







CONTEMPORARY REVIEW

# Vascular Smooth Muscle Cells in Aortic Aneurysm: From Genetics to Mechanisms

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**ABSTRACT:** Aortic aneurysm, including thoracic aortic aneurysm and abdominal aortic aneurysm, is the second most prevalent aortic disease following atherosclerosis, representing the ninth-leading cause of death globally. Open surgery and endovascular procedures are the major treatments for aortic aneurysm. Typically, thoracic aortic aneurysm has a more robust genetic background than abdominal aortic aneurysm. Abdominal aortic aneurysm shares many features with thoracic aortic aneurysm, including loss of vascular smooth muscle cells (VSMCs), extracellular matrix degradation and inflammation. Although there are limitations to perfectly recapitulating all features of human aortic aneurysm, experimental models provide valuable tools to understand the molecular mechanisms and test novel therapies before human clinical trials. Among the cell types involved in aortic aneurysm development, VSMC dysfunction correlates with loss of aortic wall structural integrity. Here, we discuss the role of VSMCs in aortic aneurysm development. The loss of VSMCs, VSMC phenotypic switching, secretion of inflammatory cytokines, increased matrix metalloproteinase activity, elevated reactive oxygen species, defective autophagy, and increased senescence contribute to aortic aneurysm development. Further studies on aortic aneurysm pathogenesis and elucidation of the underlying signaling pathways are necessary to identify more novel targets for treating this prevalent and clinical impactful disease.

**Key Words:** aortic aneurysm ■ genetics ■ mechanism ■ vascular smooth muscle cell

**A**ortic aneurysm is characterized by a permanent increase in the diameter of the aorta (>50% increase). Aortic aneurysm is the second most prevalent aortic disease following atherosclerosis and accounts for the ninth-leading cause of death overall.<sup>1</sup> The estimated incidence is 2.79 per 100 000 individuals.<sup>2</sup> Although an aortic aneurysm can remain asymptomatic for a long time, sudden rupture leads to life-threatening hemorrhage with a high mortality rate.<sup>1</sup>

The aorta is divided into the thoracic aorta (including the aorta root, ascending aorta, aortic arch, and descending thoracic aorta) and abdominal aorta (suprarenal aorta and infrarenal aorta). Aortic aneurysm can occur in the thoracic aorta (thoracic aortic aneurysm [TAA]) or abdominal aorta (abdominal aortic aneurysm [AAA]). The prevalence of AAA is about 3 times that of TAA.<sup>1</sup> TAA and AAA share many similarities but also have distinct features. In this review, we

summarize recent advancements in aortic aneurysm and mainly focus on the role of vascular smooth muscle cells (VSMCs) in this severe disease.

## PATHOGENESIS OF AORTIC ANEURYSM

The aorta can be divided into 3 layers: intima, media, and adventitia. The intima consists of an endothelium that supports the internal elastic lamina. A smooth and healthy endothelial layer is critical for the aorta to maintain an anti-inflammatory and antithrombosis phenotype.<sup>3</sup> The media is composed of several layers of VSMCs and surrounding elastic and connective tissue. The adventitia is the connective tissue surrounding the external elastic lamina to anchor the vessel and provide blood supply.<sup>4</sup> Aortic aneurysm takes place

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## Nonstandard Abbreviations and Acronyms

<b>ECM</b>	extracellular matrix
<b>LOX</b>	lysyl-oxidase
<b>MMP</b>	matrix metalloproteinase
<b>RIP</b>	receptor interacting serine/threonine kinase
<b>ROS</b>	reactive oxygen species
<b>SMCs</b>	smooth muscle cells
<b>TAA</b>	thoracic aortic aneurysm
<b>TGF-<math>\beta</math></b>	transforming growth factor- $\beta$
<b>VSMCs</b>	vascular smooth muscle cells

through progressive weakening of the aortic wall, which involves all 3 layers. Aortic dissection is caused by tearing of the intima and media, resulting in blood entering the space between the media and adventitia.<sup>4</sup> Although the pathogenesis of aortic aneurysm remains to be fully elucidated, the development of this vascular disease is highlighted by prominent inflammation, gradual loss of VSMCs, and disruption of the extracellular matrix (ECM)<sup>4</sup> (Figure 1). Here, we summarize the current well-recognized theories/pathways of aortic aneurysm pathogenesis.

