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Review

Crossing Bridges between Extra- and Intra-Cellular Events in Thoracic Aortic Aneurysms

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Thoracic aortic aneurysms (TAAs) are common, life-threatening diseases and are a major cause of mortality and morbidity. Over the past decade, genetic approaches have revealed that 1) activation of the transforming growth factor beta (TGF-β) signaling, 2) alterations in the contractile apparatus of vascular smooth muscle cells (SMCs), and 3) defects in the extracellular matrix (ECM) were responsible for development of TAAs. Most recently, a fourth mechanism has been proposed in that dysfunction of mechanosensing in the aortic wall in response to hemodynamic stress may be a key driver of TAAs. Interestingly, the elastin-contractile unit, which is an anatomical and functional unit connecting extracellular elastic laminae to the intracellular SMC contractile filaments, via cell surface receptors, has been shown to play a critical role in the mechanosensing of SMCs, and many genes identified in TAAs encode for proteins along this continuum. However, it is still debated whether these four pathways converge into a common pathway. Currently, an effective therapeutic strategy based on the underlying mechanism of each type of TAAs has not been established. In this review, we will update the present knowledge on the molecular mechanism of TAAs with a focus on the signaling pathways potentially involved in the initiation of TAAs. Finally, we will evaluate current therapeutic strategies for TAAs and propose new directions for future treatment of TAAs.

Key words: Elastin-contractile unit, Mechanosensing of SMCs, TGF-β, Extracellular matrix (ECM), Signaling pathways, Thoracic Aortic Aneurysm (TAA)

Introduction

Aortic aneurysms are characterized by an abnormal enlargement of the aortic lumen, usually asymptomatic, and are associated with a high risk of mortality from dissection and/or rupture. Aortic aneurysms can occur in the portion of the aorta above the diaphragm, termed thoracic aortic aneurysms (TAAs), or in the portion below the diaphragm, termed abdominal aortic aneurysms (AAAs). Whereas AAAs have been linked to atherosclerosis and chronic inflammation (reviewed in[1, 2]), TAAs are often associated with heritable and degenerative diseases such as Marfan syndrome (MFS) and Loeys-Dietz syndrome (LDS). MFS patients were found to have mutations in the *FBN1* gene, which encodes the extracellular matrix (ECM) protein fibrillin-1, a major component of microfibrils; structures that serves as a scaffold for elastin deposition and provides structural support and stability to elastic laminae in the aorta[3]. Heritable TAAs without syndromic features have also been reported and are classified as familial thoracic aortic aneurysms/aortic dissections (TAAD). A number of nonsyndromic TAAD genes that have been identified so far turned out to be genes involved in regulation of smooth muscle cell (SMC) contraction (reviewed in[4, 5]). Interestingly, regardless of the cause, TAAs are often accompanied by the disruption of elastic laminae. Indeed, mutations in several genes encoding for components of elastic laminae such as fibulin-4, microfibril-associated glycoprotein 2 (*MAGP2*), and lysyl oxidase (*LOX*), a cross-linking enzyme for elastin and collagen, have also been implicated in TAAs[6-8]. The discovery of gene mutations in TAAs has rapidly progressed by introduction of next generation sequencing technology combined with human genetics studies.

Most recently, it has been proposed that dysfunction of the mechanosensing in the aortic wall in

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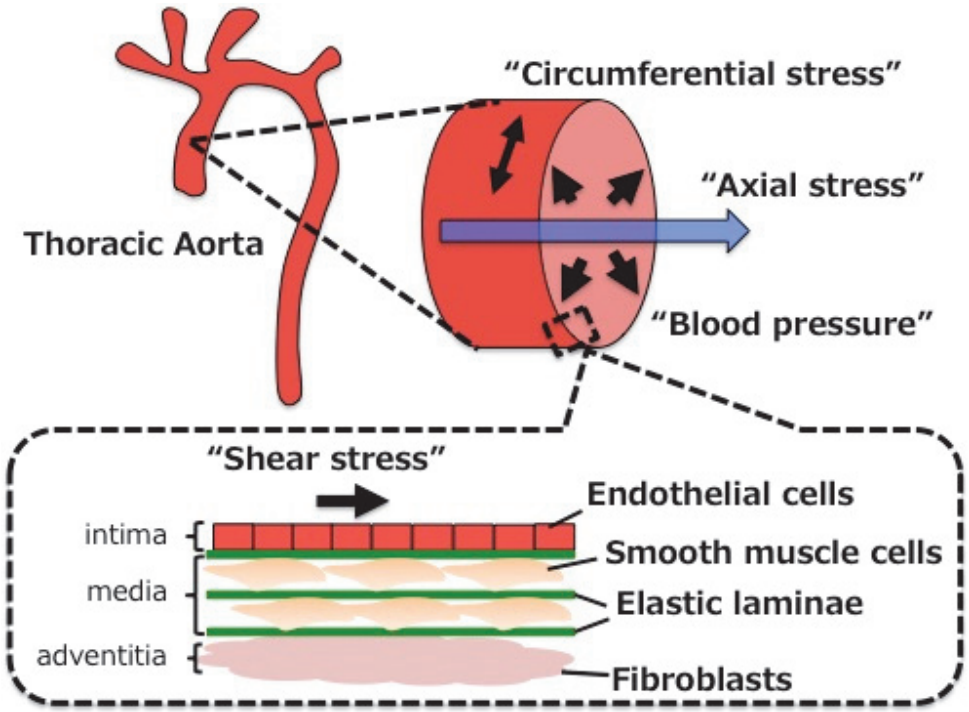

