential clinical application of TGF- β -targeting therapies in aortic diseases.

Keywords: TGF- β signaling pathway; aortic aneurysm; acute aortic syndromes; aortic dissection

1. Introduction

Aortic diseases are primarily categorized into thoracic aortic aneurysm (TAA), abdominal aortic aneurysm (AAA) and acute aortic syndromes (AAS) [1]. Aortic dissection, intramural hematoma and penetrating atherosclerotic ulcer (PAU) are the most typical AAS, each posing a risk of rupture. Aortic aneurysm is characterized by a localized dilatation of the aorta, exceeding 1.5 times the normal diameter of corresponding aortic segments in age- and sex-matched healthy individual [2]. Among AAS, aortic dissection is the most common, involving a tear in the inner layer of the aortic wall. This tear allows blood to penetrate the aortic media, splitting the intima in two longitudinally and forming a dissection flap that divides the true lumen from a newly formed false lumen [3]. Open surgical repair or endovascular surgery are recommended to prevent rupture of large aortic aneurysm and dissection [4]. However, a large number of patients with persistent aortic degeneration require further pharmacotherapy [5]. To date, despite the fact that statins and antihypertensive agents could improve the overall cardiovascular risk profile, there are no effective drugs to block the expansion of aortic aneurysms and the occurrence of aortic dissections [6].

The etiology underlying aortic aneurysms and AAS is multifactorial. Factors such as genetic changes affecting cellular function and vascular structure, inflammation, endothelial dysfunction, vascular smooth muscle cells (VSMCs) phenotypic switching, extracellular matrix (ECM) destruction and the formation of intraluminal thrombi are all considered causes of sporadic aortic diseases [7]. Yet, the molecular mechanisms underlying aortic diseases remain elusive. Thus, in-depth study in the pathological characteristics and



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potential molecular mechanisms of aortic aneurysm and aortic dissection and identifying key intervention targets are of great importance for early prevention and drug treatment.

The transforming growth factor β (TGF- β) signaling pathway plays a vital role in regulating the structure and function of the aorta. Genome-wide association analysis (GWAS) has indicated that the single nucleotide polymorphisms (SNPs) of multiple genes in the TGF- β pathway (such as *LTBP4* and *HIPK3*) are closely related to aortic dilation [8]. For example, some SNPs in *LTBP4* lead to unstable TGF- β receptors [9], and others in *HIPK3* might decrease the noncanonical TGF- β signaling [10]. But in general, these SNPs are loss-of-function mutations. Syndromic thoracic aortic aneurysm and dissection (TAAD), including Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS) and Shprintzen-Goldberg syndrome (SGS) are usually caused by sequence variants associated to the TGF- β signaling system [11]. Also, early studies suggested that excessive TGF- β pathway activation is a pathogenic factor in TAAD, with dramatically enhanced TGF- β signaling observed in the aorta of patients with familial aortic dissection and MFS [12–14]. However, the precise role of TGF- β signaling in aortic diseases, particularly whether it exacerbates or mitigates aortic dissection and aneurysms, remains a subject of debate. The main focus of this review is to present and discuss the current molecular knowledge of the effect of the TGF- β signaling pathway on aortic aneurysms and aortic dissection.

2. Overview of TGF- β Signaling Pathway

The activins, bone morphogenetic proteins (BMPs), growth differentiation factors (GDFs), müllerian inhibiting substance (MIS), nodal and TGF- β s are all members of the TGF- β superfamily. These cytokines, depending on the intrinsic properties of the target cells and the surrounding environment, can exhibit autocrine, paracrine, or endocrine functions. While different members of TGF- β superfamily might share similar functions, they can also exhibit antagonistic effects on one another [15]. TGF- β is synthesized as a proprotein that consists of a latency-associated peptide (LAP) at the N-terminus and a mature cytokine at the C-terminus, which is cut in the Golgi apparatus and secreted in a non-covalent form, known as the small latent complex (SLC), also called LAP-TGF- β . This complex can further bind to latent TGF- β binding proteins (LTBPs) to form the large latent complex (LLC), rendering TGF- β inactive. LTBPs crosslink with matrix proteins, such as fibrillin, to ensure that LLC is deposited and stored in the ECM after secretion [16, 17]. TGF- β activation is regulated by integrin proteins, particularly $\alpha\beta6$ and $\alpha\beta8$. These integrins bind to the arginine-glycine-aspartic acid (RGD) motif in the LAP segment, leading to a physical deformation of the latent complex under traction or stress, which then releases active TGF- β [18]. Interestingly, various proteinases, especially matrix metalloproteinases (MMPs), are also involved in the activation of the TGF- β complex. The conformational change induced by integrin proteins makes the complex more prone to proteolytic cleavage and latent TGF- β become activated. The most widely recognized mechanism of latent TGF- β activation via integrins involves direct activation through traction forces generated between cells and the extracellular matrix [19,20].

The TGF- β signaling pathway participates in various biological processes, including the regulation of inflammation, cell proliferation, apoptosis, migration, adhesion, ECM generation and cytoskeleton construction. Therefore, it plays a momentous role in the process of embryonic development, cell fate determination and tissue homeostasis repair. Generally, the TGF- β signaling pathway can be classified into two distinct categories: the canonical TGF- β (SMAD-dependent) signaling pathway and the noncanonical TGF- β (SMAD-independent) signaling pathway [21]. The SMADs family, as transcription factors, can directly mediate transmission of signals from the membrane to the nucleus. The canonical TGF- β signaling pathway, being SMAD-dependent, is essential for aortic development and maintaining aortic wall stability [22]. In the noncanonical TGF- β pathway, the TGF- β receptor complex transmits signals through various alternative pathways, including JNK, TGF- β -activated kinase 1 (TAK1), extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (p38 MAPK) and nuclear factor κ B (NF- κ B) signaling pathways [23].