## Genetic Risk Factors in Aortic Aneurysm

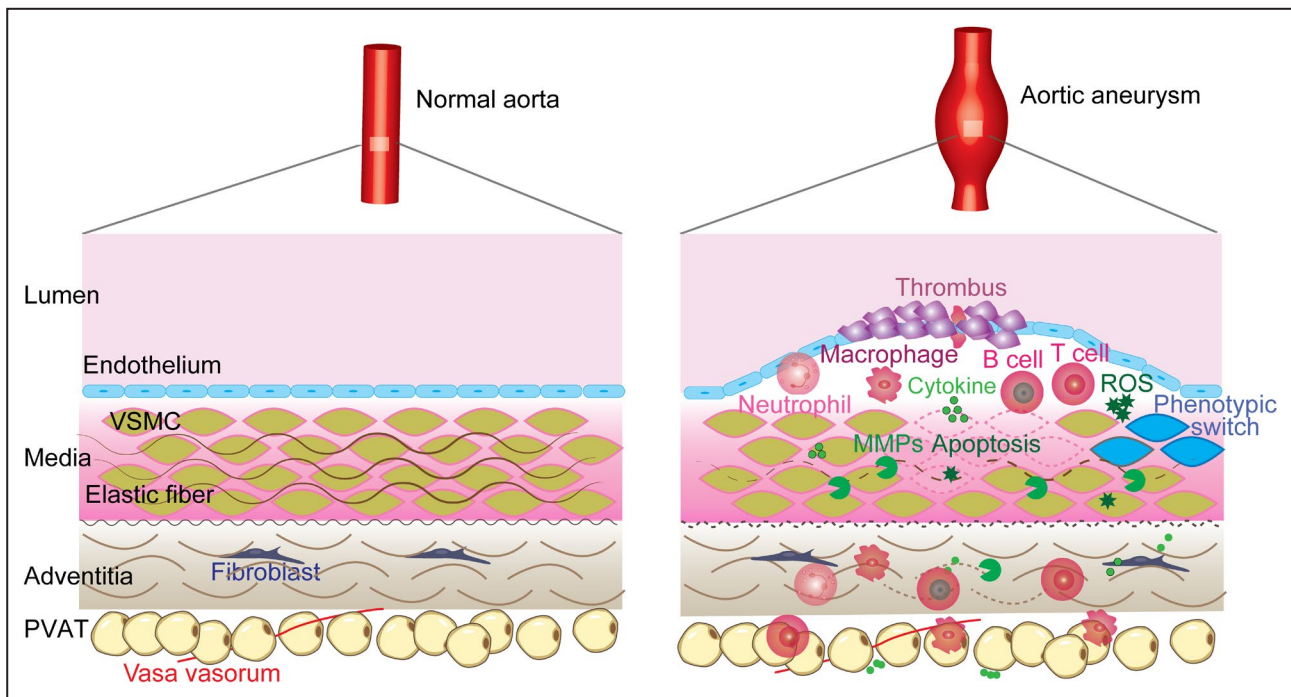
Both TAA and AAA have genetic risk factors. Typically, TAA has a more robust genetic background than AAA. About 20% of patients with TAA inherit risk mutations, while patients with AAA typically do not exhibit this pattern.<sup>5</sup> AAA shares many features with TAA, including VSMC death, ECM degradation, and inflammation. However, the pathophysiology of AAA development is distinct in many respects. The difference in pathogenesis between TAA and AAA could be attributed to their embryological origins.<sup>6</sup> VSMCs in the ascending aorta are derived from neural crest stem cells and progenitor cells in the second heart field, while VSMCs in the descending aorta are derived from somites.<sup>7</sup> This difference in developmental origin could also lead to the distinctive transcriptomic profiles in aortic VSMCs.<sup>8</sup> Linkage analysis and genome-wide association studies have provided insightful genetic information about the etiology of AAA.<sup>5</sup> A linkage study performed in 2004 identified 2 susceptible loci for AAA in the regions 19q13 and 4q13.<sup>9</sup> Large scale genome-wide association studies have identified several risk loci including rs10985349 (DAB2 interacting protein),<sup>10</sup> rs1466535 (low-density lipoprotein receptor-related protein 1), rs6511720 (low-density lipoprotein receptor [*LDLR*]), rs602633 (sortilin-1), rs4129267 (interleukin-6 receptor), rs10757274 (CDKN2BAS1/ANRIL [9p21]), rs1795061

(SET and MYND domain containing 2), rs9316871 (*LINC00540*), rs3827066 (*PCIF1/MMP9/ZNF335*), and rs2836411 (ETS Transcription Factor *ERG*).<sup>11</sup> Intriguingly, AAA risk loci overlap with several atherosclerosis risk loci, including 9p21, sortilin-1, and low-density lipoprotein receptor.<sup>5</sup> These findings suggest that AAA and atherosclerosis share common pathways during disease development.

Transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling is one of the most studied signaling pathways that play pivotal roles in vascular development and maintenance. Mutations in TGF- $\beta$  signaling pathway-related genes (TGF- $\beta$  receptor 1/2, *SMAD2/3*, or *TGFB2/3*) are associated with TAA formation in heritable connective tissue disorders, including Loeys-Dietz and Marfan syndromes, highlighting its importance in TAA development.<sup>5</sup> TGF- $\beta$  has pleiotropic effects on different cell types in the body. Three highly conserved isoforms of TGF- $\beta$  exist in humans: TGF- $\beta$ 1, TGF- $\beta$ 2, and TGF- $\beta$ 3. They share a common receptor and downstream pathway, although they have distinct tissue distribution.<sup>12</sup> TGF- $\beta$  binds to TGF- $\beta$ R1 and TGF- $\beta$ R2 and facilitates phosphorylation and subsequent activation. Downstream TGF- $\beta$  receptor signaling can be divided into 2 different pathways, a SMAD-dependent pathway and a SMAD-independent noncanonical pathway.<sup>12</sup> The role of the TGF- $\beta$  pathway in both TAA and AAA is still controversial, as the knockout of different genes and different intervention methods (knockout or neutralizing antibody) give different results.<sup>13</sup> It could be that TGF- $\beta$  has multiple downstream pathways (canonical versus noncanonical pathway) and may play different roles in different cell types and at various disease stages. These controversial results increase the difficulty of developing TGF- $\beta$ -based therapy for aortic aneurysm. The recent advancement of genetic studies in TAA and AAA has been well summarized in other publications.<sup>4,5</sup>

