Transforming Growth Factor-β and Abdominal Aortic Aneurysms

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Summary: Polymorphisms in the TGFβ signaling components are associated with AAA in some human population studies. In experimental animals TGFβ protects against AAA formation, progression and rupture. In animal models of AAA TGFβ decreases aortic inflammatory cell infiltration, extracellular matrix degradation, and vascular smooth muscle cell apoptosis. The TGFβ signaling pathway may provide a therapeutic target for AAA.

Abstract

Abdominal aortic aneurysms (AAAs) are common problems in aged people which can be associated with severe complications including aortic rupture and death. Transforming growth factor-β (TGFβ) has been implicated as causative in the development of thoracic aortic aneurysms (TAAs). In contrast current evidence suggests TGFβ inhibits AAA development. Polymorphisms in the TGFβ signaling components are associated with AAA in some human population studies. In experimental animals TGFβ protects against AAA formation, progression and rupture. In animal models of AAA TGFβ decreases aortic inflammatory cell infiltration, extracellular matrix degradation, and vascular smooth muscle cell (VSMC) apoptosis, all factors implicated in AAA pathogenesis. The TGFβ signaling pathway may provide a therapeutic target for AAA although better clarity is needed regarding the distinct roles of TGFβ in TAA and AAA.

Key words: abdominal aortic aneurysm; extracellular matrix; matrix metalloproteinase; transforming growth factor-β

1. Introduction

Aortic aneurysm is the general term for any dilation (aneurysm) of the aorta to greater than 1.5 times normal size [1]. Aortic aneurysms can be classified based on anatomic location into three types: (1) thoracic aortic aneurysms (TAAs) which involve the ascending aorta, arch or descending thoracic aorta; (2) abdominal aortic aneurysms (AAAs) which affect the abdominal aorta; and (3) thoracoabdominal aortic aneurysms (TAAAs) which involve both the thoracic and abdominal aorta [2]. This review focuses on current evidence regarding the role of transforming growth factor-β (TGFβ) in AAAs.

An AAA represents a weakened and dilated region of the abdominal aorta usually affecting the infra-renal segment [3] . Male gender and older age are important risk factors [3]. The prevalence of AAA is ~5% in men aged 65-74 years [4] and ~10% in men aged ≥75 years [5, 6]. The prevalence of AAA is four to five times lower in women than in men [7], but the outcome of the disease is worse in women than in men [8, 9].

Significant shortfalls exist in current AAA management strategies. In particular, the absence of effective drug therapies for AAA means that patients with early stage AAAs are managed conservatively requiring repeat imaging on a 6–12 monthly basis until the threshold diameter for repair is reached. Up to 70% of patients eventually require surgery which comes with associated mortality (~1-5%), major morbidity (~5-20%) and cost (~$30,000 per patient) [10-12]. There is great current interest in better understanding the pathogenesis of AAA in the hope that targets for new medical therapies to reduce AAA progression might be identified.

One area of significant current interest is the role of TGFβ in the development and progression of aortic aneurysm. TGFβ has been implicated in promoting TAA formation (previously reviewed in [13, 14]). Although AAAs share some similarities with TAAs, they are different from TAAs in disease location, embryonic origins of medial VSMCs and pathological findings [2, 3] (Table 1). TAAs may occur in the ascending aorta, arch and/or descending thoracic aorta. The embryonic origin of medial VSMCs within the ascending aorta and arch is from the neural crest, while VSMCs within the descending thoracic aorta originate from somites [15]. However, AAAs occur in the abdominal aorta, and VSMCs from this site originate from somites [16]. It has been shown that VSMCs from different embryonic origins exhibit lineage-specific

differences in the ways that they respond to TGFβ1 [17]. For example, TGFβ1 promotes growth of VSMCs derived from the neural crest, whereas TGFβ1 inhibits growth of VSMCs originating from somites [17]. AAA is an inflammatory disease with dense infiltration of macrophages and lymphocytes found within human AAA biopsies [18, 19]. In contrast, there is less inflammation within aortic biopsies from TAA patients [20] although it has been shown that inflammatory cells, including macrophage and T cells, can be identified in the aortic biopsies of some TAA patients [21]. As the pathogenesis of AAA appears to be different from that of TAA, the role of TGFβ in TAA development cannot be necessarily expected to be similar in AAA development. In contrast to its putative role in promoting TAA development, TGFβ appears to play a protective role in AAA pathogenesis.