Fig. 1. Structure of the aortic wall and hemodynamic stresses.

The aortic wall consists of three layers: intima, media and adventitia. These layers include endothelial cells (ECs), smooth muscle cells (SMCs) and elastic laminae, and fibroblasts, respectively. One risk factor for TAAs is dysfunctional mechanosensing of SMCs in the aortic wall, which leads to increased hemodynamic stress.

response to hemodynamics may be a key driver of pathogenesis of TAAs (**Fig. 1**; reviewed in[9, 10]). In particular, abnormal mechanosensing of SMCs due to the loss of elastic laminae-SMC connections (**Fig. 2**) and the resultant alteration of actin cytoskeletal remodeling, play causative roles in the formation of aortic aneurysms[11]. These observations are consistent with the concept of an "elastin-contractile unit" that is involved in the mechanosensing of SMCs and maintenance of aortic wall integrity[4, 12].

In this review, we will summarize the knowledge obtained from patients and mouse models (**Table 1-3**, respectively), and the underlying signaling pathways involved in pathogenesis of TAAs (**Fig. 3**). Finally, we will discuss current and future therapeutic strategies for TAAs.

Defective Fibrillin-1 and Activation of TGF-β Signaling in TAAs

The pathogenesis of MFS in humans and mouse models was initially suggested to be due to a weakening of the aortic wall as a result of abnormal fibrillin-1[3, 13]. Subsequently, it was proposed that increased transforming growth factor beta (TGF-β) signaling was the primary cause of aneurysm formation (reviewed in[14, 15]). TGF-β plays important roles in embryogenesis, development and normal tissue homeostasis by affecting cell proliferation and differentiation, and extracellular matrix (ECM) synthesis. Binding of TGF-β ligands to TGF-β receptors activates downstream signaling pathways, including the phosphorylation (*p*-) of Smad2 and Smad3 (known as canonical pathway), leading to the translocation of Smad4 into the nucleus and the activation of transcription of Smad-targeted genes[16]. Connective tissue growth factor (*CTGF*) and plasminogen-activator inhibitor-1 (*PAI-1*) are both well known target genes downstream of this canonical pathway and are involved in aortic wall remodeling. TGF-β also affects Smad-independent pathways (known as non-canonical pathways), which are the mitogen-activated protein kinase (MAPK) cascades that include extracellular signal-regulated kinase 1 and 2 (ERK1/2), Jun N-terminal kinase (JNK) and p38[17, 18].

Dysregulation of TGF-β activity has been implicated in the pathogenesis of MFS[19], and mutations in the genes encoding the TGF-β receptor type II (*TGFBR2*) and type I (*TGFBR1*) were identified in LDS[20, 21]. In MFS, it was proposed that defects in fibrillin-1 causes impaired tethering of the large latent complex (LLC),