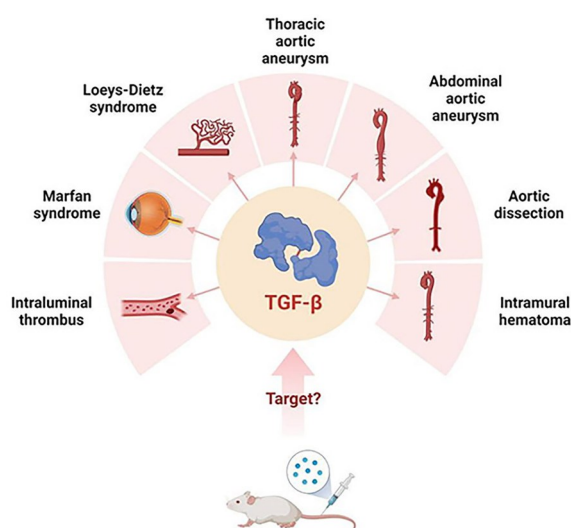


Figure 1. TGF-β's possibly related aortic diseases..

3. The Role of Canonical TGF-β Signaling Pathway in Aortic Diseases

In recent years, a growing body of evidence has suggested the dysregulation of TGF-β signaling in various aortic diseases. Multiple comprehensive reviews have analyzed and discussed the activation of TGF-β signaling in the progression of TAAD. These studies concluded that TGF-β signaling pathway is essential for vascular function: excessive activation may contribute to the development of aneurysms and dissection, while over-inhibition of TGF-β signaling may lead to the overactivation of alternative bypass pathways, thereby inducing the occurrence of aortopathy [11,22,24,25]. The activation of the canonical TGF-β signaling pathway in aortopathy relies on the binding of TGF-β to the serine/threonine kinase homodimer TGF-β type II receptors (TβRII), which promotes phosphorylation of the intracellular domain of TGF-β type I receptors (TβRI). This results in the recruitment and phosphorylation of SMAD2/3, which bind to SMAD4 and form the heterodimer complex. The complex then translocates into the nucleus and binds to SMAD response elements to induce or repress the expression of various genes encoding ECM, anti-proteases, LTBP, or others [26]. Here, we will summarize the effect of canonical TGF-β signaling pathway on the development of aortic aneurysm and aortic dissection.

3.1. Canonical TGF-β Signaling Pathway-Related Genes and TAAD

Knocking out genes such as *Tgfr1* or *Tgfr2*, which encode TβRI and TβRII, can impede the process of normal vascular morphogenesis [27] and hinder the differentiation of smooth muscle cells (SMCs), ultimately resulting in fetal death [28]. To circumvent embryonic lethality, several studies have demonstrated observable aortic pathological changes through the induction of loss of *Tgfr2* and other genes in mice at different ages [29–32]. Postnatal deletion of *Tgfr2* in SMCs leads to aortic thickening, dilatation, and thoracic aorta dissection in both C57BL/6 and *Fbn1*^{C1039G/+} (MFS model) mice. The inactivation of TβRII results in a decrease in canonical SMAD signaling, while simultaneously causing an increase in MAPK signaling [30]. SMAD2 phosphorylation is unchanged in aortic SMCs of youthful MFS mice, but SMC-specific deletion of *Tgfr2* in *Fbn1*^{C1039G/+} mice remarkably decreases the level of SMAD2 phosphorylation and aggravates the aortic lesions [31]. Chen et al. showed that inducible SMC-specific *Tgfr2* knockout significantly decreased the expression of phosphorylated SMAD2 (p-SMAD2) in aortic SMCs, inhibited TGF-β1-induced SMAD2/3 phosphorylation, and eventually resulted in the development of aortic aneurysms in *ApoE*^{-/-} mice [33]. Additionally, a previous study reported that postnatal deletion of *Tgfr2* in SMCs caused severe aortopathy in all areas of the aorta, including aortic dilation, elastolysis, dissection, matrix accumulation, wall thickening and ulceration [32]. These studies strongly suggest that physiological TβRII signaling is crucial for maintaining postnatal aortic health.

Smads deletions can cause aortic aneurysm as well. Pluijm et al. identified that *Smad3* deficiency in mice caused spontaneous dilation of the ascending aorta and an increased incidence of thoracic aortic

aneurysm. *Smad3*^{-/-} aortas exhibited elevated levels of nuclear p-SMAD2 and p-ERK, indicative of upregulated TGF- β receptor activation, yet without activating transcription of downstream TGF- β -targeted genes [34]. In calcium chloride-induced AAA mouse model, *Smad3* deficiency was found to promote AAA formation, characterized by thickened abdominal aortic wall, fragmented elastic fiber and reorganized collagen fibers, and vascular remodeling [35]. These findings provide support for the pivotal functions of SMAD3 in maintaining the integrity of the vessel wall during the development of aortopathy. Aside from *Smad3*, deletion of *Smad4* in SMCs promotes the development of both thoracic and abdominal aortic aneurysms along with reduced canonical TGF- β signaling. The phenomenon is mainly attributed to the upregulation of MMP-12, which is one of the proteases essential for elastin degradation [36]. Another study also reported that inducible *Smad4* deletion in SMCs specifically provoked aortic aneurysms, suggesting that TGF- β signaling disruption triggers interleukin-1 β as a hazard signal for the onset of aortic diseases [37].

