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SPOTLIGHT REVIEW

From genetics to response to injury: vascular smooth muscle cells in aneurysms and dissections of the ascending aorta

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Abstract

Vascular smooth muscle cells (vSMCs) play a crucial role in both the pathogenesis of Aneurysms and Dissections of the ascending thoracic aorta (TAAD) in humans and in the associated adaptive compensatory responses, since thrombosis and inflammatory processes are absent in the majority of cases. Aneurysms and dissections share numerous characteristics, including aetiologies and histopathological alterations: vSMC disappearance, medial areas of mucoid degeneration, and extracellular matrix (ECM) breakdown. Three aetiologies predominate in TAAD in humans: (i) genetic causes in heritable familial forms, (ii) an association with bicuspid aortic valves, and (iii) a sporadic degenerative form linked to the aortic aging process. Genetic forms include mutations in vSMC genes encoding for molecules of the ECM or the TGF-β pathways, or participating in vSMC tone. On the other hand, aneurysms and dissections, whatever their aetiologies, are characterized by an increase in wall permeability leading to transmural advection of plasma proteins which could interact with vSMCs and ECM components. In this context, blood-borne plasminogen appears to play an important role, because its outward convection through the wall is increased in TAAD, and it could be converted to active plasmin at the vSMC membrane. Active plasmin can induce vSMC disappearance, proteolysis of adhesive proteins, activation of MMPs and release of TGF-β from its ECM storage sites. Conversely, vSMCs could respond to aneurysmal biomechanical and proteolytic injury by an epigenetic phenotypic switch, including constitutional overexpression and nuclear translocation of Smad2 and an increase in antiprotease and ECM protein synthesis. In contrast, such an epigenetic phenomenon is not observed in dissections. In this context, dysfunction of proteins involved in vSMC tone are interesting to study, particularly in interaction with plasma protein transport through the wall and TGF-β activation, to establish the relationship between these dysfunctions and ECM proteolysis.

Keywords Extracellular matrix • TGF-β • Contractile proteins • SMC tensegrity • Plasminogen activation • Epigenetics • Protease nexin-1 • Endocytosis • Haemodynamics

This article is part of the Spotlight Issue on Novel concepts for the role of smooth muscle cells in vascular disease.

1. Definition of aneurysms

Aneurysms are morphologically defined as localized dilations of the arterial wall with a focal loss of wall parallelism and functionally defined as a progressive loss of the arterial wall's ability to withstand the wall tension generated by high intraluminal pressure, leading to intramural (dissection, AAD) or complete acute rupture. Aortic aneurysms, whatever their localization, ascending aorta TAA or abdominal aorta (AAA) share common pathophysiological features but differ by aetiologies and specific haemodynamics.[1] Since withstanding wall tension is mainly the function of the insoluble fibrillary extracellular matrix (ECM) synthesized and matured by vascular smooth muscle cells (vSMCs) in the wall, ECM degradation by proteolytic enzymes is a common mechanism in aneurysmal pathogenesis. In this micro-environment, intrinsic frailty of the ECM, loss of vSMCs, and increased permeability to plasma zymogens, directly or indirectly potentiate the proteolytic injury. In parallel, vSMCs also respond to these injuries using their functional and epigenetic plasticity (nuclear reprogramming, phenotypic shift).[2] AAAs are mainly of atherothrombotic origin,[3] whereas the ascending aorta is resistant to atheroma and devoid of intraluminal thrombus formation, most likely due to the

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high shear and its washing effect by systolic ejection (*Figure 1*). Therefore, with the exception of specific rare auto-immune aetiologies such as Takayasu's or Horton's diseases, TAADs are characterized by the absence of myeloid cell diapedesis within the aortic tissue. Thereby TAA & D offer a unique opportunity to study the predominant roles of vSMCs in aneurysmal pathologies (i) as initiators of aneurysm formation by expression of specific genetic molecular defects; (ii) by their ability to promote blood-borne proteolytic injuries in the absence of inflammation; and (iii) to limit the risk of acute rupture, by an adaptive response of the canonical TGF-β/Smad2 pathway in vSMCs, which may help to prevent dissections through synthesizing more fibrillar ECM, along with locally secreting antiproteases,[4] and clearance of protease/antiprotease complexes[5] (*Figure 2*). The wall histopathology, involving ECM breakdown, vSMC loss co-localized with areas of mucoid degeneration, is common to TAA and dissection (AAD) whatever their aetiologies. Areas of specific mucoid degeneration are characterized by accumulation of highly hydrophilic glycosaminoglycans (GAGs). Mucoid degeneration is also observed during the aging process in human aorta.[6] However, the description of the involvement of vSMCs in TAA needs to assimilate first the evolutive role of SMCs in phylogenesis, and therefore the physiology, of the arterial part of the circulation.

2. Role of vSMCs in structure/function of the arterial system in mammals

Phylogenetically, the circulatory system evolved from a low-pressure closed circulating system animated by an archaic heart in fish, to a more recent, highly pressurized (potential energy) arterial system with organ-regulated directional blood flow (vasomotricity) propelled through the conductance arterial tree by the pumping action of the left ventricle. The teleonomy of arterial vSMC evolution is to structure the wall of conductance arteries and to assist the metabolic autonomisation of organ function through resistance arteries. As a main determinant, vSMC tone defines peripheral resistances to blood flow, generating arterial blood pressure.

In parallel, wall topophysiology and structure also evolved in conductance arteries in order to respond to the acquired pressure load, from a thin cellular structure to a thick matrix-rich, layered structure. In the aorta, vSMCs assume the functional roles of both producing aortic tone in response to sympathetic stimuli, and synthesizing and modelling the ECM. Biomechanically, the tensile stress supported by the wall is proportional to pressure and radius and inversely proportional to wall thickness (Laplace's low, $T = P.r/2\,h$). Since progressive physiological dilation of the aorta is observed with age in animals[7] and humans,[8] tensional stress increases with aging, independently of pressure. To respond to tensile stress, the aortic wall is structured in three spatially organized layers, from inside to outside: the intima, the media and the adventitia. The medial layer displays spatial and functional connectivity between vSMCs and ECM, assuming the function of supporting the phasic haemodynamic load (the content) within the arterial system (the container).[9] vSMC differentiation and survival is dependent on cell adhesion to matrix,[10] creating tensegrity[11] within the cell, via ECM, intracellular cyto- and nucleoskeletal interactions,[12] largely dependent on local haemodynamic parameters, the cardiac cycle and the impedance to phasic flow. In the ECM, the insoluble macro-fibrils, collagens, mainly provide the resistance to rupture whereas elastin provides the resistance to dilation.[13]