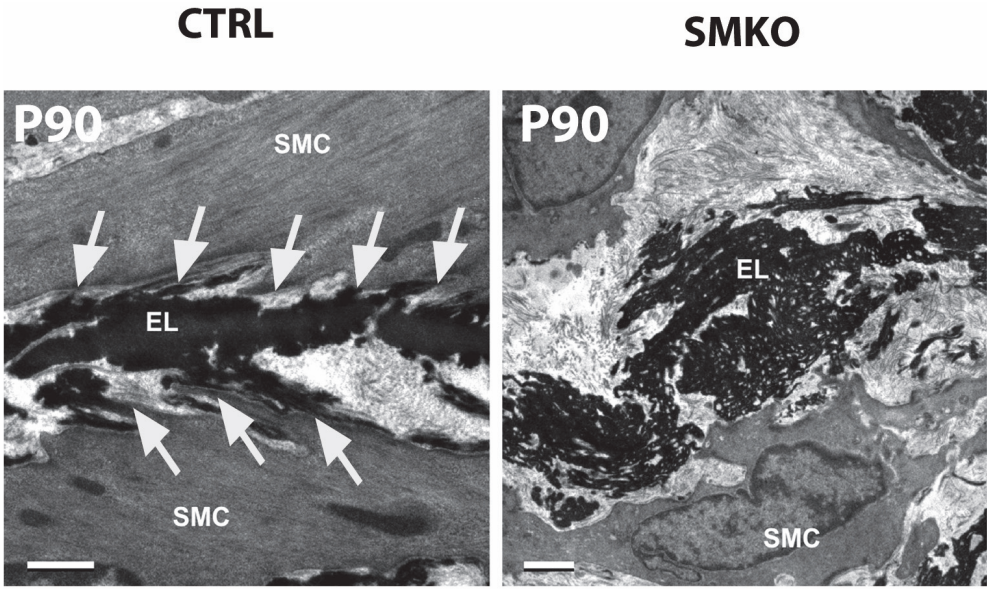

Fig. 2. Elastin-contractile unit.

Electron microscopy images from CTRL and *Fbln4*^{SMKO} (SMKO) ascending aortas at P90. Elastic laminae (EL)-SMC connections are well formed in CTRL aortas (white arrows), whereas the elastic laminae are disrupted and not connected to SMCs in SMKO aorta. Scale bars = 1 μm. Figure from Yamashiro *et al*, "Abnormal mechanosensing and cofilin activation promote the progression of ascending aortic aneurysms in mice" Sci. Signal. 20 Oct 2015: vol. 8, Issue 399, pp. ra105 DOI: 10.1126/scisignal.aab3141. Reprinted with permission from AAAS.

which is composed of proTGF-β dimers covalently bound to latent TGF-β binding proteins (LTBPs)-1, -3, or -4[22], to microfibrils[23]. Active TGF-β is released from the LLC by activators such as integrin $\alpha v\beta 6$[24, 25], thrombospondin-1 (TSP1)[26], matrix metalloproteinases (MMPs)[27, 28] and reactive oxygen species (ROS)[29]. It was hypothesized that mutations in fibrillin-1 disrupt binding of the LLC to fibrillin-1 and increase bioavailability of TGF-β in the aortic wall[23, 30, 31].

Several mouse models of MFS have provided some clues regarding the molecular pathogenesis of thoracic aortic diseases[32]. *Fbn1*$^{mgR/mgR}$ mice, which have only 20% of the amount of normal fibrillin-1, were established as the first MFS mouse model[33]. *Fbn1*$^{C1039G/+}$ mice, which harbor a disease-causing missense mutation in fibrillin-1, were also generated and recapitulated the aortic aneurysm phenotype[34]. Both types of MFS mice showed upregulation of *p*-Smad2/3 (canonical pathway) and *p*-Erk1/2 (noncanonical pathway) as well as fragmentation of elastic laminae. The severity of the aortic aneurysm, however, differed between these MFS mice; *Fbn1*$^{C1039G/+}$ mice exhibit slowly progressing aortic root aneurysms but rarely showed dissection or rupture, whereas *Fbn1*$^{mgR/mgR}$ mice showed a more severe phenotype with rapidly enlarging aortic root aneurysms and frequent dissections and/or ruptures. *Fbn1*$^{-/-}$ mice exhibit the most severe aortic phenotype and die postnatally within the first two weeks[35]. Mutations in genes encoding proteins in the TGF-β signaling pathway, including the TGF-β ligand *TGFB2*[36, 37], *SMAD3*[38] and *SMAD4*[39] were identified and shown to predispose affected individuals to thoracic aortic diseases. Interestingly, the causative mutations in these genes were shown to be loss-of-function mutations; however, paradoxical activation of TGF-β signaling was observed in the aorta of these MFS-related mouse models.

Surprisingly, treatment of these MFS mice with TGF-β neutralizing antibodies prevented progression of TAAs in some studies[40, 41], while promoting aneurysm expansion in others[42]. In addition, SMC-specific *Tgfbr2* disruption in *Fbn1*$^{C1039G/+}$ mice showed activation of the non-canonical pathway and acceleration of aneurysm growth[43]. It is interesting to note that *Ltbp3* deficiency prevented the aneurysm phenotype in *Fbn1*$^{mgR/mgR}$ mice with reduced disruption of elastic fibers and decreased Erk1/2 and Smad2/3 activation[44]. Thus, it is plausible that improper localization of the LLC to microfibrils mediated by LTBP3 contributes to progression of TAA in MFS.

Since the indentification of fibrillin-1 as a gene responsible for syndromic TAAs, substantial progress has been made in identifying the altered signaling pathways in this disease, however, the mechanism by

Table 1. Summary of selected time points with significant findings on MFS and TGF-β related TAAs studies.