3.2. TGF- β Neutralizing Antibodies and Aortic Diseases

Based on the activation of TGF- β signaling in the pathogenesis of aortopathy, the therapeutic efficacy of TGF- β neutralization was observed when the intervention was initiated at P28, P45, or P49 of age in MFS mice [14,38]. However, treatment with TGF- β neutralizing antibody (1D11) from P16 onward exacerbated arterial disease [39]. Apart from that, the application of 1D11 increased the incidence of aortic aneurysm rupture [40]. The detrimental impact of TGF- β neutralizing antibodies on AAA has also been observed. Since the elevated level of TGF- β 1 had been detected in the aneurysmal tissue from AAA patients and mice [41], TGF- β neutralizing antibodies were utilized. Unexpectedly, it exacerbated angiotensin II (Ang II) or elastase-induced AAA [42]. In a similar context, after intraperitoneal injection of another TGF- β neutralizing antibody, 2G7, in Ang II micro-pump-induced AAA mice, the serum levels of TGF- β 1 and TGF- β 3 decrease, and the adventitia of abdominal aorta further expand [43]. Based on these data, it has been proposed that the detrimental effect of TGF- β neutralizing antibodies is associated with the recruitment of macrophages, monocyte invasiveness, activation of MMP-12, and promotion of matrix degradation, which can be partially alleviated by *Mmp12* knockout or acute monocyte depletion. In other words, these tactics are unable to significantly change aneurysm incidence, yet they notably alleviate the severity of aneurysms [44]. Moreover, using neutralizing antibodies to inhibit TGF- β resulted in an enhanced infiltration of leukocytes in both the aortic wall and intraluminal thrombus, thereby leading to persistent dilation of the aortic lumen and aggravation of the ECM degradation. Therefore, the early inhibition of IL-1 β or the suppression of monocyte-dependent responses could significantly mitigate the severity of AAA [45]. TGF- β neutralizing antibodies can also promote the apoptosis of VSMCs, resulting in substantial loss of medial smooth muscle cells [46], potentially increasing the risk of aortic dissection and rupture. Cyclosporine A (CsA), known to induce TGF- β 1 production, maintains vascular elastic fiber integrity, reduces inflammation, increases α -SMA-positive cell content and eventually prevents AAA. However, these protective effects of CsA are disappeared after the administration of TGF- β neutralizing antibody [47]. Intriguingly, there were significant differences in aortic pathological damage caused by systematic inhibition of TGF- β with neutralizing antibody and SMC-specific knockout of *Tgfb2*. The severity of AAA increases after TGF- β neutralizing antibody administration, but the severity of TAA appeared unaffected. In contrast, SMC-specific *Tgfb2* knockout prevented further expansion of the abdominal aortic adventitia but increased the incidence of intramural hematoma in the thoracic aortic wall considerably [43]. Hence, identifying the distinction of the two TGF- β inhibition methods and understanding the specific mechanisms of TGF β 1, 2 and 3 is beneficial for future research in this field.

3.3. TGF- β Overexpression and AAA

Early studies found that local overexpression of TGF- β in the aorta, using adenovirus-mediated delivery, delayed the expansion of AAA. Dai et al. reported that overexpressing active TGF- β 1 in already-formed experimental AAA by endovascular gene delivery could reduce the accumulation of monocytes, macrophages and T cells, inhibit MMPs activity, increase collagen and elastic proteins level, and promote the proliferation of VSMCs [48]. Additionally, heart-specific overexpression of TGF- β 1 upregulated plasma concentration of TGF- β 1 and cardiac phosphorylated SMAD2, and prevented aortic dilation [49]. Bai et al. illustrated that in a calcium chloride-induced AAA mouse model, injection of TGF- β 1 hydrogel into the vessel wall elevated

SMAD2 phosphorylation, and reduced pathological damage to the aneurysm. Most crucial of all, hydrogels could be effectively and easily delivered between the medium and adventitia in swine aortas. This observation suggested that this technique held promise as a potential drug delivery method and therapeutic option [50]. In addition, in a pseudoaneurysm model induced by angioplasty, administration of TGF- β nanoparticles increases the expression of TGF- β 1 and phosphorylation SMAD2, limiting the formation of pseudoaneurysms. Conversely, inhibition of T β RI signaling with SB431542 increases the incidence of pseudoaneurysms, indicating that TGF- β signaling deficiency is closely related to the formation of large pseudoaneurysms [51]. Kojima et al. explored AAA alleviation by overexpressing TGF- β through efferocytosis-stimulating therapy, and demonstrated that administration of the CD47 antibody (MIAP410) into AAA mice promoted the clearance of apoptotic cells, upregulated TGF- β and SMAD3 expression in the vascular media, reduced the accumulation of free apoptotic bodies and inflammatory aggregates in the aortic lesion, and relieved aortic dilation [52]. If proven safe and effective, this method could represent a novel non-surgical approach for treating aortic disease. Furthermore, since the safety of stem cell therapy for AAA has been confirmed, and a multicenter, double-blind, phase III clinical trial has illustrated that local injection of chondrocytes with high expression of TGF- β could remarkably alleviate arthritis, exploring stem cell therapy with high expression of TGF- β might offer new intervention for AAA [53,54].