This review briefly summarizes TGFβ signaling pathways and then focuses on data suggesting a protective role of TGFβ in AAA formation, progression and complications. Finally possible mechanisms underlying the putative protective effect of TGFβ in AAA are described including areas requiring further research.

2. TGFβ and its receptors

The TGFβ superfamily of growth factors comprises at least 30 genes in mammals, including 3 TGFβ isoforms, 4 activin β chains, 10 bone morphogenetic proteins, and 11 growth and differentiation factors [22]. These growth factors regulate many cellular functions including cell growth, adhesion, migration, differentiation and apoptosis [22]. This review focuses on the three TGFβ isoforms, *i.e.*, TGFβ1, TGFβ2 and TGFβ3.

Active TGFβs are homodimeric proteins of 25 kDa [23-25]. TGFβs are synthesized as large precursor molecules that are cleaved at a conserved RXXR motif into two fragments: TGFβ and latency associated peptide (LAP). After cleavage, LAPs are still able to bind to TGFβs and this leads to a biologically inactive latent complex [24, 26]. Latent TGFβs are stored at the cell surface and in the extracellular matrix and are converted to active TGFβ [23] by the effects of multiple proteins including thrombospondin-1 [27] and integrins [28].

The effects of TGFβs are mediated by binding to their receptors. Three classes of TGFβ receptor (TβR) isoforms have been characterized [26, 29]: Type I (TβRI), type II (TβRII) and type III (TβRIII) receptors. TGFβ isoforms bind to TβRII, which induces a hetero-oligomerization between TβRII and TβRI, and subsequently, downstream signaling is initiated [26, 29]. Betaglycan, also known as TβRIII, is a membrane-anchored proteoglycan that has no signaling structure but acts to present TGFβs to TβRII [26]. Soluble forms of TβRIII are released from the cell surface by endogenous proteases and can act as a TGFβ inhibitor by sequestering TGFβ [30].

TGFβs have similar biological properties [23]. Studies investigating the biological effects of different TGFβ isoforms demonstrate a considerable overlap of their activities [31]. Specificity of the action of TGFβ isoforms in different cell types seems to be determined by the expression and/or activation of intracellular signaling molecules as well as by distinct expression of the TβR subtypes [26, 29, 32]. In addition, TGFβ1 and TGFβ3 bind TβRII without needing TβRI; however, TGFβ2 interacts only with TβRII and TβRI heterodimers [31]. TGFβ isoforms diverge in their ability to bind to receptors in a manner that correlates with their potency of biological effects. For example, TGFβ1, TGFβ2 and TGFβ3 have similar ability to bind to TβRIII but differ in their ability to bind to TβRII and TβRI. TGFβ1 and TGFβ3 are more potent than TGFβ2 in binding to TβRII and TβRI which correlates with their higher growth inhibition effect compared with TGFβ2 [32].

3. Brief summary of TGFβ signaling

TGFβs can activate different pathways, including Sma and Mad Related Family protein (Smad), mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K) pathways (Figure 1).

3.1 Smad pathway

Latent TGFβs are activated, *e.g.* by reactive oxygen species (ROS), to become active TGFβs. The active TGFβ binds to TβRII, which recruits and phosphorylates TβRI [29]. The phosphorylated TβRI phosphorylates and activates receptor-regulated Smads (R-Smads), including Smad2 and Smad3. Activated R-Smads form

heteromeric complexes with common-partner Smads (Co-Smads), e.g. Smad4, which translocate efficiently to the nucleus, where they regulate, in co-operation with other transcription factors, co-activators and co-repressors, the transcription of target genes [33, 34].