Year	Description	Reference
1991	FBN1 (encoding fibrilin-1 protein) gene mutations cause Marfan syndrome.	Dietz *et al*[3]
1997	Fibrillin-1 deficiency recapitulated vascular phenotype of Marfan syndrome in mice.	Pereira *et al*[32]
1999	Dysfunction of fibrilin-1 mimic Marfan syndrome, generating *Fbn1*$^{mgR/mgR}$ mice.	Pereira *et al*[33]
2003	Identified the upregulation of TGF-β activity in Marfan syndrome.	Neptune *et al*[19]
2004	Missense mutation of fibrillin-1 mimic Marfan syndrome, generating *Fbn1*$^{C1039G/+}$ mice.	Judge *et al*[34]
2004-2005	Identification of *TGFBR1* and *TGFBR2* mutation driven Marfan syndrome.	Mizuguchi *et al*[20] Loeys *et al*[21]
2006	Angiotensin receptor blockade as therapeutic target in mice.	Habashi *et al*[40]
2010	Identification of *SMAD3* mutation cause aortic aneurysm.	Van de Laar *et al*[38]
2012	Identification of *TGFB2* mutation driven Marfan syndrome.	Lindsay *et al*[36]
2015	Ltbp3 deficiency prevents aneurysm phenotype in *Fbn1*$^{mgR/mgR}$ mice.	Zilberberg *et al*[44]

Table 2. Summary of selected time points with important findings in familial TAAs studies.

Year	Description	Reference
2006	Mutation in *MYH11* (encoding smooth muscle myosin heavy chain) cause a familial TAAD.	Zhu *et al*[45]
2007	Mutations in *ACTA2* (encoding α-SMA) lead to familial TAAD.	Guo *et al*[48]
2010	Mutations in *MLCK* (myosin light chain kinase) cause familial TAAD.	Wang *et al*[52]
2013	*PRKG1* variant (p.R177Q) cause familial TAAD.	Guo *et al*[53]
2016	*Foxe3* deficiency reduced SMCs density and mutations predispose to TAAs.	Kuang *et al*[54]
2017	Disruption of *Acta2* in SMCs activate ROS and NF-kB signaling, leading *At1r* expression.	Chen *et al*[51]

Table 3. Summary of selected time points with significant findings linked to TAAs and fibulin-4, fibulin-5 and LOX mediated elastic fibers disruption.

Year	Description	Reference
2002	Inactivation of *LOX* leads to aortic aneurysms in mice.	Maki *et al*[6]
2002	Fibulin-5 is essential for elastic fiber assembly.	Yanagisawa *et al*[67] Nakamura *et al*[68]
2006	*ELN* (encoding elastin protein) mutations cause aortic disease in patients with cutis laxa.	Szabo *et al*[80]
2006	Fibulin-4 knockout mice abolished elastogenesis and are embryonic lethal.	McLaughlin *et al*[7]
2006	Fibulin-4 is necessary for elastic fiber formation and connective tissue development.	Hucthagowder *et al*[77]
2007	Fibulin-4 knockdown mice showed dilatation, tortuous ascending aorta.	Hanada *et al*[79]
2007	Mutations in *FBLN4* cause aortic aneurysm.	Dasouki *et al*[78]
2010	Smooth muscle specific deletion of *Fbln4* cause TAAs. Generating *Fbln4*^{SMKO} mice.	Huang *et al*[83]
2013	Losartan prevent aortic aneurysm in *Fbln4*^{SMKO} mice.	Huang *et al*[84]
2015	Abnormal mechanosensing in SMCs initiate aneurysm formation in *Fbln4*^{SMKO} mice.	Yamashiro *et al*[11]

which fibrillin-1 controls the bioavailability of TGF-β signaling has not been determined. Additionally, it is not known whether upregulation of TGF-β signaling pathway is the primary driver for TAA pathogenesis, or how loss-of-function mutations in TGF-β components lead to heightened TGF-β activity.