3.4. Underlying Mechanisms of Improving AAD via TGF- β /SMAD Pathway

TGF- β signaling is of utmost importance in maintaining the equilibrium between ECM synthesis and degradation [55]. TGF- β promotes the synthesis of various elastic proteins by SMCs and stimulates the synthesis of collagen by fibroblasts. Furthermore, TGF- β inhibits MMP-9 secretion by monocytes or macrophages, preventing excessive degradation of ECM under the condition of AAA, thus maintaining vascular structural stability [56]. SMC-specific deletion of *Tgfb2* in *ApoE*^{-/-} mice on a hypercholesterolemic diet promotes extensive reprogramming of contractile medial SMCs to mesenchymal stem cell (MSC)-like cells and leads to development of aortic aneurysms, which is mainly controlled by krüppel-like factor 4 (KLF4) [33]. Postnatal deletion of *Tgfb2* in SMCs decreases the expression of contractile-associated proteins smooth muscle protein 22 (SM22) and smooth muscle myosin heavy chain (SMMHC), inability to maintain contractile phenotype, and increases proliferation capacity, which may potentially increase the susceptibility of the vessel wall to complications such as dissection and dilatation [30]. However, Dichek DA et al. reported that postnatal SMC-specific *Tgfb2* deletion did not alter contractile protein abundance, but resulted in severe endothelial dysfunction and increased contractility of the aorta [32,57]. The authors suggested that this paradox may be due to different aortic mRNA collection sites (total aortic mRNA versus aortic medial mRNA). The *TGFBR1*^{A230T} mutation, which has been newly identified as a mutation associated with LDS, impairs contractile transcript and protein levels, and function of cardiovascular progenitor cell (CPC)-VSMCs via decreasing SMAD3 phosphorylation [58]. Meanwhile, SMAD3 deficiency in CPC-VSMCs notably disrupts canonical TGF- β signaling, thus reducing contractile function and downregulating the expression of contractile-related genes, such as SM α -actin, myosin heavy chain 11, calponin-1 [58,59]. Analogously, the deficiency of microRNA-21 in SMAD3 heterozygous (*Smad3*^{+/-}) mice increases the expression of SMAD7 and suppresses the activation of canonical TGF- β signaling, thereby promoting VSMC phenotype switching from the contractile phenotype to the synthetic phenotype and accelerating aneurysm and dissection [60]. Another study showed that the matrix protein cellular communication network factor 2 (CCN2) has the ability to interact with the TGF- β 1-TGF- β receptor complex, thereby enhancing TGF- β 1-mediated SMAD3 phosphorylation and improving the contractile function of SMCs. Additionally, the deletion of CCN2 triggered SMC reprogramming, thus exacerbating the development of Ang II-induced AAA [61]. GDF11, belonging to the TGF- β superfamily, can hamper SMC phenotype switching and keep its contractile state via inhibition of MMP activity and activation of the canonical TGF- β signaling pathway. Consequently, GDF11 serves as a protective factor against the development of thoracic aortic aneurysms [62]. Interestingly, the mechanisms of SMC phenotype switching in a disease context differ from those during the differentiation of SMCs from CPCs. CPC-VSMCs maintain the contractile phenotype mainly by relying on SMAD3-dependent TGF- β signalling, whereas many other factors besides SMAD3 play a role in phenotype switching of SMCs in a disease context. Besides inducing medial degeneration, disruption of the TGF- β signaling brings on a

large number of monocytes and macrophages to accumulate outside the blood vessels, thus promoting inflammation infiltration and accelerating vascular damage. The specific deletion of *Smad4* in SMCs induces the recruitment of monocytes and triggers inflammation in the adventitia through the activation of the IL-1 β /C-C motif chemokine ligand 2 (CCL2)/C-C motif chemokine receptor 2 (CCR2) axis. Inhibiting this axis by knockout of IL-1 β receptor 1 or *Ccr2*, or using an anti-IL-1 β antibody (canakinumab), blocks pro-inflammatory monocyte infiltration and alleviates the aortic pathology and aneurysm dilation caused by the inactivation of *Smad4* in SMCs [38]. Similarly, in patients with TAAD resulting from rs12455792 variant of *SMAD4*, macrophage recruitment and M1 type inflammatory response increase in the aortic tissue [63]. These results suggest that SMC phenotype switching, endothelial dysfunction and monocyte inflammation, which were induced by disruption of TGF- β signaling, promote the development of aortic diseases.

4. The Role of Noncanonical TGF- β Signaling Pathway in Aortic Diseases

Apart from the canonical SMAD pathway, TGF- β family cytokines also activate non-SMAD signaling pathways, including TAK1, ERK, JNK, p38 MAPK and NF- κ B signaling pathways. Typically, canonical TGF- β signaling is weakened while noncanonical TGF- β signaling is activated. The loss of SMAD4 leads to diminished canonical TGF- β signaling, concurrent activation of the JNK pathway, tissue proteinase S (CTSS) and MMPs, resulting in increased elastin degradation [36]. Likewise, the SMC-specific knockout of *Tgfb2* leads to the downregulation of SMAD2 phosphorylation and the upregulation of p38 phosphorylation in the thoracic aorta. Also, the MAPK pathway is abnormally activated [30]. Unlike the canonical TGF- β signaling pathway, noncanonical TGF- β signaling is believed to promote the progression of aortic dilation, aneurysm and dissection. In MFS mice, the selective inhibition of ERK1/2 has been shown to alleviate the progression of aneurysms, while *Smad4* deletion exacerbates aortic diseases. Moreover, *Smad4* deficiency increases the activation of JNK1, and a JNK antagonist ameliorates aortic growth in MFS mice [64]. One possible explanation is that the ERK pathway inhibits the transcription of SMAD-mediated smooth muscle contraction-related genes and promotes the expression of cell cycle and apoptosis-related genes by Notch 3 in MFS [65]. All of these studies suggest that the canonical and noncanonical TGF- β signaling pathways exert divergent regulatory effects on aortic diseases.