Outward adventitial intersititial pressure is low: 10 mmHg. Therefore, an important transmural pressure gradient (100 mmHg) exists between the intraluminal arterial blood pressure (130/80 mmHg) generated by the peripheral resistance, and adventitial interstitial pressure, creating a unidirectional outward hydraulic conductance across the arterial wall. This hydraulic conductance is responsible for advective/convective radial mass transport of soluble plasma molecules and macromolecules through the arterial wall. This biomechanical phenomenon is termed «outward convection». Outward convection is dependent, on the one hand, on haemodynamic factors, including pressure and shear[14,15] and, on the other hand, on the porosity of the arterial wall, partly determined by the integrity of the elastic network[16] and by vSMC tone[17,18] which limit advection across the wall. In this paradigm, pressurized water and blood-borne components percolate through the wall components, creating potential interactions with vSMCs and/or the ECM and leading to retention, proteolysis, clearance and metabolism of plasma proteins or blood particles, or to their exfiltration towards the adventitia for recycling.[9,19] For example, free water molecules will be retained more in hydrophilic areas of the aortic wall than in hydrophobic ones (elastin), and could contribute to the swelling of the GAG-rich mucoid degenerative areas, potentially inducing intramural delamination.[20]

3. Nosology and aetiologies of TAADs

TAAs are non-atheromatous aneurysmal diseases related to three main aetiologies: monogenic diseases, associated with bicuspid aortic valves (BAV) and sporadic (also termed degenerative), associated with aging.[21] These different aetiologies are characterized by the age of clinical expression: younger in genetic forms, middle age in association with BAV, older in sporadic forms. Up to 30% of cases of TAAs are associated with underlying mutations in single genes. These mutations are predominantly inherited in an autosomal dominant manner and may (syndromic forms) or may not be associated with other systemic manifestations.

When compared with TAAs, characterized by a progressive dilation of the aorta, dissections are acute events, defined by intramural rupture, with or without a subjacent aneurysm, usually of small dilation. The initial intimal tear, causing blood leaks within the external part of the media, can take place in the ascending aorta (Type I or A dissections) or just below the ostia of the left subclabvicular artery developing in the descending thoracic aorta (Type III or B dissections). Type II is the extension of Type I to the thoracic descending aorta, with possible re-entry in the abdominal aorta. Types II and III dissections could generate circulating, partially circulating or thrombosed false channels which may impact the evolution of the disease.[22,23] When compared with dilated aortic tissue, the dissected tissue is also characterized by areas of GAG-rich mucoid degeneration, which pave the way for initial tears and haemorrhagic suffusion and diffusion towards the external part of wall, the site of developing vasa vasorum. In this context the specific role of GAG accumulation, particularly in the tissue environment of vasa vasorum, has been recently highlighted in AAD.[24] Tobacco, hypertension and intensive tonic physical effort (weightlifting) are risk factors for acute dissections.

4. Experimental models

Experimental animal models of aortic aneurysms and dissections have been recently reviewed.[25] Experimental models of progressive TAA are

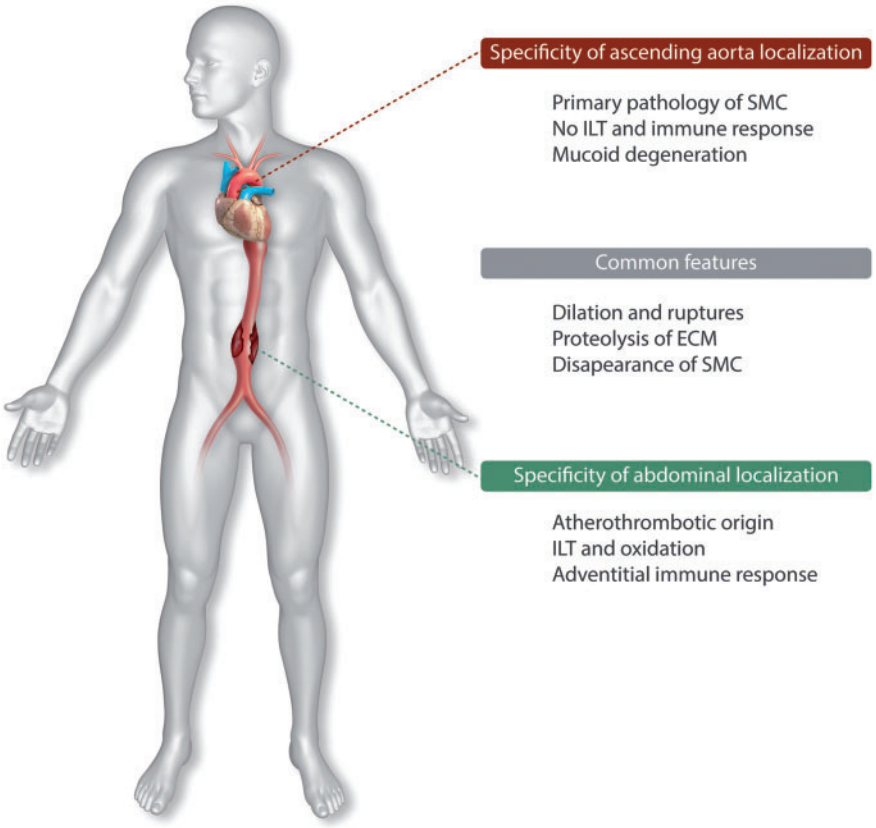

Figure 1 Common features and specificities of TAA vs. AAA.