## Inflammation in Aortic Aneurysm

Inflammation is a hallmark of aneurysm development, although it has been more extensively studied in AAA. Accumulating studies on AAA in both human samples and mouse models indicate that inflammation is a necessary process underlying AAA.<sup>4</sup> Samples from patients with AAA show massive inflammatory cell infiltration.<sup>14</sup> Both innate immune cells (mast cells, macrophages, and neutrophils) and adaptive immune cells (dendritic cells, B cells, and T cells) have been shown to be related to AAA. Neutrophil infiltration occurs in the early phase of AAA development, and neutrophils are important sources of matrix metalloproteinase (MMP)-2 and MMP-9. Depleting neutrophils by an antineutrophil antibody<sup>15</sup> or inhibition of neutrophil recruitment by intraperitoneal injection of a neutralizing antibody against



**Figure 1. Pathogenesis of aortic aneurysms.**

The healthy aorta (left) consists of intimal, medial, and adventitial layers. Vascular smooth muscle cells (VSMCs) and extracellular matrix (ECM) maintain integrity of the aortic wall. Aortic aneurysm lesions (right) are characterized by thrombi, infiltration of inflammatory cells (neutrophils, macrophages, B and T cells), degradation of the ECM, VSMC phenotypic switching and apoptosis, excessive production of cytokines, reactive oxygen species (ROS), and matrix metalloproteinases (MMPs). PVAT indicates perivascular adipose tissue.

family with sequence similarity 3, member D<sup>16</sup> reduces AAA formation in the elastase mouse model, underscoring the importance of neutrophils in AAA development. Monocytes are recruited into the aortic wall by chemotactic cytokines, including C-C motif chemokine ligand 2 and interleukin-6<sup>17</sup> during AAA development. In both human and mouse AAA lesions, macrophages produce MMPs, cytokines, and chemokines.<sup>18</sup> In addition, macrophages also exert reparative functions in the aortic wall.<sup>18</sup> As aortic aneurysms evolve, LY6C<sup>low</sup> monocytes or segregated-nucleus-containing atypical monocytes with reparative potential are recruited to the lesions and exhibit anti-inflammatory and pro-resolving effects to strengthen aortic wall integrity.<sup>18</sup> The role of macrophages in AAA has been nicely reviewed<sup>18</sup> and will not be discussed in detail here.

In TAA development, the role of inflammation is less well studied. In a mouse thoracic aortic dissection model ( $\beta$ -aminopropionitrile+angiotensin II), neutrophil depletion by an anti-Gr-1 antibody decreases the dissection incidence, indicating that neutrophils promote TAA development. Interleukin-6 is also elevated in human patients with TAA, and interleukin-6 knock-out inhibits elastase-induced TAA in C57BL/6 mice.<sup>19</sup> In summary, inflammation representing a critical response to vascular injury is an essential component for aortic aneurysm development and progression. A deeper understanding of this process will help to

identify novel potential therapeutic targets for aortic aneurysm treatment.

### ECM Disruption in Aortic Aneurysm

As a critical component of the healthy vascular wall, the ECM is synthesized and secreted by different vascular cell types, including VSMCs, endothelial cells and fibroblasts.<sup>20</sup> The aortic ECM mainly consists of collagen, elastin, proteoglycans, and glycoproteins,<sup>21</sup> providing the aorta with the expansion capacity and tensile strength. Besides this mechanical function, the ECM also modulates VSMC proliferation, adhesion, and migration.<sup>22</sup> The balance among synthesis, deposition, and degradation maintains ECM homeostasis.<sup>4</sup> The critical role of ECM disruption was revealed by the impact of ECM-related protein mutations on TAA development.<sup>23</sup> ECM degradation is mediated by various proteinases, including MMPs, cathepsins, a disintegrin and metalloproteinases, and a disintegrin and metalloproteinase with thrombospondin motifs.<sup>20</sup> In total, 23 different MMPs have been discovered and can be divided into collagenases, matrilysins, gelatinases, stromelysins, and membrane-type MMPs. Tissue inhibitors of metalloproteinase directly bind to and inhibit different groups of MMPs in the vascular wall. In both TAA and AAA, MMPs play a pivotal role in ECM remodeling.<sup>4</sup> In the circumstance of aortic aneurysm, besides VSMCs, endothelial cells, and fibroblasts, infiltrated

immune cells secrete additional MMPs that further aggravate extracellular matrix degradation.<sup>20</sup> Overall, the imbalance between ECM proteases and protease inhibitors leads to the development of aortic aneurysm.

## ANIMAL MODELS FOR MECHANISM STUDIES

Animal models provide valuable tools to understand disease mechanisms and test novel therapies before clinical trials. There have been multiple animal models developed to study aortic aneurysm *in vivo*.<sup>24</sup> Although animal models cannot perfectly recapitulate all the features of human disease, they are still useful in testing scientific hypotheses. We have incorporated a comparison among different animal models in Figure 2.