There is another type of Smad, *i.e.*, inhibitory Smad (I-Smad). I-Smads include Smad6 and Smad7. They negatively regulate TGFβ/Smad signaling by preventing activation of Smad2 and Smad3 [35]. Induction of Smad6 and Smad7 [34, 36, 37] expression by TGFβ represents an auto-inhibitory feedback mechanism. Smad6 and Smad7 can inhibit the activation of R-Smads by competing with R-Smad for type I receptor interaction or by recruiting specific ubiquitin ligases to the activated receptor complex thereby targeting it for proteosomal degradation [22, 38, 39].

Smad ubiquitination-regulatory factor1 (Smurf1) and Smurf2 antagonize TGFβ signaling by interacting with Smads and targeting them for degradation [40].

3.2 MAPK pathway

TGFβ receptors can activate TGFβ-activated kinase-1 (TAK1) [41], which can further activate mitogen-activated protein kinase kinase 6 (MKK6) or MKK3 to activate p38. Alternatively, TAK1 can activate MKK4 which leads to c-Jun N-terminal kinases (JNKs) activation. In addition, TGFβ receptors activate Ras which can further activate extracellular signal-regulated kinase (ERK) [31, 42].

3.3 PI3K pathway

TGFβs via their receptors activate PI3K, as indicated by phosphorylation of its effector protein kinase B (Akt) [43, 44]. This activation can be directed by RhoA [43] or other proteins, e.g. epidermal growth factor [44].

3.4 Interaction between the TGFβ pathways

There is cross talk between the different signaling pathways which mediate TGFβ effects. For example, inhibition of p38 MAPK inhibits TGFβ1-induced R-Smad activation [45]; c-jun inhibits Smad2 signaling [46]; The ERK pathway can attenuate Smad accumulation within the nucleus [47]; Smad6 binds to TAK1 and

down-regulates its activity [48]; whereas Smad7 enhances JNK activation [49]. Thus, the balance among these different pathways likely defines the cellular response to TGFβ.

4. Feature of AAAs

AAAs are characterised by chronic inflammation, degradation of the aortic wall and loss of VSMCs within the medial layer [50-54], associated with progressive dilatation and eventual aortic rupture [3].

The infiltrating inflammatory cells identified in AAA biopsies are dominated by macrophages and lymphocytes [55] which produce pro-inflammatory mediators and ROS [52]. Inhibition of inflammation, *e.g.* by blocking nuclear factor-κB, inhibits the development of AAAs in mice [56], supporting a role for inflammation in AAA formation. The exact role of inflammation in AAA is controversial [57], as intense immune-suppressive treatment was associated with rapid AAA progression (13 mm/y) in a patient in whom histological analysis showed complete absence of T cells, B cells and neutrophils within the AAA wall.

AAAs are characterised by increased ECM degradation. Aneurysmal tissue shows increased levels of matrix metalloproteinases (MMPs), a family of enzymes capable of degrading the primary structural proteins of the aortic wall [58]. Pharmacological [59] or genetic [60] inhibition of MMPs can inhibit the development of AAA in experimental animals. Another cardinal feature of AAA is the depletion of VSMCs within the medial layer [53, 54]. Therapies which prevent VSMC depletion can stabilize pre-formed aneurysms in experimental animals [61].

5. TGFβ signaling in human AAA

TGFβ signaling has been shown to be down-regulated in human AAA. For example, in a small study involving biopsies from 12 AAAs and 6 control aortas, loss of one copy of TβRII exon 8 was identified in 92% of AAA patients and this was associated with the down-regulation of TβRII mRNA expression [62].

Some genetic studies have reported an association between single nucleotide polymorphism (SNPs) in TGFβ3 [63], TβRI [64], TβRII [64-66] and latent TGFβ binding protein 4 (LTBP4) [63] with AAA (Table 2). It is noted that the associations between the polymorphisms and AAA are population specific [63, 65, 66]. It

is also noted that these polymorphisms might work in concert with other polymorphisms. For example, in one study TβRI gene polymorphism (6A allele) was reported to increase the predisposition for AAA not *per se*, but only when increased angiotensin II levels were present [67].