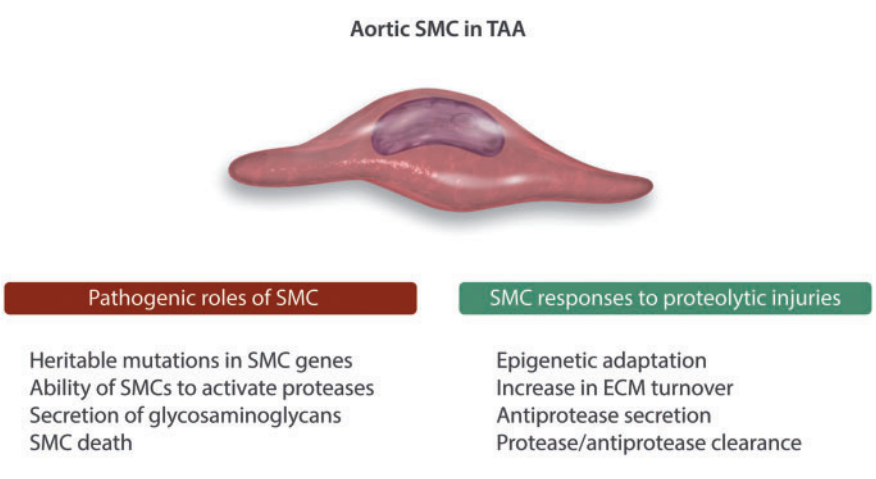

Figure 2 vSMC involvement in TAA pathogenesis and subsequent responses.

rare in rats and mice and limited to some pharmacological models and to mice genetically engineered to harbour gene mutations observed in humans with thoracic aortic disease, such as Marfan mouse models[26] (*Table 1*). The Blotchy syndrome in mice remains one of the very few available models of naturally occurring, genetically determined aneurysms.[27] The blotchy syndrome is due to a mutation in a copper transporter leading to copper insufficiency. Copper is the transition metal necessary for Lysyl-oxydase (LOX) enzymatic activity. As a translational example in humans, genetic copper deficiency is observed only in the rare condition, Menkes disease. Dissections have been modelled

in animals for many years.[28] Spontaneous dissecting aneurysms of the aorta were initially described in turkeys with stress-dependent acute hypertension.[29] The frequency of these acute dissections were increased when the turkeys were fed β-aminopropionitrile (BAPN).[30] BAPN is a toxic chemical isolated from a variety of sweet pea and responsible for clinical angiolathyrism. BAPN is a powerful inhibitor of LOX, impairing the maturation of elastin and collagen and thus sensitizing the ECM to proteolysis. Administration of BAPN in rats and mice also increases the risk of dissecting aneurysm.[31] The effect of BAPN is dependent on the dose but also on the age of administration, with younger animals (immediate post-weaning) more likely to have dissections.

More recently, Daugherty and colleagues[32] described that angiotensin II (AngII) infusion induces dissecting aneurysms in *Apoe*$^{-/-}$mice.[33–35] This model, easy to do, is not limited to models of atherosclerosis in mice. When angiotensin II is administered to mice sensitized by genetic mutations on ECM genes,[36] BAPN exposure,[37] or TGF-β antibody administration,[38] a rate of dissection of 100% can be observed. Similar results were obtained in rats (JBM personal data). In these pharmacological models, the level of circulating plasma AngII is high and the mice are moderately hypertensive, suggesting that AngII acts more by causing mechanical intimal damage, rather than targeting the medial vSMCs. Physiologically, the renin/angiotensin system is compartmented and the plasma AngII level is very low, since renin acts more at a tissue level than in plasma. Thereby, it would be interesting to compare the AngII model with a similar model of renin infusion. Nevertheless these models, associating an ECM defect or vSMC relaxation[39] with endovascular injury by AngII are particularly interesting for testing new therapeutic approaches in dissections. The current tendancy is to associate two models, a genetic defect with angiotensin II infusion in mice, or two pharmacological approaches (BAPN + AngII) in rats.

Table 1 Experimental models of TAADs

Spontaneous TAADs in animals
Turkey (aortic dissection)[29]
Blotchy mice (mutation in ATPase copper transporter)[104]
Genetic models K.O. and K.I.
Fibrillin-1 (Marfan)[105]
Coll3A1 (Ehlers-Danlos)[36]
FOXE3$^{-/-}$ (transcription factor)
LOX[106]
ADAMTS1[51]
Biglycan K.O.[107]
Filamin A[108]
Pharmacological models
Angiotensin II infusion in sensitized mice (dissections)[36]
β-aminopropionitryl (BAPN, LOX inhibitor)[109]

5. Pathogenic roles of vSMCs in TAA & D

5.1 vSMC genes predisposing to TAAs weaken ECM, disrupt vSMC tone or limit canonical TGF-β signalling

The list of genes associated with syndromic and non-syndromic ascending aortic aneurysms and dissections was recently updated.[40] It has been known for many decades that one gene in the human genome can be mutated and lead to a strong familial predisposition for TAAD. Marfan syndrome (MFS) is a condition inherited in an autosomal dominant manner with skeletal (long limbs and fingers, scoliosis, pectus deformities), connective tissue (joint laxity, striae, flat feet) and ocular (ectopia lentis) complications. Affected individuals can have cardiac features (mitral insufficiency and prolapse) but the major cardiovascular complication is progressive enlargement of thoracic aortic root aneurysms and acute ascending aortic dissections, related or not to dilation. MFS is mainly due to mutations in *FBN1*, which encodes an ECM protein called fibrillin-1 that localizes in microfibrils. Microfibrils play an important role in the aorta; they link the elastic fibres to focal adhesions on the cell surface of vSMCs. Mutations in *FBN1* lead to either less fibrillin synthesis (mutations leading to haploinsufficiency) or quantitatively decreased fibrillin-1 being incorporated into microfibrils (missense mutations).[4] Other ECM genes predisposing to TAAs include other proteins in microfibrils (*MFAP5*)[41] and loss-of-function mutations in *LOX*,[42] which is an enzyme involved in cross-linking and maturation of the ECM. In parallel, the nosology and management of vascular Ehler-Danlos syndrome, in relation to a Col3A1 mutation,[43] and the structure–dysfunction relationship created by the mutation[44] have recently progressed (*Table 2*).

Another group of altered genes that predispose to TAAs and aortic dissections are genes encoding either the major structural components of the contractile unit in vSMCs or the enzymes that control vSMC contractile tone. Smooth muscle α-actin (SM α-actin), a major protein in vSMCs, is synthesized as a monomer that polymerizes to form the thin filament in the contractile unit. Heterozygous mutations, which are overwhelmingly missense mutations predicted to lead to production of a mutant monomer, predispose to TAAs and dissections.[45] The mutant monomers alter the function of the thin filament, including decreasing the tensegrity pathways, the stability of the contractile filaments and decreasing the movement of the filament by the myosin motor, and thus are predicted to decrease force generation by the vSMCs. The thick filaments are composed of a smooth muscle-specific isoform of myosin heavy chain dimer (SM MHC; encoded by *MYH11*), and four light chains (LCs), two regulatory LCs and two essential LCs. Heterozygous *MYH11* mutations also lead to an inherited predisposition for TAAs and aortic dissections.[46] These exonic mutations, localized in the rod domain of *MYH11*, perturb the quaternary structure of the thick filament. Phosphorylation of the LC on the myosin thick filament is necessary to activate the force generating cycle of SM myosin motor heads with the actin filaments. Heterozygous loss-of-function mutations in the gene (*MYLK*) encoding the dedicated kinase phosphorylating the LC, myosin LC kinase, are a cause of heritable thoracic aortic disease.[47] A single heterozygous gain-of-function *PRKG1* mutation, p.Arg177Gln (designated R177Q), also causes a familial form of heritable thoracic aortic disease.[48] PRKG1 encodes a Type I cGMP-dependent protein kinase (PKG-1), which is activated upon binding of cGMP and controls vSMC relaxation, in part through activation of the phosphatase that de-phosphorylates the LC. In summary, mutations in *ACTA2, MYH11, MYLK*, and *PRKG1* are all predicted to disrupt force generation, especially in vSMCs, promoting TAAs and acute aortic dissections. In contrast, loss of function mutations in the NO signalling molecular pathway in vSMCs is associated with a familial form of coronary artery disease[49] and Moyamoya neurovascular disease.[50]