### Angiotensin II Infusion

Angiotensin II plus hyperlipidemia is the most commonly used aneurysm model.<sup>25</sup> This model uses a combination of continuous infusion of angiotensin II by subcutaneous implantation of osmotic pumps and hyperlipidemia by *ApoE* or *Ldlr* knockout or an adenovirus-associated viral vector expressing a gain-of-function mutation (D377Y) of mouse proprotein convertase subtilisin/kexin type 9<sup>26</sup> in C57BL/6 mice. This model is technically easy to achieve without any sophisticated surgery skills. The aortic aneurysm usually appears in

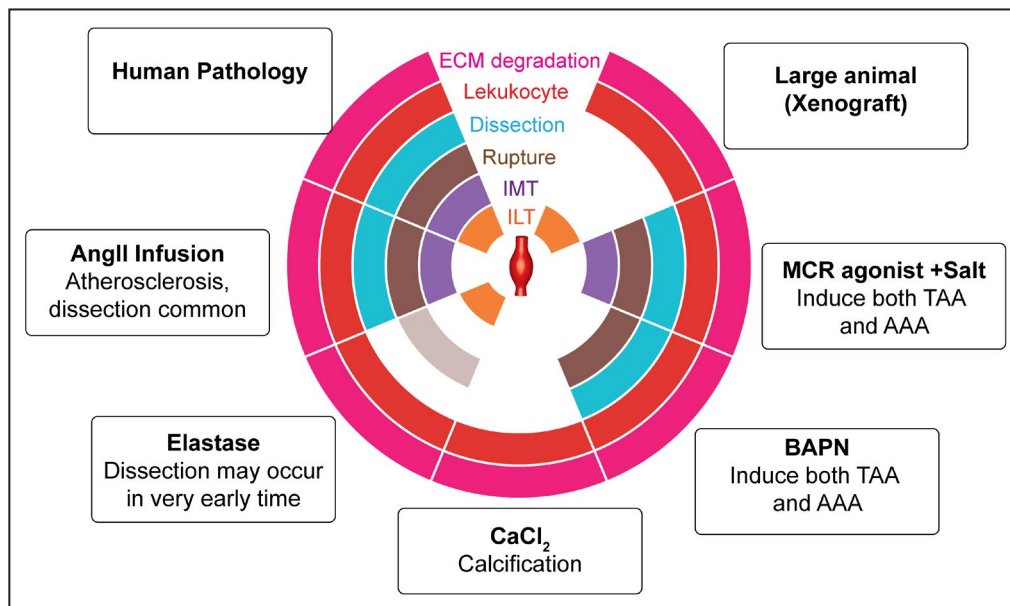
the suprarenal region, and dissection and hemorrhage can also be seen in the aortic root.<sup>25</sup> The model shows several essential features of human AAA, including atherosclerosis, medial degeneration, intramural thrombosis, leukocyte infiltration, and dissection. However, the location of AAA in this experimental model is not consistent with human AAA, which occurs more often in the infrarenal region.

### Mineralocorticoid Receptor Agonists Plus Salt

In this mouse model, the combination of deoxycorticosterone acetate and salt or aldosterone and salt can induce a high incidence of aortic aneurysm and an aortic aneurysm may occur in both thoracic and abdominal aortas.<sup>27</sup> Pathological changes are similar to the angiotensin II model, except that atherosclerosis is not present in this mouse model.<sup>27</sup>

### Elastase Model

The elastase-induced AAA model consists of application of porcine pancreatic elastase either in the lumen<sup>28</sup> or outside adventitia.<sup>29</sup> This model does not require a specific genetic background and can be used in both mice and rats. Unlike the angiotensin II model, aortic dissection is not seen in this model and rupture is also uncommon. This model is primarily driven by ECM degeneration and massive



**Figure 2. Comparison of animal models used for aortic aneurysm studies.**

Animal models have been developed to recapitulate features of human aortic aneurysms. Aortic aneurysms in rodent species can be induced by several methods, including surgical procedures, pharmacological treatments, and genetic manipulations. Each model has its advantages and drawbacks when comparing with human aortic aneurysm pathology. AAA indicates abdominal aortic aneurysm; AngII, angiotensin II; BAPN,  $\beta$ -aminopropionitrile; ILT, intraluminal thrombus; IMT, intramural thrombus; MCR, mineralocorticoid receptor; and TAA, thoracic aortic aneurysm.



leukocyte infiltration.<sup>29</sup> The limitation of this model is that atherosclerosis and intramural thrombus are not present. In addition, the severity of the aneurysm is highly dependent on the porcine pancreatic elastase batch.<sup>24</sup> The elastase model has recently been employed in TAA studies by the placement of elastase on the thoracic aortic wall,<sup>30</sup> further expanding this model's usage.

### CaCl<sub>2</sub> Model

This procedure is performed by applying cotton gauze soaked with CaCl<sub>2</sub> to the aortic wall, then adding phosphate-buffered saline on the aorta.<sup>31,32</sup> CaPO<sub>4</sub> precipitation in the aortic wall was considered the driver for AAA formation. The pathology of this model shows calcification, elastin breakdown, VSMC loss and leukocyte infiltration, but rupture, intramural thrombus, or atherosclerosis does not occur.<sup>32</sup>

### β-Aminopropionitrile Model

Lysyl-oxidase (LOX) is an enzyme secreted by VSMCs and performs the crosslinking between collagens. Loss-of-function mutation of *LOX* leads to TAA and dissection in humans.<sup>33</sup> β-Aminopropionitrile, a potent inhibitor of LOX, combined with angiotensin II or deoxycorticosterone acetate, induces a high incidence of both TAA and AAA.<sup>34</sup> In this model, aneurysm incidence can reach 100%, and rupture is frequent. Histological studies indicate elastic fiber breaks, thrombus formation, and leukocyte infiltration.<sup>35</sup>

### Large-Animal Models

Large animals are more expensive than small animals (mice or rats), but they are useful to test endovascular devices or surgical procedures. There are several different ways to generate aneurysm models in large animals: (1) aortic patch<sup>36</sup> and artificial aneurysm graft<sup>37</sup>; (2) intra-aortic porcine pancreatic elastase infusion<sup>38</sup>; and (3) aortic dissection with endovascular procedures.<sup>39</sup> Many drugs effectively preventing aortic aneurysm in animal models did not show protective effects in clinical trials. This inconsistency could partially attribute to the limitations of animal models in recapitulating human disease. Better understanding of the pathogenesis of aortic aneurysm is the key to lead to effective therapies.