Serum TGFβ1 was not associated with the presence [65] or progression [68] of AAA in previous reports. However, TGFβ1 serum concentrations might not reflect its level in aneurysmal tissues or its downstream signal pathway activity.

6. TGFβ inhibits AAA formation, progression and rupture in experimental animals

6.1 TGFβ inhibits AAA formation in mouse models

Systemic neutralisation of TGFβ activity using a blocking antibody breaks the resistance of C57BL/6 mice to angiotensin II-induced AAA formation and rupture [69]. Angiotensin II and TGFβ blocking antibody-induced AAAs appear to be mainly mediated by monocyte/macrophages [69], as depletion of monocytes decreases both macrophage infiltration and AAA formation [69].

6.2 TGFβ stabilizes pre-formed AAAs in animal models

Protecting pre-formed aortic aneurysms from expanding has clinically relevant consequences because risk of AAA rupture is proportional to aortic diameter [70]. In one previous study over-expression of TGFβ1 stabilized the aortic diameter of pre-formed AAAs in experimental animals; while in the non-treated control group, the aortic diameter continued to increase over time [71].

In another study over-expression of TGFβ1 by endovascular gene delivery stabilized pre-formed aortic aneurysms [72]. This effect of TGFβ1 was associated with preservation of medial elastin, a decrease in infiltration of macrophages and T lymphocytes, and a decrease in MMP-2 and MMP-9 expression. TGFβ1 also triggered ECM repair, as over-expression of TGFβ1 promoted a VSMC-, collagen- and elastin-rich intima [72].

TGFβ1 has been reported to mediate cyclosporine A (CsA)-induced protection from AAA induction in experimental animals. CsA is an imunosuppressive drug which induces TGFβ1 gene transcription and

activates latent TGFβ [33, 73]. Chronic administration of CsA leads to tissue accumulation in humans [74]. CsA stabilized AAAs in experimental animals [73] and this effect was mediated by TGFβ, as a TGFβ neutralising antibody abrogated the stabilizing effect of CsA [73].

6.3 TGFβ protects against AAA complications in mouse models

Blocking TGFβ activity using TGFβ antibody promoted AAA rupture within an angiotensin II-induced AAA model. This effect of TGFβ was mediated by MMP-12, as blocking TGFβ increased MMP-12 activity and MMP-12 deficiency prevented aneurysm rupture [69].

7. Putative mechanisms underlying the potential protective effect of TGFβ in AAA pathogenesis

TGFβ most likely exerts its protective effects via multiple mechanisms, including inhibiting aortic inflammatory cell infiltration, reducing ECM degradation and limiting VSMC apoptosis as well as promoting ECM formation (Figure 2). The TGFβ signaling pathway might provide a therapeutic target for AAA.

7.1 TGFβ inhibits aortic inflammatory cell infiltration

Inflammation is one of the characteristic pathological features of both human [75, 76] and experimental AAAs [77]. The aortic density of inflammatory cells is correlated with AAA diameter in humans [76]. Rapid aortic diameter enlargement is associated with more marked aortic inflammation in experimental animals [78]. Macrophages and lymphocytes [55] are the major inflammatory cells identified in AAA biopsies [68]. The inflammatory cells produce MMPs which lead to ECM degradation and AAA formation in experimental animals [79]. Administration of TGFβ neutralising antibody promoted monocyte-macrophage infiltration within experimental AAAs [69, 73] in mice and rats [73]. TGFβ appears to be able to decrease inflammatory cell recruitment and potentially the release of proteolytic enzymes which promote ECM degradation.

7.2 TGFβ promotes elastin and collagen formation

Elastic fibres in the ECM of vascular tissues provide elasticity and resilience. Elastin is cross-linked and extremely hydrophobic, which makes it one of the most stable proteins in the body [80, 81]. Inflammatory cells release MMPs that breakdown elastin to generate soluble elastin peptides [82]. These peptides are different from intact elastic fibres as they activate medial VSMCs and prompt the secretion of cytokines, chemokines, interleukins and proteinases that propagate the cycle of matrix degradation [83]. This ultimately leads to the loss of elasticity and strength of the aortic wall and its progressive dilation to form a rupture-prone sac of weak tissue.