This role of vSMC tone has been also recently highlighted by two experimental studies in mice. In the first one, Doyle and colleagues

Table 2 Genomic mutations in heritable forms of TAADs in human

Genes encoding for ECM defects
Fibrillin -1 and -2 (MFS)[4]
Microfibril-associated Protein5 (*MFAP5*)[41]
Filamin A[110]
LOX[42,111]
Menkes disease (*ATP7A copper transporter*)[112]
Elastin (*ELN*)[113] and Fibulin (FBLN),[114] (Cutis Laxa), Emilin1 (elastin microfibril interfacer 1)[115]
Collagen 1 α2, 3 α1, 5 α1, 5 α2 chains (Elhers-Danlos)
ATP7A (ATPase copper transporter, Menkes disease)[116]
Glucuronyl transferase-1 (GAG synthesis)[117]
Dermatan-sulphate proteoglycans[118], Biglycan[119]
NOTCH1[120,121]
TGF-b signalling pathways (loss of function)
TGFBR1 and TGFBR2[54,122]
SMAD3[55] *and SMAD4*[56]
TGFβ-2[52] *and -β-3*[53]
TGFβ-repressor SKI (Shprintzen-Goldberg syndrome)[123]
Contractile proteins (loss of function)
MYH-11 (myosin heavy chain)[46]
ACTA2 (SM Actin)[45]
MYLCK (myosin LC kinase)[47]
PKG (protein kinase G, gain of function)[48]
vSMC metabolism
Methionine adenyl transferase[124]
Glucose transporter *(SLC2A10)*[125]
FOXE3 (Transcription factor)[126]

explored the effects of calcium channel blockers in a genetic model of Marfan mice.[39] In this model, amlodipine and verapamil exacerbated the progression of the aortic dilation (monitored by echography) and decreased the survival rate, due to an increased frequency of acute ruptures (dissections) during the 3 months of calcium blocker administration. These clinical data were confirmed by the histology of the aortic wall, showing a more important fragmentation of the elastic fibres under calcium blocker treatment. They retrospectively confirmed these data in Marfan patients receiving calcium blockers (more surgery and more rapid progression of aortic dilation) as compared with other antihypertensive agents.

In their recent study, Oller *et al.*,[51] explored the impact of ADAMTS1 heterozygous K.O. in mice. They describe that the development of TAADs in these mice is dependent on inducible Nitric Oxide Synthase (iNOS or NOS2) overexpression by aortic vSMCs. iNOS induction is associated with a powerful inhibition of vSMC contractile tone. This experimental pathology is rescued, including angiotensin II-induced aortic dissection in this model, by inhibition of iNOS by L-NAME, a general inhibitor of NOSs, and by 1400 W (GW 274150) a specific inhibitor of iNOS. These experimental results (ADAMTS1 decreased expression and iNOS overexpression in vSMCs) were extended to a small series of TAA samples in MFS patients as compared with healthy human aortas. It is important to note that vSMCs cannot generate effective tensegrity unless the cells are anchored to the ECM. As mentioned previously, in the aorta, vSMCs are anchored to elastin by microfibrils. *FBN1* mutations promote disruption of these connections and *Fbn1* mutations in mice have been shown to decrease tensegrity of vSMCs in the aorta. The relationship between contraction/relaxation of vSMCs and outward hydraulic conductance through the wall has been recently explored *ex vivo* in rat aorta[18] showing that induced SMC tone by addition of catecholamine, decreased mass transport through the wall. The relationship between a chronic defect in vSMC tone generation and changes in arterial wall permeability to water and plasma proteins remains to be established *in vivo* in human TAAs.

Heritable alterations of the genes that encode proteins in the canonical TGF-β signalling pathway can also predispose to heritable thoracic aortic disease, including TGF-β ligands (*TGFB2, TGFB3*),[52,53] TGF-β receptors Types I and II (*TGFBR1, TGFBR2*)[54]and regulatory SMADs (*SMAD3, SMAD4*).[55,56] Although initial studies suggested that increased TGF-β signalling was the primary driver for thoracic aortic disease, the mutations disrupting the TGF-β pathway are predicted or have been shown to decrease TGF-β signalling, limiting the ability of the vSMCs to repair the wall in response to proteolytic injury.[38,57,58]

5.2 vSMCs activate blood-borne proteases

In relation to the principle of advective mass transport of plasma proteins, the difference between results of proteomic analysis of the aortic wall, in which plasma proteins represent 30% of the arterial wall proteome, and results of transcriptomics, where 100% of the mRNA represent wall cell genomics, provides evidence of plasma protein enrichment of the wall. In particular, the convection of plasma proteins is largely enhanced by the increase in wall permeability in TAA (*Figure 3*). For example, the albumin (a neutral protein synthesized by the liver and secreted into the plasma) concentration is increased by 60% within the TAA wall as compared with healthy aorta (*Figure 3*), demonstrating the change in wall permeability. This increase in permeability is determined by the wall structure and function, including endothelial integrity/desintegrity, elastic network degradation, and potentially, a decrease in wall tensegrity via a decrease in vSMC tone.[9] Conversely, percolation of blood components through the arterial wall may not be neutral, but could also impact permeability by modifying the connections between cells and matrix within the wall. In this paradigm, blood-borne components could injure the arterial wall. Thus, outward hydraulic conductance of blood-borne components is the largest common denominator of all TAAD, whatever their aetiology.