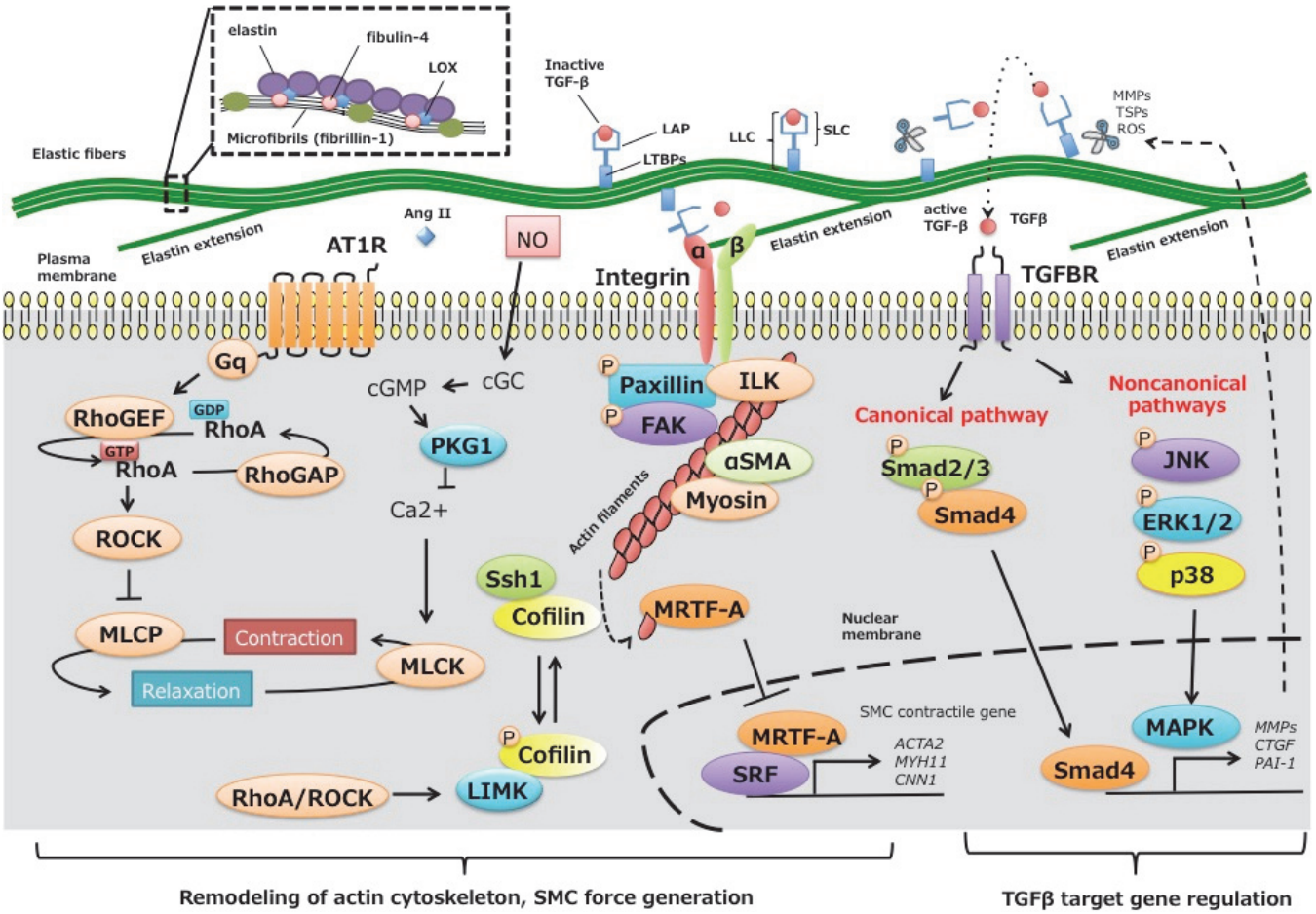

Fig. 3. Molecular signaling pathways involved in TAA formation.

The ECM protein fibulin-4 regulates lysyl oxidase (LOX) activity, which plays an important role in elastic fiber assembly. Fibrillin-1 is a major component of extracellular microfibrils. Inactive TGF-β is tethered onto microfibrils via latent TGF-β binding proteins (LTBPs). Once TGF-β is activated by one of its activators, such as MMPs, TSPs, ROS or integrin $\alpha v\beta 6$, it is released from latency-associated protein (LAP) and binds to its receptor (TGFBR), thus activating subsequent signaling pathways. Angiotensin II type 1 receptor (AT1R) and nitric oxide (NO) signaling pathways are involved in SMC contraction or relaxation. Integrins leads to an altered mechanotransduction signal and initiation of cellular responses such as cytoskeletal remodeling.

LLC (large latent complex), SLC (small latent complex), FAK (focal adhesion kinase), ILK (integrin-linked kinase), RhoGEF (Rho guanine nucleotide exchange factor), RhoGAP (Rho GTPase-activating protein), ROCK (Rho associated kinase), MLCP (myosin light chain phosphatese), MLCK (myosin light chain kinase), PKG1 (protein kinase G 1), cGC (soluble guanylate cyclase), cGMP (cyclic guanosine monophosphate), MRTF-A (myocardin-related transcription factor A), SRF (serum response factor), JNK (c-Jun NH2-terminal kinase), ERK (extracellular signal regulated kinase), LIMK (LIM kinase), Ssh1 (slingshot-1).