AAA regression is unlikely without regeneration of new elastic matrix structures. Unfortunately, both healthy and diseased post-neonatal VSMCs poorly synthesize elastic fibers [84]. TGFβ1 enhances tropoelastin mRNA and protein production in VSMCs [84] and it can also augment lysyl oxidase protein expression [84] which is critical to elastin crossing linking and fiber assembly. The TGFβ1-induced elastin-matrix regeneration by VSMCs can be enhanced by hyaluronan oligomers [84]. It has been shown that elastogenic factors, composed of hyaluronan oligomers and TGFβ1, can stimulate both healthy and aneurysmal rat aortic smooth muscle cells to enhance elastin synthesis and matrix formation [84, 85]. Importantly, these elastogenic factors also increase elastin and matrix formation by human AAA-derived VSMCs [86]. In addition, subcutaneous injection of TGFβ1 can increase collagen formation by fibroblasts *in vivo* [87] and over-expression of TGFβ1 induces procollagen and collagen synthesis in normal arteries *in vivo* [88].

7.3 TGFβ inhibits ECM degradation

Matrix degradation is one of the key features of AAA. MMPs play an important role in this process. MMP-9 is believed to play a leading role in ECM degradation and AAA formation. Macrophages, abundant within AAAs, are a major source of MMP-9 [89]. Macrophage-derived MMP-9 promoted aneurysm formation within the $CaCl_2$-induced mouse model of AAA [79].

TGFβ1 inhibited TNF-α-induced MMP-9 expression [90] and advanced glycation end products (AGEs)-induced MMP-9 activity in macrophages [91]. *In vivo*, TGFβ blocking antibody increased MMP-9 expression

in rats [74]; while over-expression of TGFβ1 reduced MMP-9 within experimental AAAs, which was associated with stabilization of pre-formed AAAs and preservation of medial elastin [72]. Thus, the TGFβ-mediated down-regulation of MMP-9 in macrophages may play an important role in the prevention or stabilization of AAAs [72].

MMP-2 is also implicated in ECM degradation in AAA formation. Mesenchymal cell-derived MMP-2 contributed to the aneurysm formation in $CaCl_2$-induced AAAs in mice [79]. Over-expression of TGFβ1 reduced MMP-2 which was associated with stabilization of pre-formed AAAs and preservation of medial elastin [72].

TGFβ inhibits MMP-12. MMP-12 deficiency inhibits elastin degradation and aneurysm severity. TGFβ controls the progress towards aneurysm rupture through inhibition of MMP-12 activity in experimental models [69]. In addition, TGFβ regulates the expression of tissue inhibitor of metalloproteinases (TIMPs) which inhibit MMP activity. For example, TGFβ enhances mRNA expression of TIMP-1 [92] and TGFβ antibody decreases TIMP-1 mRNA expression [74].

7.4 TGFβ inhibits VSMC apoptosis

One of the key features of human AAA is the depletion of VSMCs within the medial layer [53, 54]. Endovascular smooth muscle cell therapy stabilised aneurysm diameter of pre-formed aneurysms, and this protective effect was associated with increase expression of TGFβ1 mRNA, but not TGFβ2 or TGFβ3 mRNA within the intima [93]. Treatment with a TGFβ neutralising antibody induced a significant decrease in VSMCs within the medial and adventitia layer of pre-formed AAAs within an animal model [73].

7.5 Possible role of TGFβ in modulating hemodynamic forces relevant to AAA pathogenesis

Shear stress appears to play an important role in the pathogenesis of AAA based on studies in experimental animals [see a recent review 94]. High shear stress has been reported to limit AAA growth [95, 96]. The mechanisms underlying this putative protective role of high shear stress include: (1) inhibition of aortic VSMC apoptosis; (2) preservation of aortic elastin and collagen; (3) an increase in vascular progenitor

cell numbers within the aorta; (4) a decrease in aortic macrophage infiltration; and (5) an increase in the aortic expression of heme oxygenase 1 (an anti-inflammatory enzyme) [94].