## ROLE OF VSMCS IN AORTIC ANEURYSM

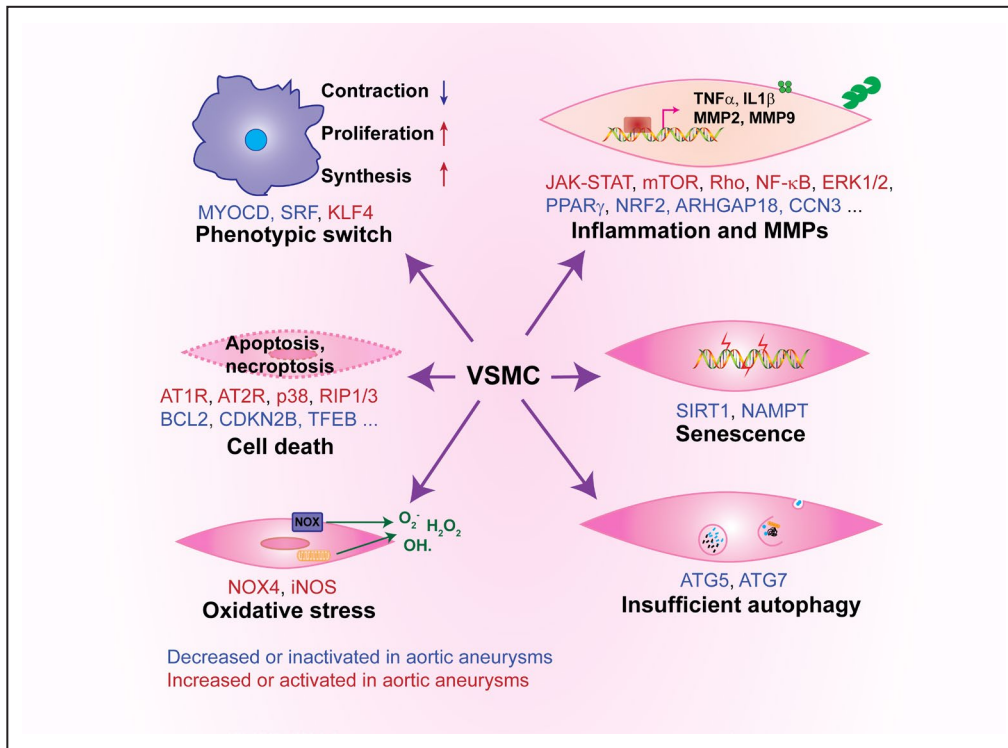
The loss of organized structure of the aorta leads to a weakened vessel and subsequent dilatation of the aorta. Although other cell types, including endothelial cells, neutrophils, macrophages, and lymphocytes, are

involved in aortic aneurysm development, VSMC dysfunction is crucial for the loss of structural integrity in the aortic wall.<sup>20</sup> VSMC apoptosis and ECM degeneration are the hallmarks of aortic aneurysm.<sup>20</sup> Here, we mainly focus on discussing the role of VSMCs in aortic aneurysm (Figure 3).

### Phenotypical Switching and Reprogramming of VSMCs

Healthy VSMCs maintain a quiescent and contractile phenotype, but they switch to a proliferative, synthetic, and migratory phenotype in response to various pathological stimuli.<sup>40</sup> The phenotypic switching of VSMCs has been studied extensively in atherosclerosis.<sup>41,42</sup> Transcription of contractile genes (transgelin, Calponin 1 and actin alpha 2 smooth muscle) is mainly controlled by myocardin along with serum response factor.<sup>43</sup> On the contrary, Krüppel-like factor 4 is a critical transcription factor promoting VSMC phenotypic switching from a contractile to synthetic phenotype through several mechanisms, including by directly binding to G/C repressor elements and by inhibiting serum response factor binding to CARG-boxes in the target gene promoter.<sup>44,45</sup> In addition, epigenetic regulation and chromatin remodeling also participate in the VSMC phenotypic switching.<sup>46,47</sup> For example, H3K4 dimethylation, H4 acetylation, H3K79 dimethylation, and H3K9 acetylation are required for the myocardin-serum response factor complex to access the promoter of VSMC-specific markers and contractile genes.<sup>48</sup> Treating VSMCs with dedifferentiating stimuli (platelet-derived growth factor-BB, injury, oxidized phospholipids) removes these histone modifications, leading to transcriptional suppression of these VSMC-specific genes.<sup>46</sup> The phenotypic switching of VSMCs has also been documented in both TAA and AAA development. Phenotypic modulation takes place in early aneurysm development in both human aneurysm samples and mouse models.<sup>47,49</sup> Recently, VSMC clonal expansion was observed in angiotensin II-induced aortic aneurysm by using *Myh11-CreERT2/Rosa26* Confetti mice. Proliferative VSMCs showed a decreased expression of smooth muscle cell (SMC) markers and increased expression of phagocytic markers.<sup>50</sup> In summary, VSMC phenotypic switching leads to vascular dysfunction and contributes to the pathogenesis of aneurysm formation.

