ESC European Society of Cardiology
European Heart Journal (2023) **44**, 1262–1264
https://doi.org/10.1093/eurheartj/ehac771

EDITORIAL

The emerging role of endothelial cells in the pathogenesis of thoracic aortic aneurysm and dissection

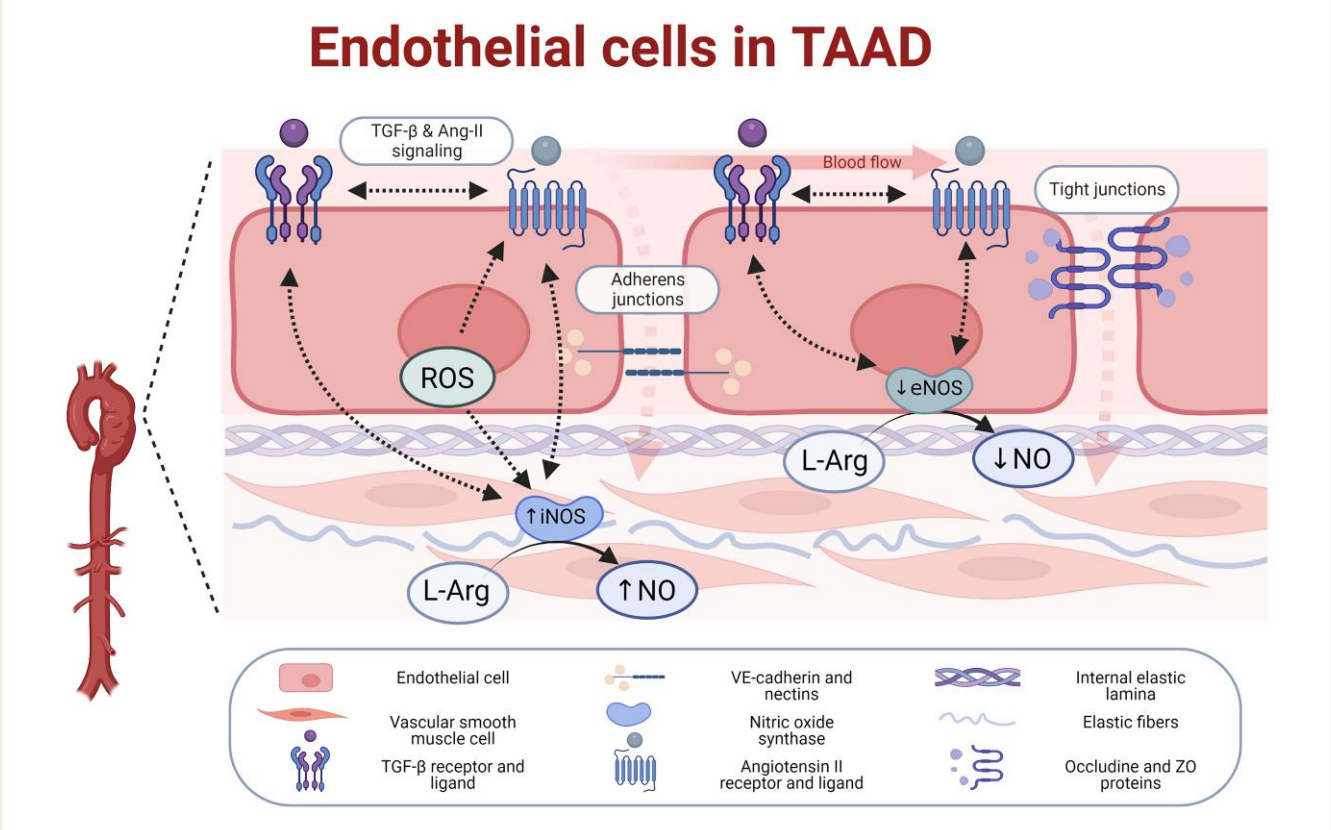
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Online publish-ahead-of-print 18 January 2023

This editorial refers to 'Targeting endothelial tight junctions to predict and protect thoracic aortic aneurysm and dissection', by X. Yang *et al.*, https://doi.org/10.1093/eurheartj/ehac823.