TGFβ1 mRNA and protein expression increases in response to steady laminar shear stress (20 dynes/cm^2) in bovine aortic ECs [97]. The increase in TGFβ1 mRNA correlates with the shear stress intensity within the physiologic range (5-40 dynes/cm^2). Shear stress (28 dynes /cm^2) also induces TGFβ1 mRNA and protein expression in human VSMCs isolated from umbilical arteries [98]. *In vivo*, increased shear stress enhances TGFβ1 mRNA and protein levels in rabbit common carotid arteries subjected to balloon injury [99]. Shear stress can activate latent TGFβ1 released by platelets and fibroblasts, and the extent of activation is correlated with the shear stress intensity [100]. The increase in active TGFβ1 induced by shear stress is transmitted to downstream signal components. For example in cultured human aortic ECs exposed to moderate shear stress (10 dynes/cm^2) for 20 hours, increased Smad2 phosphorylation and nuclear translocation have been reported [101].

Exogenous TGFβ1 has been reported to stimulate VSMC proliferation at low concentrations but inhibit VSMC proliferation at higher concentrations [102]. In the context of high shear stress TGFβ1 appears to inhibit VSMC proliferation. Application of shear stress has been reported to inhibit the proliferation of human VSMC and this effect was blocked by TGFβ1 antibody [98]. Addition of TGFβ1 blocking antibody has been reported to promote the proliferation of VSMCs exposed to conditioned media from ECs exposed to shear stress. This effect of TGFβ1 blocking antibody appears to be lost when VSMCs are exposed to the conditioned media from ECs not subjected to shear stress [102].

It is thus possible that increased TGF β1 expression mediates some of the putative protective effects of high shear stress on AAA pathogenesis. Further direct evidence is however needed.

8. Future directions

The protective effect of TGFβ in AAAs in humans is not yet verified and future research in which interventions promoting TGFβ1 overexpression are assessed in patients would be required to resolve this. For example, it is possible that clinical trials can be designed to investigate the benefit of endovascular delivery of

TGFβ1 to AAAs or the effect of pharmacological modulation of TGFβ. Cyclosporine appears to upregulate TGFβ in pre-clinical studies although this is likely to be too toxic to be used for the treatment of small AAAs [73].

Importantly a clearer understanding of the reasons for the distinct effects of TGFβ in TAAs and AAAs is needed. The mechanisms underlying the biphasic effects of TGFβ need to be investigated. For example, TGFβ decreases MMP-9 in macrophages in AAAs, however, it increases MMP-9 in other cell types including human meningeal cells [103], fibroblasts [104], keratinoctes [105] and tumour cells [106-108]. In addition, TGFβ can both decrease [73] and increase VSMC apoptosis [20], and it can both inhibit [85] and promote [109] VSMC proliferation. The effects of TGFβ appear to be context dependent. For example, TGFβ can both inhibit and promote the proliferation of valve interstitial cells under normal physiological conditions and in the early stages of repair, respectively [110, 111]. The identification of mechanisms which determine why the effects of TGFβ change in different environments is probably critical if therapies targeting TGFβ are to be developed.

9. Conclusion

Current evidence suggests that TGFβ protects experimental animals against AAA formation, progression and complications. The underlying mechanisms are thought to be due to the ability of TGFβ to inhibit inflammatory cell infiltration, ECM degradation and VSMC apoptosis, as well as the ability of TGFβ to promote ECM formation. The TGFβ signaling pathways might provide a therapeutic target in AAA.

Disclosure statement

None.