In recent years, single-cell RNA sequencing has expanded the toolbox to understand cell heterogeneity in complex tissues. In a Marfan syndrome mouse model (*Fbn1*<sup>C1039G/+</sup>), single-cell RNA sequencing combined with an SMC lineage tracing study has shown a specific SMC population in the diseased aorta marked by decreased expression



**Figure 3.** The role of VSMCs in aortic aneurysms.

VSMCs play a critical role in the development of an aortic aneurysm. In pathological conditions, VSMCs undergo phenotypic switching, cell death (apoptosis and necroptosis), oxidative stress, inflammation, senescence, and insufficient autophagy, contributing to aortic aneurysm development. Critical signaling pathways have been identified to mediate VSMC dysfunction in aortic aneurysms. The schematic illustration also shows the genes/pathways either decreased/inactivated (blue) or increased/activated (red) in aortic aneurysm development. ARHGAP18 indicates Rho GTPase activating protein 18; AT1R, angiotensin II receptor type 1; AT2R, angiotensin II receptor type 2; ATG5, autophagy related 5; ATG7, autophagy related 7; BCL2, B-cell lymphoma 2; CCN3, cellular communication network factor 3; ERK1/2, extracellular signal-regulated kinases 1/2; iNOS, inducible nitric oxide synthase; JAK, Janus kinase; KLF4, Krüppel like factor 4; mTOR, mammalian target of rapamycin; MYOCD, myocardin; NAMPT, nicotinamide phosphoribosyltransferase; NF-κB, nuclear factor kappa B; NOX4, nicotinamide adenine dinucleotide phosphate oxidase 4; NRF2, nuclear factor erythroid 2-related factor 2; PPAR $\gamma$ , peroxisome proliferator-activated receptor-gamma; RIP1/3, receptor interacting serine/threonine kinase 1/3; SIRT1, sirtuin1; SRF, serum response factor; STAT, signal transducer and activator of transcription; and TFEB, transcription factor EB.

of SMC markers (myosin heavy chain 11 and actin alpha 2 smooth muscle), increased ECM synthesis (collagen type I alpha 1 chain and lumican), elevated dedifferentiation (Krüppel-like factor 4, and increased proliferation.<sup>51</sup> In a different aortic aneurysm mouse model (hypercholesterolemia+*Tgfr2* ablation), a similar strategy was used, and the results demonstrated that in aortic aneurysm formation, a subset of VSMCs transform to mesenchymal-like stem cells and then give rise to various types of cells, including adipocytes, chondrocytes, osteoblasts, and macrophage-like cells.<sup>52</sup> This process is dependent on Krüppel-like factor 4, as *Klf4* knockout largely abrogates aortic aneurysm development in mice.<sup>52</sup> Maintaining the contractile force of VSMCs is essential for aortic function and structure. The implication of force generation in

the development of TAA is evidenced by genome-wide association studies showing that mutations of genes encoding filaments (actin alpha 2 smooth muscle and myosin heavy chain 11), kinases (myosin light chain kinase and protein kinase cGMP-dependent 1), ECM glycoproteins (*LOX*, fibrillin 1, or microfibril associated protein 5) are associated with TAA.<sup>5</sup> Thus, disruption of VSMC force generation would lead to TAA and aortic dissection.

### Inflammation and MMPs in VSMCs

Similar to atherosclerosis, inflammation is always present in the aneurysmal lesion as an immune response to vascular injury.<sup>18</sup> VSMCs can secrete multiple cytokines (interleukin-6, monocyte chemoattractant protein-1, interleukin-1 $\beta$ , and tumor necrosis

factor- $\alpha$ )<sup>53</sup> and chemotactic factors to recruit inflammatory cells to the aortic wall.<sup>4</sup> Multiple signaling pathways, including Janus kinase-signal transducer and activator of transcription,<sup>54</sup> mammalian target of rapamycin,<sup>55</sup> nicotinamide adenine dinucleotide phosphate oxidase 4,<sup>56</sup> nuclear factor kappa B,<sup>57</sup> switch/sucrose nonfermentable-related, matrix-associated, actin-dependent regulator of chromatin, subfamily d, member 1,<sup>58</sup> extracellular signal-regulated kinases 1/2<sup>59</sup> and Rho kinase<sup>60</sup> promote VSMC inflammation and aortic aneurysm progression. By contrast, several signaling pathways, including Rho GTPase activating protein 18,<sup>61</sup> nuclear factor erythroid 2-related factor 2,<sup>62</sup> cellular communication network factor 3<sup>63</sup> and peroxisome proliferator-activated receptor- $\gamma$ ,<sup>64</sup> attenuate VSMC inflammation and AAA progression. Inflammatory VSMCs further aggravate local inflammation, forming a vicious cycle.<sup>20</sup>

Several MMPs from VSMCs are implicated in the development of aortic aneurysm. MMP-2 and MMP-9 are the most studied MMPs produced by VSMCs.<sup>20</sup> MMP-2 is constitutively expressed in VSMCs, while MMP-9 expression is inflammation inducible.<sup>65</sup> Transcriptome analysis of VSMCs isolated from human AAA samples shows increased MMP-2 and MMP-9, which can further be augmented by recruited macrophages.<sup>66</sup> The increases in MMP-2 and MMP-9 contribute to ECM degeneration in the aortic wall. Besides MMP-2 and MMP-9, VSMC-derived MMP-3 can be activated by macrophage-derived netrin-1 and promotes AAA formation in mice.<sup>67</sup> Thus, it is critical to restore the balance between ECM proteases and protease inhibitors in VSMCs to inhibit aortic aneurysm formation and progression.