Alteration of SMC Contractile Apparatus in TAAs

Familial thoracic aortic aneurysms and dissections (familial TAAD) are autosomal dominant disorders and refer to an inherited predisposition to thoracic aortic disease in the absence of syndromic features. Mutations in the *MYH11* gene, which encodes for the thick filaments in the smooth muscle-specific isoform of myosin heavy chain, were identified in familial TAAD with patent ductus arteriosus (PDA)[45, 46]. These mutations led to deletion of the C-terminal region of *MYH11* and were predicted to decrease myosin motor activity. A rare variant of $MYH11^{R247C}$ was also reported in TAAD and mice carrying homozygous $Myh11^{R247C}$ were generated[47]. The $Myh11^{R247C/R247C}$ mice exhibited decreased aortic contraction but no aortic aneurysms; however, they developed severe neointima formation after injury due to an increased proliferation of SMCs. In addition, *ACTA2*, which encodes the SMC-specific isoform of α-actin, was also identified as a causal gene in familial TAAD[48]. Although *Acta2* null mice did not develop aortic aneurysms, they showed compromised vascular contractile force,

tone and blood flow[49], as well as increased neointima formation after vascular injury due to proliferation of SMCs and activation of focal adhesion kinase (FAK)[50]. Furthermore, it has recently been shown that *Acta2*-null mice have increased angiotensin Ⅱ (Ang Ⅱ) signaling in a ligand-independent manner. Loss of SM-actin led to an increase in ROS generation and an upregulation of Ang Ⅱ type 1a receptor (*Agtr1a*) expression, thereby increasing the sensitivity to Ang Ⅱ by 100-fold in SMCs[51]. These studies indicate that mutations in SMC contractile genes not only affect contractile force generation but also alter the intrinsic properties of SMCs.

Other mutations linked to familial TAAD are dominant negative mutations in the gene encoding for the myosin light chain kinase (*MYLK*), which controls SMC contraction[52]. One PRKG1 variant (p.R117Q), which encodes a type I cyclic guanosine monophosphate (cGMP)-dependent protein kinase (*PKG1*) that is activated upon binding of cGMP and controls SMC relaxation, was also identified as a causal mutation in TAAD[53]. Additionally, in the forkhead family of transcription factors, forkhead box E3 (*FOXE3*) mutations have been reported in TAAD. *Foxe3*$^{-/-}$ mice had decreased SMC density in the aortic media and increased SMC apoptosis leading to dysfunction of the aortic wall[54]. Mechanistically, these mutations lead to reduction of SMC contraction.

Rare variants in *MFAP5* (encoding Microfibril-Associated Glycoprotein 2, *MAGP2*) and *MAT2A* (encoding methionine adenosyltransferase 2A) have also been found in TAAD[8, 55]. *MFAP5* is a component of elastic fibers and associates with the microfibrils. Although the *Mfap5* knockout alone did not show an obvious phenotype, double knockout mice for *Mfap5* and *Mfap2*, which encode an evolutionary-related protein known as *MAGP1*, caused age-dependent aortic dilation[56]. *MAT2A* is involved in the synthesis of s-adenosylmethionine, which serves as a methyl-group donor for methylation reaction *in vivo*. In both cases, the alteration of these genes caused haploinsufficiency or loss-of-function and predispose the affected individuals to TAAD.

In the aortic wall, endothelial cells (ECs) and SMCs constantly interact with each other, either directly or in a paracrine fashion. Nitric oxide (NO) is involved in vascular tone[57] and is produced from L-arginine by a calcium-dependent endothelial nitric oxide synthase (NOS-3; known as eNOS). NO regulates the degree of SMC contraction by stimulating soluble guanylyl cyclase (sGC), which generates cyclic GMP and activates protein kinase G, thereby activating myosin light chain phosphatase (MLCP) and causing SMC relaxation[58]. Endothelial dysfunction causes altered NO production, increased aortic wall stiffness and increased pulse wave velocity[59]. Such endothelial dysfunction has been reported in MFS patients[60, 61] and in the *Fbn1*$^{C1039G/+}$ mice[62]. However, how the dysregulation of endothelial cells and NO signaling contribute to the development of TAAs remains unexplored.