Since the hydrophobic, fibrillar ECM is insoluble, the convection and interactions of plama zymogens with the wall components are of particular pathophysiological interest. We observed in human TAA tissues, whatever the underlying aetiology, that prothrombin[21] and plasminogen[59,60] were present in greater concentrations when compared with healthy aortic wall and could be activated in the TAA wall. Plasminogen is of particular interest because it can be activated by vSMC membranes, via an S100A4/annexin A2 heterotetramer, exposing terminal lysine-binding sites and forming ternary complexes with t-PA. A role for urokinase (u-PA) cannot be excluded.[61] Plasmin is able to induce vSMC detachment from ECM (anoikis) by degrading fibronectin, one of the main adhesion proteins for vSMC integrins.[62] Plasmin is the main enzyme able to mobilize TGF-β from its storage site associated with latent TGF-β-binding proteins in the ECM. Furthermore, plasmin is able to convert inactive pro-MMPs into active MMPs. In our studies, we observed that all components of the fibrinolytic system were upregulated in the TAA media, including S100A4 (mRNA and protein, providing evidence of vSMC phenotypic switching), t-PA (mRNA and protein), u-PA (mRNA and protein), and plasminogen (plasmin activity and plasmin–antiplasmin

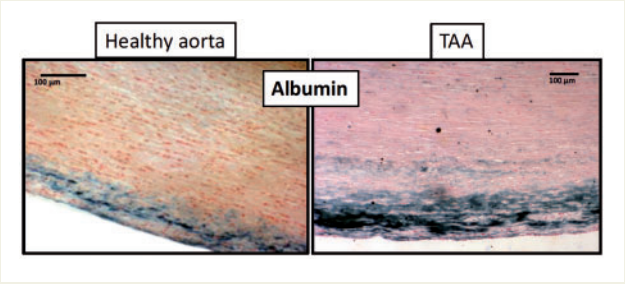

Figure 3 Neutral albumin staining (in blue) in the luminal media of healthy aorta vs. TAA.

complexes).[59] In contrast, plasminogen mRNA is not detected in the aortic wall, whereas the protein is, further supporting the hypothesis that it is outwardly convected from the blood.

5.3 vSMCs extracellularly accumulate GAGs

One of the common characteristics of TAA, dissections and aging in the aorta is the presence of mucoid ECM accumulation (MEMA),[63] composed of alcianophilic GAGS. This osmotically active GAG accumulation evolves towards extracellular watery vacuoles, originally improperly named 'cysts' (but there are no 'cysts' because there are no capsule membranes). This mucoid degeneration is not specific of aortopathy and can also be observed in tendinous and cartilaginous, hydrophilic, GAG-rich tissues. Aortic GAG chain synthesis by vSMCs is under the control of the canonical TGF-β pathway, involving smad2 linker region phosphorylation by ERK.[64] This signalling pathway activates the expression of GAG chain-synthesizing enzymes: xylosyltransferase, chondroitin sulphate synthase, and chondroitin sulfotransferase-1. GAGs are highly hydrophilic and their accumulation in specific areas leads to localized interstitial fluid retention potentially favouring the risk of dissection. Moreover areas of MEMA are able to retain specific MMPs, particularly MMP-3 (stromelysin) and MMP-7 (matrilysin), which possess a domain which interacts with the anionic structure of GAGs.[65]

5.4 vSMC death

Cell adhesion and tensegrity[66] are requisites for cell survival within tissues. This concept is particularly relevant for vSMC differentiation, including acquisition of actomyosin complexes, allowing contractility in response to sympathetic and other stimuli, and survival within the aortic wall tissue. There is a direct relationship between tensegrity and the ability of vSMCs to resist and survive in a microenvironment of high mechanical stress. Conversely, any injury to, or defects of, the molecular tensegrity cascade[67] from fibrillar ECM, adhesive intermediate proteins such as fibronectin, integrins, and focal adhesion kinases (FAK), cytoskeleton and actomyosin can induce partial or complete loss of tensegrity causing anoikis/apoptosis[10] of vSMCs. In this context the activity of focal adhesion complexes, involving FAK, Src, talin, vinculin, paxillin, stimulates MEK and ERK signalling pathways and therefore promotes cell survival and growth by inhibiting anoikis signalling.[68] Conversely defects in the tensegrity molecular cascade, endocellular (decrease in vSMC tone of either genetic or pharmacological origin) or extracellular (ECM defect of either genetic or pharmacological origin, pericellular proteolysis), promote cell apoptosis/anoikis by PI3K/AKT and NFkB pathways. In this context, a polycationic micro-environment is highly cytotoxic, promoting anoikis/apoptosis,[69] whereas poly-anions, including negatively charged polysaccharides, are protective.[70] As described earlier, activation of plasminogen on vSMC membranes by t-PA or u-PA/u-PAR, may promote cell detachment and therefore apoptosis/anoikis, mainly by degrading fibonectin which is the principal pericellular adhesive protein for vSMCs.[62] Conversely, the secretion and pericellular retention of tissue anti-proteases, including Protease Nexin-1 (PN-1)[71]or plasminogen activator inhibitor (PAI-1),[72] protect against apoptosis/anoikis.

6. vSMC responses to proteolytic injury

6.1 Transforming growth factor-β

TGF-β is a ubiquitous protein with a molecular mass of 25 kD which plays a role in tissue repair. In the aortic wall, TGF-β is secreted from vSMCs as a latent complex of TGF-β, the latency-associated peptide (LAP, interfering with αv integrin), and a molecule of latent-binding protein (LTBP).[73] This inactive complex is stored within the ECM (fibrillin, fibronectin, and elastin) and is released by proteolytic activity, mainly involving plasmin and MMPs, but is also released when vSMC contractile forces stretch the ECM via integrins.[74,75] Activation of TGF-β allowing receptor binding requires its dissociation from LAP. SMCs also possess TGFReceptors which are coupled to the canonical SMAD2 intracellular pathway, involving SMAD2 phosphorylation and nuclear translocation, inducing expression of numerous genes (genes coding for ECM, antiproteases, LTBP, etc). The majority of these cascade proteins present several isoforms. Canonical TGF-β extra- and intracellular pathways are physiologically involved in the protection and/or restoration of the mechano-protective function of ECM during proteolytic injury and degradation. Because of the cyclic haemodynamic stretching of the aortic ECM during the cardiac cycle, and the contractile tone of the aortic vSMCs, TGF-β release by the aortic ECM is physiologically stimulated, as a guardian of aortic ECM integrity. When ECM disruption and/or proteolytic injury, and/or specific defects in ECM interactions occur, the balance between latent, ECM-retained forms of TGF-β and active form is altered in favour of the latter, as in TAA (*Figure 4*).