## Oxidative Stress in VSMCs

Oxidative stress is high in plasma and aneurysmal segments from subjects with AAA.<sup>68</sup> Reactive oxygen species (ROS) contribute to AAA development by influencing VSMC inflammation, MMP activation, and apoptosis. Antioxidants including vitamin E, edaravone, ursodeoxycholic acid, lipoic acid, apocynin, and folic acid inhibit aortic aneurysm in mice.<sup>69</sup> Consistent with pharmacological inhibition, genetically engineered transgenic mouse models demonstrated that excessive oxidative stress promotes AAA development. Nicotinamide adenine dinucleotide phosphate oxidases produce ROS in cells.<sup>56</sup> Deletion of the nicotinamide adenine dinucleotide phosphate oxidase subunit, *p47phox* attenuates angiotensin II-induced AAA in *Apoe*<sup>-/-</sup> mice.<sup>70</sup> Inducible nitric oxide synthase deletion reduces ROS and inhibits CaCl<sub>2</sub>-induced AAA.<sup>71</sup> On the other hand, catalase (a critical H<sub>2</sub>O<sub>2</sub> scavenger) overexpression in VSMCs protects against AAA induced by either angiotensin II<sup>72</sup> or CaCl<sub>2</sub><sup>73</sup> in mice.

ROS is also elevated during TAA development.<sup>74</sup> VSMCs from Loeys-Dietz or Marfan syndrome mice exhibited reduced mitochondrial respiration and an increased ROS level.<sup>75</sup> *Nox4* deletion ameliorates aortic root dilatation in Marfan syndrome (*Fbn1*<sup>C1039G/+</sup>) mice.<sup>74</sup> Although the actual mechanisms of how ROS is generated and how ROS affects VSMC biology are not fully clear, it is well recognized that excessive ROS leads to VSMC dysfunction and subsequent aortic wall disruption in aortic aneurysm development.<sup>4</sup>

## VSMC Loss in Aortic Aneurysm

The loss of VSMCs is a common phenomenon in aneurysmal lesions,<sup>20</sup> where VSMC death may occur through multiple signaling pathways. The elucidation of these pathways can help us develop drugs targeting VSMCs to maintain normal VSMC population and function in the diseased aorta.

### Apoptosis

Apoptosis is mediated by 2 distinct pathways, the intrinsic pathway (mitochondria mediated) and extrinsic pathway (death receptor mediated).<sup>76</sup> Apoptosis is critical for body homeostasis by removing dysfunctional or senescent cells. However, excessive VSMC apoptosis during human and mouse aortic aneurysm development results in weakening of the aortic wall.<sup>20</sup> VSMC apoptosis could be induced by excessive ROS, cytokines, or modified lipoproteins.<sup>77</sup> In Marfan syndrome, angiotensin II can induce VSMC apoptosis via angiotensin II receptor types 1 and 2.<sup>78</sup> Using an in vitro Marfan syndrome vascular cell model derived from human induced pluripotent stem cells, inhibition of the p38 pathway and Krüppel-like factor 4 knock-down were found to reduce SMC apoptosis.<sup>79</sup>

Based on these findings, inhibition of VSMC apoptosis has been shown to be effective in preventing aortic aneurysm in murine models.<sup>60,80–84</sup> 2-Hydroxypropyl-beta-cyclodextrin inhibits AAA development in both  $\beta$ -aminopropionitrile and angiotensin II-induced AAA<sup>80</sup> via upregulation of B-cell lymphoma 2 in a transcription factor EB-dependent manner. KMUP-3, a xanthine derivative, suppresses VSMC apoptosis in the angiotensin II-induced AAA model.<sup>81</sup> A recent study revealed that APLN-NMeLeu9-A2, an apelin analog, prevented angiotensin II-induced AAA formation via reducing VSMC apoptosis in *Ldlr* knockout mice on a high-fat diet.<sup>83</sup> Moreover, deletion of cyclin-dependent kinase inhibitor 2B (*Cdkn2b*), a tumor suppressor gene, promotes p53-dependent VSMC apoptosis and induces larger aortic aneurysms in the AAA elastase mouse model.<sup>82</sup> The pan-caspase inhibitor Q-VD-OPh diminished VSMC apoptosis, inflammation, and AAA formation in the angiotensin II mouse

model.<sup>84</sup> Although apoptosis is the cause of VSMC loss in aortic aneurysm, it is still challenging to target apoptosis as a potential treatment because apoptosis itself has important homeostatic functions that are necessary to maintain proper function and vascular health. The unwanted adverse effects of inhibiting apoptosis need to be carefully considered when pursuing novel targets and approaches for aortic aneurysm treatment.