4.1. MAPK Signaling Pathway

The MAPK family includes three key kinases: ERK, JNK, and p38 [66]. ERK1/2 activation participates in non-canonical TGF- β signalling, which is excessively increased in MFS, and has been recognized as a critical factor in the cultivation of aortic pathology [64]. Increase of phosphorylated ERK and activation of the ERK pathway can be detected in the aortas of AAA patients, MFS mice, elastase and Ang II-induced AAA mice. Such activation of the ERK pathway promotes the production and release of MMPs and plasminogen activator, which degrade ECM and accelerate aortic dilation [39,67–70]. Notably, *Erk1^{-/-}* mice exhibit resistance to AAA development, and inhibiting ERK activation blocks AAA formation [68, 69]. Besides, the specific inhibition of TGF- β -mediated ERK signaling activation by losartan has been shown to delay aortic aneurysm expansion, while, enalapril, another agent capable of attenuating TGF- β signaling in the aorta, demonstrates less efficacy [71]. Nettersheim et al. reported that nitro-oleic acid reduces aortic dilation and wall stiffening in MFS via inhibition of aortic ERK1/2 phosphorylation without altering TGF- β 1 or T β RI/II expression [72]. Additionally, some drugs, such as enzastaurin, hydralazine and cycloastragenol significantly reduce aortic root growth in MFS mice or Ang II-treated *ApoE^{-/-}* mice by inhibiting ERK activation [73]. Women diagnosed with MFS face a high risk of experiencing aortic dissection during pregnancy. Habashi et al. proposed that oxytocin mediated the increased risk of aortic dissection during pregnancy through phosphorylation of ERK. Therefore, treatment with oxytocin receptor antagonist, ERK kinase inhibitor (trametinib) or hydralazine substantially decrease pregnancy-associated aortic disease [74]. These studies suggest that the activation of ERK pathway promotes the occurrence and development of aortic dilation and dissection. Therefore, targeting TGF- β 1-mediated ERK signaling could represent a more efficacious therapeutic strategies for aortic diseases.

High levels of phosphorylated JNK have been observed in human AAA tissues [75]. Activated JNK signaling is known to stimulate the secretion of MMPs by monocytes and macrophages, especially MMP-2,

which could exacerbate aortic ECM degradation. In addition, activated JNK constricts the biosynthesis of ECM through downregulating ECM synthetic enzymes to hinder collagen deposition, thus participating in aortic structural damage [76,77]. The activity of JNK is regulated by various signaling molecules. For example, the loss of ephrin-B2 in smooth muscle cells enhances the internalization of platelet-derived growth factor receptor β , subsequently activating the JNK pathway. This activation leads to increased aortic root diameter and vessel wall defects [78]. Also, a recent study demonstrated that prostaglandin E receptor 4 (EP4) in SMCs increased IL-6 secretion, triggered inflammatory cell infiltration via activating the TAK1-NF- κ B/JNK/p38 pathway, leading to exacerbating AAA [79]. Furthermore, knockout of microRNA-33 in macrophages inactivates JNK, reduces the expression of MMP-9, suppresses matrix degradation and attenuates AAA [80]. Comparative studies indicate that administration of JNK inhibitor, SP600125, could alleviate Ang II- and calcium chloride-induced aortic dilation, medial thinning and elastic plate rupture [76, 81]. All these studies demonstrate that JNK-targeted therapy has the potential to offer alternative therapeutic approaches for the treatment of AAA.

It has been reported that an increase of the p38 MAPK signaling activity aggravates the progression of aortic diseases as well. Granata et al. used an MFS patient-derived human induced pluripotent stem cell (hiPSC) model to recapitulate the major phenotypes of MFS vascular pathology and identified a key role for p38 MAPK in disease development. They found that p38 MAPK inhibition by SB203580 increased fibrillin-1 deposition and promoted SMC proliferation, while inhibition of canonical SMAD signaling or ERK1/2 had no significant effect, highlighting a crucial role of p38 MAPK in MFS [82]. Similarly, SMC-specific deletion of *Mapk14* (*p38a*), which is the primary isoform of p38 family in SMCs, has been shown to improve AAA formation, as well as vascular senescence and inflammation [83]. Another study indicates that sodium-glucose cotransporter 2 (SGLT-2) inhibitor, empagliflozin, significantly inhibits Ang II-induced AAA formation, independently of blood pressure effects via inactivation of p38 MAPK signaling pathway [84]. All of these studies indicate the beneficial effect of p38MAPK inhibition on aortic aneurysm and dissection.