For instance, recent studies[76,77] have explored this molecular diversity in the context of Marfan models. A first study of the New York Marfan group, using a fibrillin-1- deficient model,[78] explored the specificity of LTBP isoform binding to microfibrils, and demonstrated that LTBP-1 mainly binds to fibronectin, whereas LTBP-2 and -3 mainly bind to fibrillin.[76] In a second study, the same group demonstrated, in the same model, that FBN mutations disrupt the interaction of LTBP-3 with fibrillin, causing aneurysm formation, elastin degradation, and lethal dissections, whereas genetic deletion of LTBP-3 rescues this morbid phenotype. Of note, in this model, fibrillin deficiency is associated with experimental TAAD but not with a defect in elastin maturation.[78] These important studies, limited to Marfan mice, but not extended to TAAD, illustrate the complexity introduced by molecular isoforms in the genetics as well as pathophysiology, and the potential functional overlap of different molecules, and the necessary condition of proteolysis to produce elastin breakdown.

In our first study on the tissue TGF-β pathway in human healthy aorta and in TAA, we observed an increased TGF-β1 storage in TAA within the ECM without changes in TGF-β1synthesis, but an increase in LTBP synthesis.[79] In healthy ascending aorta as in TAA, TGF-β accumulates in

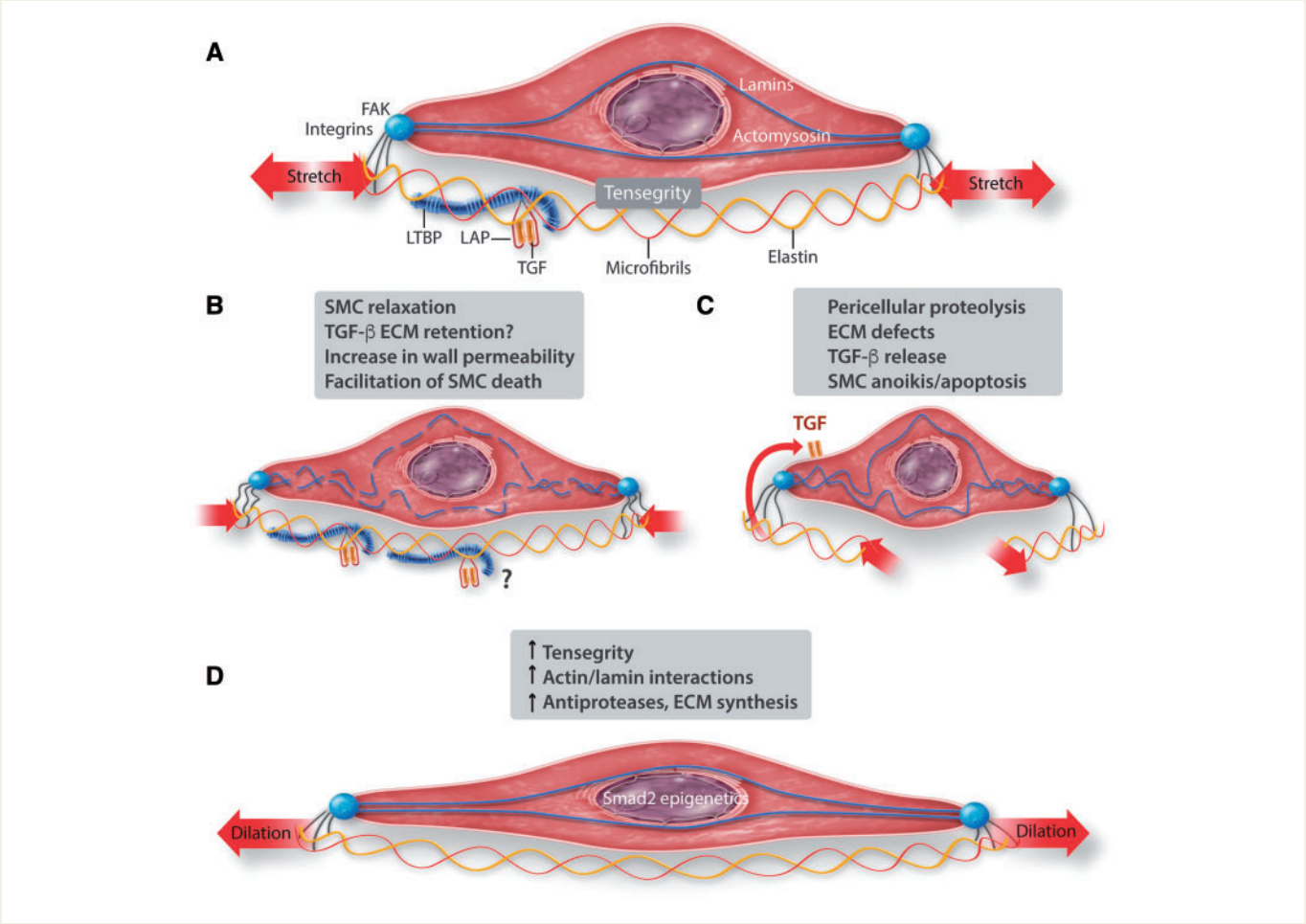

Figure 4 Different impacts of vSMC tensegrity on the main molecular components of TAA and AAD pathophysiology (ECM, TGF-β canonical pathway, SMC cytoskeletton and nucleoskeletton: *(A)* physiological tensegrity involving intracellular components and interactions within SMC, and ECM submitted to cyclic hemodynamic stretching; *(B)* impact of SMC relaxation on tensegrity and consequences. The behaviour of TGF-β in this context remains to be defined (**?**); *(C)* Defect in ECM (enzymatic, genetic, and pharmacologic) released active TGF-β; *(D)* Progressive dilation increases stretching and induces chromatin remodelling via tensegrity-induced cyto/nucleoskeletton more interactions.

the external third of the media, with a gradient similar to that of vSMC myosin where the concentration is greatest in proximity to the adventitial sympathetic contractile stimuli.

In this context, it has been demonstrated that TGF-β overexpression is protective, limiting aneurysmal progression in rats.[57] More recently, injections of TGF-β-neutralizing antibodies promoted aortic dissections and death in response to AngioII infusion in mice.[38] These early results have now been confirmed by different groups.[80–82] These repeated experimental observations, associated with clinical observations and therapeutic assays, lead to the conclusion that strategies aimed at inhibiting canonical TGF-β-dependent signalling are unlikely to provide any benefit to patients with TAADs[58].