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Figure legends

Fig. 1 Illustration of the TGFβ signaling pathway. TGFβs bound to LAPs are not active. Dissociation of LAPs from TGFβs leads to activation of TGFβs. The active TGFβ binds to TβRII, which then recruits and phosphorylates TβRI. The phosphorylated TβRI activates R-Smads. Activated R-Smads form heteromeric complexes with Co-Smads, which translocate to the nucleus, where they regulate the transcription of target genes. TGFβs can also activate Smad-independent pathways, including MAPK (ERK, p38 and JNK) and PI3K pathways. Akt: protein kinase B; Co-Smads: common-partner Smads; ERK: extracellular signal-regulated kinase; I-Smad: inhibitory Smad; JNK: c-Jun N-terminal kinases; LAPs: latency associated peptides; MAPK: mitogen-activated protein kinases; MKK: protein kinase kinase; PI3K: phosphoinositide 3-kinase; R-Smads: receptor-regulated Smads; Smad: Sma and Mad Related Family protein; TAK1: TGFβ-activated kinase-1; TβRI, type 1 transforming growth factor-β receptor; TβRII, type 2 transforming growth factor-β receptor; TGFβ: transforming growth factor-β.

Fig. 2 Mechanisms implicated in the putative protective effect of TGFβ in AAA. TGFβ prevents aortic inflammatory cell infiltration, and thus inhibits MMP generation and consequent ECM degradation. In addition, TGFβ can inhibit VSMC apoptosis and increase elastin and collagen formation. AAA: abdominal aortic aneurysm; ECM: extracellular matrix; MMP: matrix metalloproteinase; TGFβ: transforming growth factor-β; VSMC: vascular smooth muscle cell.

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Table 1 Similarity and difference between AAAs and TAAs

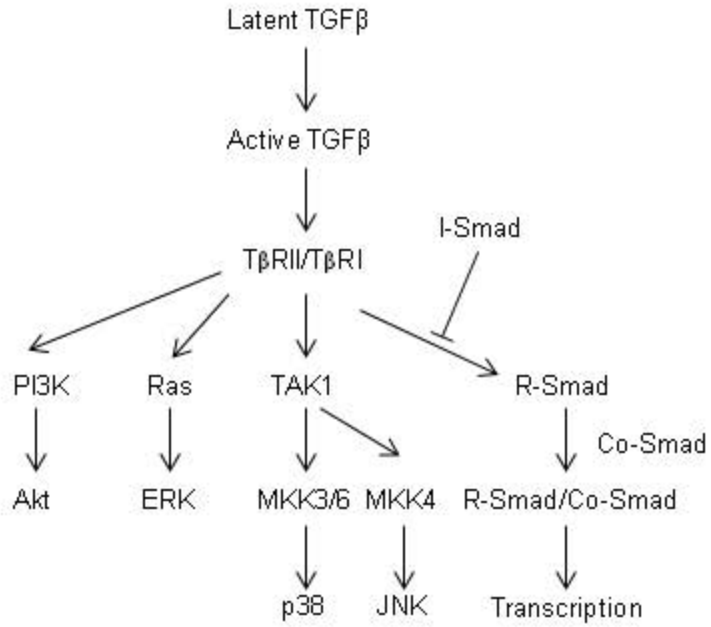
Features	AAAs	TAAs
Loss of VSMCs	Yes	Yes
Increased MMPs	Yes	Yes
Increased ECM degradation	Yes	Yes
Age is risk factors	Yes	Yes
Locations	Abdominal aorta	Ascending aorta, arch and descending thoracic aorta
Origins of VSMCs	VSMCs of abdominal aorta originate from somites	The embryonic origin of medial VSMCs in the ascending aorta and arch arise from neural crest, and VSMCs in descending thoracic aorta from somites.
Increased inflammatory cell infiltration	Yes	Minimal