4.2. NF- κ B Signaling Pathway

It is known that under stimuli, such as reactive oxygen species (ROS), the NF- κ B pathway is activated, which subsequently triggers a series of inflammatory reactions, including the increased expression of MMPs and monocyte chemoattractant protein 1 (MCP-1). During the formation of AAA, the process of pathological vascular remodeling is primarily triggered by the infiltration of macrophage. The epigenetic enzyme JMJD3 facilitates NF- κ B-mediated transcription of inflammatory genes and enhances monocyte and macrophage infiltration into the aortic wall. Inhibition of JMJD3 prevents AAA formation and decreases macrophage inflammation [85]. Abnormal amino acid metabolism has also been associated with vascular diseases. One study found that the level of 3-hydroxyanthranilic acid (3-HAA) rises in the plasma and aorta of AAA. 3-HAA activates MMP2 exceptionally by increasing the phosphorylation of NF- κ B, thereby promoting AAA formation [86]. In addition, some studies have identified an association between membrane attack complex (MAC) and AAA. MAC can activate NF- κ B and increase the expression of MMP-2 and MMP-9, thus accelerating the development of AAA [87]. Furthermore, evidence suggests that the activation of mesenchymal cells is closely related to vascular inflammation and the formation of AAA. The absence of NF- κ B signaling in mesenchymal cells can appreciably inhibit monocytes recruitment and vascular inflammation, thereby blocking Ang II-induced AAA formation [88]. In addition, Liu et al. has found that eosinophils could block the activation of NF- κ B and polarization of macrophages and monocytes to protect against AAA [89]. These studies collectively demonstrate that NF- κ B-mediated vascular inflammation plays a pivotal role in the progression of AAA.

Apart from promoting the infiltration of inflammatory cells, the TGF- β 1/ROS/NF- κ B signaling pathway also induces SMC senescence and phenotype switching. In aortic tissue harvested from the MFS patients, TGF- β 1 has been found to induce VSMC senescence by activating NF- κ B signaling through the generation of ROS. This effect can be suppressed by NF- κ B inhibitor (SC-514) [90]. Likely, the histone deacetylase SIRT1 has been observed to blocks vascular cell senescence and AAA formation via impeding the binding of NF- κ B to the MCP-1 promoter [91]. The NF- κ B pathway is known to promote SMC phenotypic switching, as evidenced by the downregulation of SM22 α . Inhibition of NF- κ B or ROS has been shown to partially

mitigate the promoting effect of SM22 α deficiency on AAA formation and reduce the maximal diameter of the aorta [92]. Besides, the loss of α -actin in SMCs leads to NF- κ B-dependent abnormal dilation of the mouse aortic root [93]. Liang et al. reported that Ang II promotes SMC proliferation, migration, apoptosis, and MMPs' expression. However, these phenomena are alleviated by inhibiting the NF- κ B signaling pathway [94]. In conclusion, TGF- β 1/ROS/NF- κ B signaling pathway induces monocytes and macrophages inflammation and SMC dysfunction, thus promoting aortic diseases.

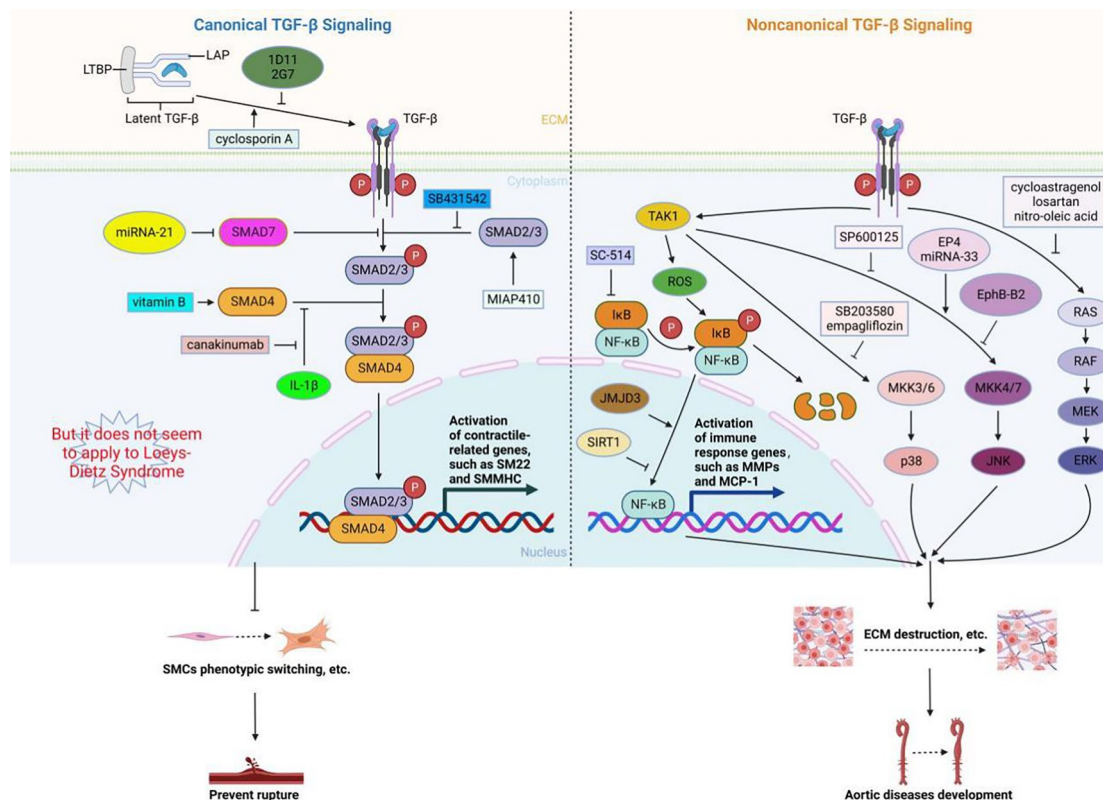


Figure 2. The role of canonical and noncanonical TGF- β signaling pathways in aortic diseases..