Disruption of Elastic Fibers in TAAs

Elasticity is provided by elastic fibers, which play a crucial role by maintaining structural integrity in the medial layer of the aorta. The major components of elastic fibers are polymerized elastin and microfibrils (consisting predominantly of fibrillin-1). Normally, monomers of elastin, known as tropoelastin, form small aggregates (known as coacervates), that are transported to and deposited onto microfibrils. These elastin aggregates are then cross-linked by lysyl oxidase (LOX) to form mature, insoluble elastic fibers[63]. Fibulins (FBLNs) play a critical role in elastic fiber assembly, and to date, seven members of the FBLN family have been identified[64, 65]. Among these members, *FBLN3*, *4* and *5* possess high homology to each other and are involved in elastic fiber assembly[7, 66-68]. An immuno-electron microscopy (EM) study showed that fibulin-4 is localized on microfibrils and fibulin-5 on elastin[69]. Subsequent research revealed that fibulin-5 promotes coacervation of tropoelastin and its deposition onto microfibrils[70] by interacting with LTBP-4, thereby leading to cross-linking and elastic fiber assembly[71, 72]. In addition, Lox and Loxl1 (lysyl oxidase-like protein 1) are recruited to elastic fibers in a fibulin-4-dependent and fibulin-5-depednet manner, respectively[73, 74]. Inactivation or loss-of-function mutations of LOX reduces the crosslinking of collagen and elastin and causes aortic aneurysms[6, 75].

Although elastic fiber disruption was frequently observed in the aneurysmal wall of MFS patients, MFS mouse models[76] and *FBLN4* deficiency[77-79], an aneurysm phenotype was uncommon in patients with mutations in *ELN* (encoding elastin protein)[80]. In addition, *Eln* deficiency in mice led to increased SMC proliferation and thickening of the aortic wall with narrowing of the lumen[81]. Similarly, aortic aneurysms were never observed in mice deficient in Fibulin-5 (*Fbln5*) or in cutis laxa patients with *FBLN5* deficiency[67, 82]. These observations suggest that a disrupted elastin core is not sufficient to cause TAAs and that elastin and microfibrils have distinct roles in protecting the vessel wall from the development of TAAs.

Loss of Elastin-Contractile Units Results in Abnormal Mechanosensing of SMCs in TAAs

SMC-specific deletion of *Fbln4* in mice (*Fbln4*^{SMKO}), showed ascending aortic aneurysms with marked disruption of elastic fibers, thickened medial wall, increased phosphorylation of ERK1/2 signaling and decreased expression of SMC differentiation markers[83]. In addition, angiotensin-converting enzyme (ACE) was highly expressed in the aneurysmal walls and subsequent activation of angiotensin II signaling in the aortic wall was responsible for driving the aneurysm phenotype[84]. In this *Fbln4*^{SMKO} model, aneurysms are completely prevented by administration of an ACE inhibitor or angiotensin II type 1 receptor (AT1R) blockade (ARB) within the first month of life. ARB treatment initiated after the establishment of an aneurysm did not reverse the aneurysm phenotype, indicating that the signals required for maintenance of aneurysms might be independent of angiotensin II- AT1R[84]. Furthermore, the actin depolymerizing factor cofilin, which severs polymerized actin and triggers disassembly of actin fibers, was activated (=dephosphorylated) by its phosphatase slingshot-1 (Ssh1), resulting in accelerated actin remodeling[11, 85]. In the *Fbln4*^{SMKO} aneurysmal wall, the ratio of monomeric actin (G-actin) to filamentous actin (F-actin) was significantly increased compared to control mice[11]. The increased G-actin potentially affects aneurysm expansion by sequestering myocardin-related transcription factor A (MRTF-A) in the cytoplasm and inhibiting its binding to the transcriptional co-activator serum response factor (SRF), which induces the transcription of SMC contractile genes, including *Acta2*, *Myh11* and *Cnn1* (*calponin 1*)[86]. Similarly, mice that have integrin-linked kinase (ILK) deletion in vascular SMCs (*SM22Cre*$^{+}$*Ilk*$^{Fl/Fl}$) showed aneurysmal dilatation, alteration in RhoA/Rho-associated protein kinase (ROCK) signaling, decreased F-actin and abnormal localization of MRTF-A[87]. ILK is located at focal adhesions and links the ECM to the actin cytoskeleton via β1- and β3-integrins. Since integrin cytoplasmic domains lack actin-binding sites and enzymatic activity, signaling is propagated through a series of linker proteins including vinculin, paxillin, talin, α-actinin and kinases such as FAK and ILK (reviewed in[88-90]). Therefore, deletion of ILK in vascular SMCs may lead to the impaired activation of RhoA/ROCK and down-regulation of SMC contractile genes due to reduced nuclear MRTF-A.