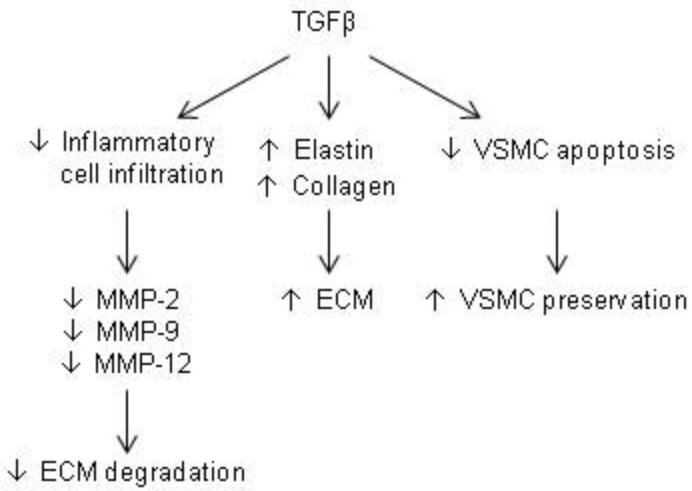
AAAs: abdominal aortic aneurysms; ECM: extracellular matrix; MMPs: matrix metalloproteinases; TAAs: thoracic aortic aneurysms; VSMC: vascular smooth muscle cells.

Table 2 Polymorphisms of TGFβ signaling components are associated with human AAA in some studies

Author, Year	Gene	SNP	Associated allele	Number of subjects	Controls	Population	Conclusion
Lucarini, 2009 [58]	TβRI	6A	6A	201	252	Italy	TβRI 6A allele is not associated with the susceptibility to AAAs. However, the contemporary presence of ACE DD genotype and TβRI 6A allele, increase the predisposition to the disease.
Baas, 2010 [9]	TβRI	rs1626340	A	736	1024	Netherlands	These 3 SNPs out of 32 SNPs tested are associated with AAAs after correction for multiple testing
	TβRII	rs1036095	C				
		rs4522809	A				
Golledge, 2009 [30]	TβRII	rs1078985	C	640	1071	Australia	C allele is weakly associated with AAAs in the Australian cohort; however, this association does not hold after adjusting for multiple testing and is not validated in the New Zealand cohort.
				654	389	New Zealand	
Biros, 2011 [12]	TβRII	rs764522	G	610	1065	Australia	G alleles of both SNPs are associated AAAs even after adjusting for multiple test in this meta-analysis. The association is mainly driven by findings in the Netherlands group, , as this association is lost if the Netherlands group is removed.
		rs1036095	G	601	608	New Zealand	
				693	943	Netherlands	
Thompson, 2010 [90]	LTBP4	rs2077407	T	580	2752	UK	T allele in LTBP4 rs2077407 is associated with the presence of AAAs in the UK cohort; but this association is confirmed in other cohorts.
		-4234A>G	G	369	358	WA, Australia	
		10384G>A	A	295	159	Queensland, Australia	
		21011A>T	T	652	474	New Zealand	5 SNPs in LTBP4 (-4234A>G, 10384G>A, 21011A>T, 25859C>T and 32603C>G) and A allele of TGFβ3 -614G>A are associated with a decreased growth in the UK cohort.
		25859C>T	T				
		32603C>G	G				
	TGFβ3	-614G>A	A				Meta analysis of AAA size and growth rates in larger AAAs (≥ 45 MM), demonstrated a significant association with the LTBP4 21011A>T genotype (a 2% decrease in AAA diameter, or a 0.53 mm/year reduction in AAA growth rate, per T allele)

AAA: abdominal aortic aneurysm; ACE: angiotensin converting enzyme; SNP: single nucleotide polymorphism; TGFβ: transforming growth factor-β; LTBP: latent TGFβ binding proteins; TβR: TGFβ receptor.


Latent TGFβ
Active TGFβ
I-Smad
TβRII/TβRI
PI3K
Ras
TAK1
R-Smad
Co-Smad
Akt
ERK
MKK3/6
MKK4
R-Smad/Co-Smad
p38
JNK
Transcription


TGFβ
↓ Inflammatory cell infiltration
↑ Elastin
↑ Collagen
↓ VSMC apoptosis
↓ MMP-2
↓ MMP-9
↓ MMP-12
↑ ECM
↑ VSMC preservation
↓ ECM degradation