5. TGF- β Signaling Pathway and Heritable TAAD

Pathogenic variants in numerous genes, including those within the TGF- β signaling pathway, as well as genes related to extracellular matrix protein, and vascular smooth muscle contraction components or cytoskeleton genes, have been discovered to be associated with an increased risk of heritable TAAD [95,96]. Sequence variants related to the TGF- β signaling pathway have been identified as the underlying cause of syndromic TAAD, encompassing conditions such as MFS, LDS, and SGS. In this review, we will focus on current molecular knowledge and the effect of TGF- β signaling pathway on MFS and LDS.

5.1. Marfan Syndrome (MFS)

MFS is a genetic disorder affecting the connective tissue, primarily manifesting in cardiovascular abnormalities such as aortic aneurysm and dissection, ocular complications like ectopia lentis, pulmonary issues including pneumothorax, and skeletal system disorders characterized by elongated limbs and fingers, scoliosis, and pectus deformities. As a hereditary disease, MFS is primarily caused by heterozygous mutations in the fibrillin-1 gene (*FBNI*). Fibrillin-1 monomers undergo self-assembly to form microfibrils with a diameter of 10 nm. These microfibrils, constituting part of the extracellular matrix in the aortic wall, play a key role in the regulation of TGF- β bioavailability in the extracellular matrix through interacting with latent TGF- β -binding proteins [97]. Thus, mutations in the fibrillin-1 gene not only leads to structural weakness in connective tissue, but also results in an increase in TGF- β signaling. Both of these factors contribute to the complex pathogenesis observed in MFS. Deletion or mutation in *Fbn1* increases the

activation of TGF- β signaling in murine models of MFS [13,14,98]. Although the role of TGF- β in MFS pathogenesis is still debated, early investigations have demonstrated that the overactivation of TGF- β signaling exerted a causal effect on progressive aortic root enlargement and the formation of aortic aneurysm in mice with non-lethal MFS (*Fbn1*^{C1039G/+} mice). This conclusion was derived from the observation that TGF- β antagonists, such as TGF- β -neutralizing antibody or the angiotensin II type 1 receptor (AT1) blocker, could effectively inhibit the development of aneurysms [14]. Another report also showed that treatment with anti-TGF- β antibody in *Fbn1*^{C1039G/+} mice at 4 weeks old could prevent TAA progression [38]. By investigating the effects of TGF- β on the development and advancement of aortic aneurysm in mice with progressively severe MFS (*Fbn1*^{mgR/mgR}), Ramirez et al. revealed that the impact of TGF- β on the formation and progression of aortic aneurysm varied, with some cases showing a protective effect while others demonstrated a detrimental effect. The researchers reached the conclusion that TGF- β hypersignaling played a secondary role in driving the progression of aneurysms in individuals with MFS. This conclusion was based on the observation that blocking TGF- β in young MFS animals during the early stages of the disease (2 weeks old/P16) worsened the aneurysm, whereas treatment administered at later stages proved to be beneficial [39]. Similarly, loss of physiological TGF- β signaling induced by disruption of *Tgfb2* gene exacerbates the degree of TAA and aortic dissection in *Fbn1*^{C1039G/+} mice [32]. In addition, Galunisertib, a highly effective small-molecule inhibitor of T β RI, fails to mitigate the disrupted architecture of the medial wall in MFS mice [99]. These findings suggest that the impairment of basal TGF- β signaling can lead to the development of aortic dilatation and dissection.

The underlying causes for the divergent outcomes observed in aortopathy following TGF- β inhibition remain unclear. However, a growing body of research suggests that the noncanonical TGF- β /ERK signaling pathway plays a significant role in driving the progression of aortic aneurysms in mice with MFS [64,71]. Both ERK 1/2 and SMAD2/3 are activated in MFS mice, and nitro-oleic acid or recombinant angiogenic factor with G patch and FHA domains 1 (AGGF1) protein attenuates TAA, accompanied by the inhibition of ERK1/2 and SMAD2/3 phosphorylation [72,100]. Selective inhibition of ERK1/2 activation ameliorates aortic dilatation but has no effect on SMAD2 activation. Inhibition of canonical SMAD4 exacerbates the aortopathy and causes premature mortality in mice with MFS [65]. Park JH et al. reported MFS mice treated with galunisertib or losartan completely blocked SMAD2 phosphorylation. But only losartan exhibited remarkable inhibition of aortopathy and a marked decrease in ERK1/2 phosphorylation [99]. A preventive treatment with TGF- β inhibitor peptide (P144) before the onset of the aortic aneurysm (at the age of 4 weeks in MFS mice) fully prevented the formation of the aneurysm and reduces the nuclear translocation of p-ERK1/2, but did not significantly inhibit p-SMAD2 activation [101]. Vitamin B mixture has been shown to restore canonical TGF- β signaling and mitigates aortic dilatation in MFS mice [102]. Given the pleiotropic effects of TGF- β in the aortic wall and various tissues, direct inhibition of TGF- β may not be suitable for MFS and could even have harmful effects. Therefore, alternative targets need to be identified. Lim et al. proposed that IL-11 induced by activation of TGF- β 1/T β RII/SMAD2 signaling pathway may potentially play a vital role in noncanonical TGF- β /ERK-mediated aortic dilatation and dissection. They concluded that specifically targeting IL-11-induced ERK activation, either through genetic means or with a neutralizing antibody, rather than the overall TGF- β signaling pathway, had uniquely benefit as this approach effectively limited aortopathy in MFS mouse model and without influencing SMAD4-targeted gene transcription [103–105]. Therefore, the IL-11 emerges as a potential therapeutic target for aortopathy in individuals with MFS.