6.2 Epigenetic adaptation of vSMCs in TAA

6.2.1 Epigenetic modifications

Epigenetic modifications can be defined as the introduction of new stable heritable traits independently of changes in the DNA sequence. In many cardiovascular disorders, significant epigenetic modifications have been shown to affect disease development or progression. Epigenetic modifications encompass different mechanisms: modifications of DNA-associated histones, DNA methylation and non-coding RNA-mediated modifications. These mechanisms target DNA molecules, transcriptional machinery or transcription products, resulting in modulation of gene expression and consequent protein synthesis.

6.2.2 Histones

An epigenetically-determined constitutive overexpression of active Smad2 in vSMCs was identified in TAAs.[83] By CHIP (CHromatin Immuno Precipitation) assays on human tissues and cells, it was shown that this constitutive overexpression involved the use a Smad2 alternative promoter that was related to the acetylation of histone, the recruitment of histone acetyl transferase, a shift of Myc repressor transcription factor to P53 activator, where the TRRAP co-factor facilitated the formation of this new molecular complex.[84] The epigenetic nature of this phenotypic change was confirmed by the demonstration that this phenotype (constitutive overexpression of Smad2 as compared with vSMCs derived from healthy aorta) is conserved, in primary culture of vSMCs derived from TAA tissue, throughout successive passages. Moreover, vSMCs derived

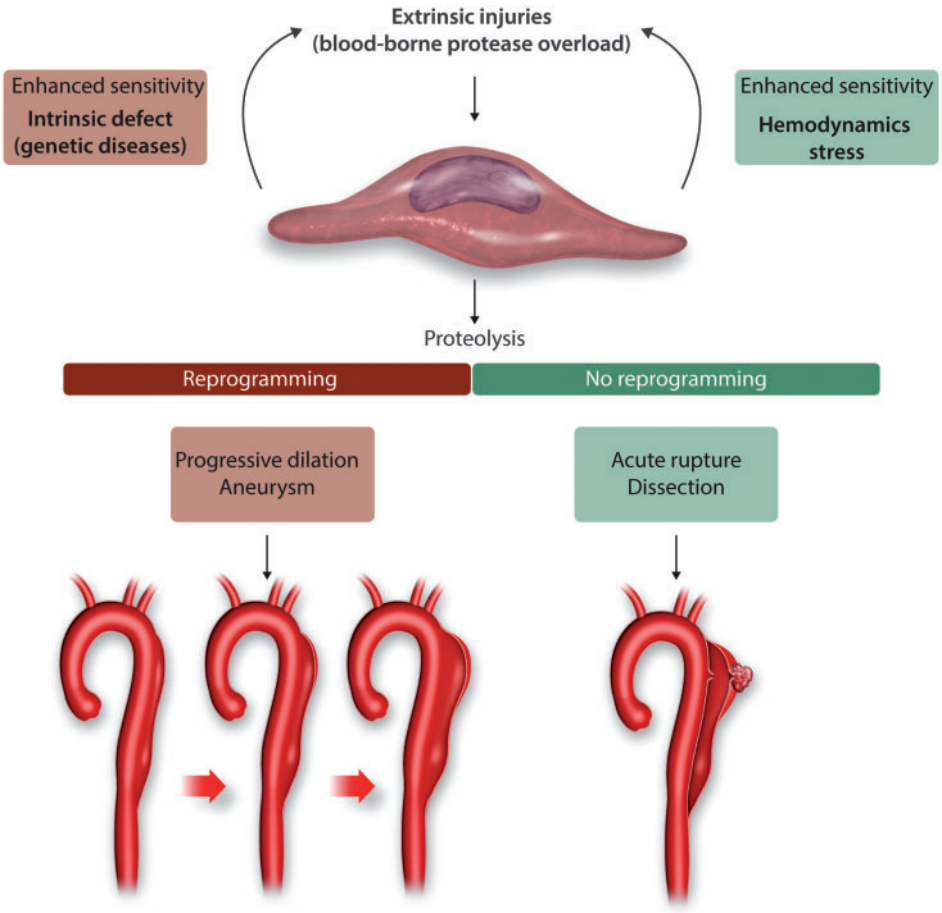

Figure 5 Schematic representation of the pathophysiological determinants of TAA and AAD and the central role of vSMCs.

from TAA no longer responded to exogenous TGF-β1, whereas those derived from healthy aorta did. In parallel, TAA-derived vSMCs are more resistant to plasminogen-induced anoikis than healthy aorta-derived cells. This chromatin remodelling is potentially related to the modifications in mechanical strain which occur in TAAs. Indeed, the chronic dilation characteristic of TAAs is responsible for an increase in the wall tension according to Laplace's law. The nuclear envelope is mechanically coupled to other vectors of mechanotransduction (tensegrity) via vSMC adhesion to the ECM, coupling of integrins to intracellular actin and linkers of nucleoskeleton to cytoskeleton.[85,86] It has been proposed that the mechanical environment impacts the chromatin state of vSMCs thereby controlling vascular gene expression and function.[87] It is likely that the increased wall tension in TAAs modifying mechanotransduction between matrix and nuclei will have an impact on the chromatin remodelling in vSMCs, although this issue has never been directly addressed. Interestingly, this chromatin remodelling is observed only during progressive chronic dilation, i.e. a progressive increase in wall tension, but not during acute intramural rupture.[88] Thereby chromatin remodelling in vSMCs may be a hallmark of TAAs as compared with aortic dissection.

6.2.3 DNA methylation

This is carried out by DNA methyl transferase which covalently, but reversibly, binds a methyl group to the DNA base cytosine, which limits the transcription factor accessibility to the methylated sequence. Therefore, DNA methylation usually leads to repression of gene transcription. One study reports identification of abnormal DNA methylation in vSMCs from TAAs associated with BAV,[89] but not in those from TAAs associated with TAV. No vSMCs from healthy aorta were studied here.

6.2.4 Non-coding RNAs

Non-coding RNAs are defined as RNA molecules lacking protein-coding potential. They are generally classified according to their size: small non-coding RNAs contain <200 nucleotides (miRNAs) while long non-coding RNAs contain at least 200 base pairs. These epigenetic aspects are more well-documented than DNA methylation. Different studies underline the impact of miRNA-29a and b on aneurysms in general, and more particularly on non-syndromic and Marfan TAA and dissections. The non-coding RNAs and their impact on vSMCs are reviewed in this issue (Leeper N.J. and Maegdefessel L.).