Interestingly, disruption of elastic laminae-SMC connections was observed in *Fbln4*^{SMKO} aortas, along with a remarkable, moth eaten-like, irregular appearance of the elastin located between the SMC layers (**Fig. 2**). In wild-type aortas, extensive connections exist between the elastic laminae and SMCs via elastin extenstions and cell surface receptors, such as integrin receptors. The elastin extensions attach to the cell surface at the sites of membrane-associated dense plaques; sites where intracellular actin filaments attached to cell membrane[91]. This "elastin-contractile unit" of the aorta plays a critical role in the proper transmission of the mechanical force between elastic laminae and SMCs (**Fig. 2**). Disruption of genes involved in the extracellular or intracellular portion of the elastin-contractile unit have been shown to lead to aortic aneurysms in humans and mice (reviewed in[4]). In *Fbln4*^{SMKO} aortas, mechanosensitive molecules such as early growth response 1 (Egr1), ACE and TSP1, all of which were shown to respond to pressure overload of the aorta, were highly up-regulated, and phosphorylation of cofilin was significantly decreased (=activated) in the aneurysmal wall[11]. These observations suggested that a loss of elastin-contractile units resulted in abnormal mechanosensing of the *Fbln4*^{SMKO} aortas. The fact that down-regulation of Ssh1 by a phosphatidylinositol-3 kinase (PI3K) inhibitor led to an increase in phosphorylated cofilin and prevented the aneurysms expansion in *Fbln4*^{SMKO} mice, indicates that abnormal mechanosensing may be driving the aneurysmal phenotype. Similarly, it was reported that impaired microfibril-cardiomyocyte connections in *Fbn1*$^{mgR/mgR}$ mice caused down-regulation of phosphorylated FAK and affected intracellular signaling in the heart[92]. Furthermore, compound heterozygous mice for *Fbn1* and *Itgb1* (encoding the integrin β1 gene) developed cardiomyopathy while *Fbn1*$^{+/-}$ mice appeared normal[92]. We speculate that in the aorta, the elastin-contractile units composed of elastin extensions, SMC receptors and actin filaments, form a structural and functional unit that transmits mechanical stress from the ECM to the SMCs, as well as maintains cellular tension through actin cytoskeletal remodeling.

Prospective Strategies for Treatment of TAA

Current therapeutic strategies to treat TAAs are limited to surgical endovascular procedures such as stent grafts and aortic replacements[93]. So far, effective therapeutic strategies based on the etiology of each TAA type have not been established. Further understanding of the underlying mechanism of TAAs is required to establish an effective treatment for TAAs.

In the *Fbn1*$^{C1039G/+}$ mice, treatment with losartan (ARB) prevented aortic root enlargement from exceeding normal levels and recovered pathologic changes, such as elastic fiber fragmentation, in the medial layer[40]. The first prospective trial was reported in 2008; losar-

tan treatment significantly reduced dilatation of the aortic root growth in young children with severe MFS[94]. Although this trial was a small cohort study with only 18 patients, the success of using losartan to prevent TAA in MFS patients led to the initiation of randomized trials of losartan worldwide. In 2013, an open-label, randomized controlled trial was conducted as a series of double-blind trials, which assessed 233 MFS patients over the age of 18 years. This trial reported that losartan reduced the aortic dilatation rate in the ascending aorta in patients who had not undergone aortic root replacement, and in the ascending aorta in patients who had undergone aortic root replacement[95]. Subsequent analyses revealed that MFS patients with *FBN1* haploinsufficiency seem to be more responsive to losartan therapy for the inhibition of aortic root growth compared with dominant-negative patients[96]. The largest randomized trial, which enrolled 608 patients with MFS between the ages of 6 months to 25 years, demonstrated that both groups treated with losartan or atenolol (β blocker) showed a decrease in the growth of aortic root with no significant difference between the groups[97]. Recent trials from European countries comparing losartan to β blockers or placebo reached similar conclusions[98, 99].

A more recent study in *Fbn1*$^{mgR/mgR}$ mice showed that neither losartan nor TGF-β neutralizing antibodies prevented aneurysm formation; however, a combination of both treatments starting at postnatal day (P)16 and P45, respectively, effectively prevented aortic aneurysms in these mice[42]. Other potential therapeutic targets that have been identified include MMPs and PI3K. Inhibition of MMP activity by doxycycline and deletion of *Mmp2* gene attenuated aneurysm formation in the *Fbn1*$^{C1039G/+}$ and *Fbn1*$^{mgR/mgR}$ mice[100], and two PI3K inhibitors, Wortmannin and LY294002, independently prevented TAA progression in the *Fbln4*^{SMKO} mice[11]. It is therefore likely that a multidrug regimen targeting various molecular pathways will be required to prevent TAAs.

Conclusion

For the past 20 years, hyperactivation of TGF-β signaling pathways, disruption of the vascular SMCs contractile apparatus and impairment of ECM synthesis have been identified as causal events for TAAs. Molecular signaling pathways have been linked to initiation of TAAs although it is still debated whether these pathways converge into a common pathway or are independent of each other (**Fig. 3**). Currently, we have not established effective therapeutic strategies based on the etiology of each TAA type. Accumulating recent reports suggest that studying a better understanding of the mechanobiology of SMCs will shed light on advanced therapeutic strategies based on the underlying pathophysiology of TAAs.

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Conflicts of Interest

None.

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