### Necroptosis

Necroptosis is another kind of programmed cell death. Unlike apoptosis, necroptosis does not involve caspases and usually induces local inflammation.<sup>85</sup> Necroptosis has been implicated in atherosclerosis, ischemia-reperfusion injury and myocardial infarction.<sup>85</sup> Receptor interacting serine/threonine kinase 1 (RIP1), RIP3, and mixed lineage kinase domain-like pseudokinase are the primary effectors of necroptosis.<sup>85</sup> Increased necroptosis was found in both human and murine AAA.<sup>86</sup> RIP1 inhibition with necrostatin-1s or *Rip3* genetic deficiency protects mice from elastase-induced AAA.<sup>86,87</sup> Consistently, the dual inhibitor of RIP1/RIP3, GSK2593074A, inhibits angiotensin II- and calcium-induced AAA in mice.<sup>88,89</sup> These studies reveal the potential application of necroptosis inhibitors in treating aortic aneurysm.

### VSMC Senescence

AAA incidence increases dramatically with advancing age, indicating AAA to be an aging-related pathological process.<sup>1</sup> On the cellular level, features of VSMC senescence include DNA damage, telomere shortening, epigenetic changes, loss of proteostasis, abnormal nutrient sensing, and mitochondrial dysfunction.<sup>90</sup> Current evidence shows that VSMC senescence is involved in aortic aneurysm development.<sup>91</sup> The mammalian sirtuins (sirtuins1–7) are highly conserved deacetylases and promising targets for antiaging therapy.<sup>92</sup> Age-related sirtuin1 was observed to be decreased in human AAA samples. VSMC-specific *Sirt1* knockout and transgenic mice consistently show that sirtuin 1 significantly attenuates AAA formation via inhibition of the p21 and nuclear factor kappa B pathways in angiotensin II and CaCl<sub>2</sub> aortic aneurysm mouse models.<sup>91</sup> Nicotinamide phosphoribosyltransferase and the nicotinamide adenine dinucleotide fueling system replenish the substrates used by sirtuin proteins. This system is impaired in the aorta from patients with dilated aortopathy, and VSMC-*Nampt* knockout mice show premature vascular senescence and dissection.<sup>93</sup> These studies indicate that aged VSMCs are more susceptible to pathological stimuli, leading to an increased risk of aortic aneurysm in aging individuals.

### VSMC Autophagy

Autophagy is an evolutionarily conserved process for cells to remove unwanted protein and organelles. Impaired autophagy in cells may lead to endoplasmic reticulum stress, inflammation, and even cell death.<sup>94</sup> Enhanced autophagy in VSMCs shows protective effects on various vascular diseases, including vascular calcification,<sup>95</sup> neointimal formation,<sup>96</sup> and atherosclerosis.<sup>96</sup> In response to increased ROS, cytokines, metabolic stress, or growth factors,<sup>95</sup> autophagy is activated in VSMCs in aortic aneurysm.<sup>50</sup> The importance of autophagy in maintaining VSMC homeostasis is further demonstrated by VSMC-specific knockout of autophagy machinery proteins. Deletion of autophagy related 5 (*Atg5*) in VSMCs increases the incidence of abdominal aortic dissection and promotes vascular inflammation in the angiotensin II aneurysm mouse model.<sup>50</sup> Autophagy related 7 (*Atg7*) knockout in VSMCs exacerbates adverse cardiac remodeling and dissecting abdominal aortic aneurysm in mice after angiotensin II treatment.<sup>97</sup>

Dysfunction and loss of VSMCs are common features in aortic aneurysm development.<sup>20</sup> This section briefly summarizes our knowledge about VSMC phenotypic switching, inflammation, oxidative stress, death, senescence, and autophagy, and their roles in aneurysm development. Importantly, these different pathways are not separate from each other, or rather, there is often crosstalk and cell signal convergence of these pathways. For example, autophagy is closely related to senescence, as aging cells usually show impaired autophagy and defective autophagy results in accelerated senescence.<sup>98</sup> Defective autophagy leads to enhanced VSMC phenotypic switching<sup>50</sup> and cell death.<sup>94</sup> A deeper understanding of these complex pathways and their interactions is key to finding safe and efficient therapies for treating aortic aneurysm.

### CONCLUSIONS AND PERSPECTIVES

The development of aortic aneurysm is a complex process involving multiple cell types. Beyond this, previously defined cell types such as VSMCs or macrophages also consist of several distinct subtypes.<sup>52</sup> They may exert different roles in the initiation and progression of the disease. The recent application of single-cell RNA sequencing technology largely expanded our understanding of cellular heterogeneity. In aortas from elastase-induced AAA mice, at least 4 VSMC clusters and 5 monocyte/macrophage clusters were identified.<sup>99</sup> Furthermore, human aortic aneurysm samples also show heterogeneity with different VSMC clusters (contractile, stressed, and proliferating).<sup>100</sup> This heterogeneity must be taken into consideration when developing new drugs and approaches to avoid non-specific adverse effects.



Aortic aneurysm remains a significant cause of death because of its high mortality once rupture takes place. Open surgery and endovascular repair remain the major treatments for aortic aneurysm. These procedures have specific limitations, including the feasibility of aortic aneurysm surgery for each patient and surgery-related complications. Unfortunately, no drugs to date have been shown to be effective in clinical trials, although novel diagnostic and therapeutic strategies are still under development. In both human and animal aortic aneurysms, VSMC phenotypic switching, inflammation, oxidative stress, cell death, autophagy, and senescence are involved in the development and progression of disease. Currently, many of the detailed mechanisms underlying aortic aneurysm still come from animal models. Each animal model has its advantages and pitfalls, and therefore it is always important to consider its relevance to human conditions and pathophysiology. Further studies are necessary to overcome these limitations for improved research translation and subsequent clinical application for treating aortic aneurysm.

## ARTICLE INFORMATION

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### Disclosures

None.

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