6.3 Increase in antiprotease secretion by vSMCs

The canonical TGF-β/Smad 2 pathway controls the expression of numerous genes, including genes responsible for ECM synthesis and maturation. It can be triggered either by TGF-β1 release from its ECM

storage site and/or by the epigenetic overexpression and nuclear translocation of Smad2. Connective Tissue Growth Factor gene is the archetypal gene controlled by TGF/Smad2 pathway directly involved in ECM synthesis and modelling.[90] We observed an increase in fibronectin turnover and LTBP accumulation in TAA as compared with healthy human aorta. Because we were interested in plasminogen activation by vSMCs in TAA, we studied the impact of the constitutive overexpression of Smad2 on antiprotease synthesis and accumulation. We previously showed that PN-1 a tissue inhibitor of thrombin, plasmin, t-PA and u-PA, is highly expressed by vSMCs in response to TGF-β1.[91] We also observed a high concentration of PN-1 in TAA media as compared with healthy aorta. We demonstrated that this PN-1 enrichment is under the control of Smad2 both in aortic tissue and in cultured vSMCs. By CHIPS assay we observed a highly significant increase in Smad2 binding to the PN-1 promoter, leading to overexpression and accumulation of the protein. Similar results, but less specific of Smad2, were reported for the PAI-1 promotor. These epigenetic modifications make TAA-derived cultured vSMCs more resistant to plasminogen-induced anoikis[62] than healthy aorta-derived cells.

6.4 vSMCs clear protease/antiprotease complexes

It is well accepted that vSMCs in the arterial wall are able to ingest macromolecules by endocytosis,[92,93] via scavenger receptors, and to engulf particles[94] and cells by phagocytosis.[95] LRP-1 (LDL receptor-related protein-1) is a major scavenger receptor for modified LDL in vSMCs,[92] but also an endocytic receptor for protease/antiprotease complexes.[96] We recently observed that circulating plasminogen, which had entered the aortic wall and had been converted into plasmin on vSMC contact, can bind to tissue PN-1 present in the pericellular GAG environment. Plasmin/PN-1 complexes can then be endocytosed by vSMCs in an LRP-1 dependent manner, whereas plasmin alone cannot.[5] LRP-1 is also able to engulf MMP/TIMP complexes,[97] so that this system may be involved the *in situ* clearance of protease/antiprotease complexes by vSMCs. Therefore, this clearance function of vSMCs, which is dependent on the *in situ* presence of antiproteases, may limit proteolytic injury within the aortic wall. Also, in keeping with this hypothesis, a decrease in LRP-1 expressed by vSMCs in genetically modified mice leads to aortic aneurysm formation.[98,99] In parallel, a mutation on the LRP-1 gene has been recently published in a chinese Marfan-like family[100] and an LRP-1 variant has been associated with AAA in humans.[101]

6.5 vSMCs induce inward neo-angiogenesis

Neo-angiogenesis from the adventitia to the medial layer is another consequence of both outward convection of mediators through the aortic wall, and the avascular nature of the aortic tissue. In the ascending aorta, only the external quarter of the media is vascularized, i.e. contains arterioles, capillaries and venules. However, there are no lymphatics in the medial layer. This external physiological vascularisation arises from the adventitia and is relatively scarce in normal aorta, but can extend across the full medial thickness in TAA in humans. As we have demonstrated in initial human aortic atheroma,[102] this inward neo-angiogenesis, generated by sprouting of endothelial cells from the adventitia or external media, is related to the outward convection of angiogenic mediators synthesized and secreted by vSMCs. In contrast to atheroma,[102] the inward neo-angiogenesis present in TAA is not related to lipid mediators or VEGF overexpression and secretion by vSMCs. Similarly, the markers of hypoxia, specifically HIF and sirtuine-1, are not altered in TAA when compared with control aorta. In contrast other angiogenic mediators are increased in the media of TAA when compared with healthy aorta[60] (angiopetin-1 and -2 (proteins and mRNA), thrombospodin-1 (TSP-1) and -2, platelet-derived endothelial growth factor and IGFBP-1. Although TSP-1 and angiopoietin-1 overexpression is clear, the stimuli have not yet been determined. Neo-angiogenesis seems to be more pronounced in degenerative and Marfan TAA, whereas, in degenerative forms, a patchy intimal proliferation of SMCs is observed.

7. Aneurysms vs. dissections

Similar activation of plasminogen and the presence of areas of mucoid degeneration are observed in both chronic dilation (TAA) and acute dissection. However, in the aorta after dissection no epigenetic overexpression of Smad2 is observed, which limits the activation of TGF-β pathway and the ability of vSMCs to prevent acute intramural rupture (PN1 secretion. . .) is thus limited[88] (*Figure 5*).

8. Conclusions

As described earlier, TAA in humans is a model for the interaction between vSMCs and outwardly-convected plasma components. Syndromic or non-syndromic monogenic diseases secondary to mutations in a gene altering the contractile apparatus of the vSMC, an ECM protein, or a protein of the canonical TGF-β signalling pathway have the potential to sensitize the aortic wall to plasma-borne, proteolytic injury.[4] Disruption of the ECM or TGF-β signalling or an excess of vSMC relaxation in the aorta may lead to aneurysm formation involving the aortic root. As first illustrated by Leonardo da Vinci, the sinuses of Valsalva are the site of physiological vortexing of blood during diastole, in relation to the closing of the aortic valve, blood stagnation in the aorta, and coronary inflow. This blood stagnation and vortexes could potentially enhance outward hydraulic conductance of plasma components through the aortic wall.

In contrast, in TAA associated with BAVs, haemodynamic modifications may promote aneurysmal development on the outer curvature of the ascending aorta. Morphological ovalization of the aortic ring associated with BAVs alters the blood flow patterns in the ascending aorta, creating a hot spot of velocity vector dispersion (Tranverse Wall Shear Stress[103]) and mechanical impedance on the convex side of the ascending aorta.

vSMCs, as the main mesenchymal cells of the aortic wall, play a central role in the genesis and evolution of TAA and D, involving different vSMC functions and different extracellular and intracellular signalling pathways within the aortic wall. vSMC defects may be pathogenic in TAA and D, but TGF-β activation and vSMC responses to aneurysmal injury may also represent repair mechanisms. Despite important progress in understanding TAA & D pathophysiology, some interactions between vSMC physiology and pathology, remain to be further explored. This may be crucial for the development of new therapeutic approaches, potentially involving other antihypertensive compounds associated with higher sympathetic activity, blockade of plasmin generation, and specific inhibition of iNOS signalling, for preventing the development of aneurysms of the ascending aorta.

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