5.2. Loeys-Dietz Syndrome (LDS)

Loeys-Dietz syndrome (LDS) is characterized by the presence of aortic and branch vessel aneurysms and dissections, distinctive facial features such as hypertelorism, arterial tortuosity, and bifid uvula. Individuals with this syndrome are particularly susceptible to arterial dissection or rupture at a young age [106]. LDS is usually induced by pathogenic variants in multiple genes, including TGF- β ligands (*TGFB2*, *TGFB3*), receptors (*TGFBRI*, *TGFBRII*), or intracellular signaling mediators SMAD3 and SMAD2 [2,107–112]. Almost all sequence variants related to LDS indicate that development of aortic disease can be linked to the deficiency of TGF- β signaling. However, pathogenic variants in *TGFBRI* or *TGFBRII* have been shown to upregulate the level p-SMAD2 in the aortic wall [110]. Consistently, SMAD2 and ERK phosphorylation in

aortic media are evidently increased in knock-in mice with loss-of-function mutations (*Tgfb1*^{M318R/+} and *Tgfb2*^{G357W/+}) at end-stage aneurysmal samples. Furthermore, overall activation of TGF-β signature is also observed in the aortic tissues with mutations in *SMAD3* or *TGFB2/3* [108,111,113]. A recent study identified a novel *TGFBR1* variant (*TGFBR1*^{A230T}) causing LDS, revealing that the *TGFBR1*^{A230T} mutation reduced phosphorylated SMAD3 but does not affect SMAD2 or ERK activation [58]. Several reviews have summarized extensive investigations and proposed some hypotheses to explain this paradox [26,114–117]. Nevertheless, further investigation is required to provide a conclusive explanation for the variations in TGF-β signaling in relation to the development of aneurysms.

6. Conclusions

TGF-β signaling dysregulation plays a vital role in the occurrence and development of aortic diseases. Pathogenic sequence variants in TGF-β signaling-related genes are implicated in hereditary TAAD. In general, SMAD-dependent canonical TGF-β signaling pathways not only promote aortic development and maintain aortic structural homeostasis under normal physiological conditions, but also reduce the incidence of aortic diseases under pathological conditions by inhibiting aortic dilation. The protective mechanisms include promoting extracellular matrix synthesis, activating regulatory T cells, inhibiting inflammation and matrix degradation, and improving VSMCs phenotype switching. On the contrary, TGF-β, through noncanonical signaling pathways, exacerbates aortic injury and the development of aortic aneurysm and dissection. In the clinical setting, the primary approaches targeting TGF-β involving inhibiting TGF-β receptor signal transduction, attenuating its activation and expression, and blocking TGF-β binding to its receptor [118]. However, there are significant challenges in advancing these treatments clinically. For instance, individuals treated with Fresolimumab, a TGF-β-blocking antibody, have experienced the development of acanthoma [119]. Given the complexity of TGF-β's role in aortic diseases, precise and targeted therapies need to be developed. Exploring effective and safe intervention methods to activate canonical TGF-β signals or inhibit non-canonical TGF-β signals can provide potential new treatment strategies for aortic diseases.

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Nonstandard Abbreviations and Acronyms.

3-HAA	3-hydroxyanthranilic acid
AAA	abdominal aortic aneurysm
AAS	acute aortic syndromes
AGGF1	angiogenic factor with G patch and FHA domains 1
Ang II	angiotensin II
AT1	angiotensin II type 1 receptor
BMP	bone morphogenetic protein
CCL2	C-C motif chemokine ligand 2
CCN2	cellular communication network factor 2
CPC	cardiovascular progenitor cell

Continued

CsA	cyclosporine A
CTSS	tissue proteinase S
ECM	extracellular matrix
EP4	prostaglandin E receptor 4
ERK	extracellular signal-regulated kinase
FBN1	fibrillin-1
GDF	growth differentiation factor
GWAS	genome-wide association analysis
hiPSC	human induced pluripotent stem cell
KLF4	krüppel-like factor 4
LAP	latency-associated peptide
LDS	Loeys-Dietz syndrome
LLC	large latent complex
LTBP	latent TGF- β binding protein
MAC	membrane attack complex
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein 1
MFS	Marfan syndrome
MIS	müllerian inhibiting substance
MMP	matrix metalloproteinase
MSC	mesenchymal stem cell
NF- κ B	nuclear factor κ B
p-SMAD2	phosphorylated SMAD2
RGD	arginine-glycine-aspartic acid
ROS	reactive oxygen species
SGLT-2	sodium-glucose cotransporter 2
SGS	Shprintzen-Goldberg syndrome
SLC	small latent complex
SM22	smooth muscle protein 22
SMC	smooth muscle cell
SMMHC	smooth muscle myosin heavy chain
SNP	single nucleotide polymorphism
T β R	TGF- β receptor
TAA	thoracic aortic aneurysm
TAAD	thoracic aortic aneurysm and dissection
TAK1	TGF- β -activated kinase 1
TGF- β	transforming growth factor β
VSMC	vascular smooth muscle cell

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