Graphical Abstract



Schematic overview of the different important endothelial signaling pathways and molecular processes involved in the pathogenesis of thoracic aortic aneurysm and dissection

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.
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Thoracic aortic aneurysm (TAA), leading to acute thoracic aortic aneurysm dissection (TAAD), is a life-threatening condition for which no curative medical therapy is available yet. TAA(D) can present as a non-syndromic or syndromic condition (e.g. Marfan syndrome or Loeys-Dietz syndrome), with syndromic forms being more rare but displaying TAAD at relatively young ages and small aortic diameters. Histologically, TAA tissue samples are characterized by medial degeneration, which predisposes the aortic wall to TAAD, i.e. a disruption of the intima that leads to separation of the media.

Each of the aorta's three tissue layer has its characteristic components. In the media, vascular smooth muscle cells (VSMCs) are the predominant cell type, whereas in the adventitia fibroblasts are assigned a prominent role. The intima, on the other hand, is composed of a single layer of endothelial cells (ECs). Although these three cell types are considered the key structural and functional constituents of the aortic wall, recent single-cell RNA sequencing (scRNAseq) experiments have pinpointed mesenchymal stem cells, pericytes, and immune cells as additional players in both normal and aneurysmal aortic walls.[1]

Historically, VSMCs have been considered the most important cell type in the pathogenesis of TAA(D). Dysregulation of the so-called phenotypic switch between the contractile and synthetic state of these VSMCs is hypothesized to be a key process in aortic aneurysm formation. The following lines of evidence support this hypothesis: (i) pathogenic variants in genes encoding components (e.g. *ACTA2*, *MYH11*) or regulators (e.g. *PRKG1*, *MYLK*) of the contractile apparatus are well-established causes of non-syndromic hereditary forms of TAA(D);[2] (ii) recent scRNAseq experiments in diseased aortas of Marfan mice and patients (with Marfan referring to a syndromic form of TAA(D) that is caused by pathogenic variants in the extracellular matrix protein fibrillin-1) revealed the presence of disease-specific VSMC clusters;[3,4] (iii) extracellular matrix deficiency and VSMC dysfunction trigger altered transforming growth factor-beta (TGF-β) signalling — moreover, pathogenic loss-of-function variants in the genes coding for the ligands (*TGFB2/3*), the receptors (*TGFBR1/2*), and downstream effectors (*SMAD2/3*), which underlie a syndromic form of TAA(D) called Loeys-Dietz syndrome, themselves cause VSMC dysfunction; and (iv) VSMCs of different embryonic origins contribute to a different extent to the pathogenesis of TAA(D). For example, in an Loeys-Dietz syndrome *Tgfbr1*-knock in mouse model, it was demonstrated that secondary heart field-derived, but not their neighbouring cardiac neural crest-derived VSMCs, showed impaired Smad2/3 activation in response to TGF-β.[5]

In this issue of the *European Heart Journal*, Yang *et al.* demonstrate that tight junction dysfunction plays a role in the pathogenesis of TAAD.[6] In the aortic intima, ECs are connected to each other by both adherens junctions, composed of VE-cadherin and nectins, and tight junctions, comprising >40 transmembrane proteins [e.g. claudins, occluding and junctional adhesion molecules (JAMs)] or cytoplasmic actin-binding proteins [e.g. zona occludens (ZO) proteins]. Although Yang *et al.* are the first to establish a role for tight junction abnormalities in the aetiology of TAAD, dysfunction of EC-related processes has been linked to TAA(D) before.

First, limited genetic evidence implies endothelial dysfunction as causal in aortic and arterial aneurysm/dissection. Pathogenic variants in *ROBO4*, encoding a transmembrane receptor, predispose individuals to bicuspid aortic valve and TAA.[7] Endogenous ROBO4 silencing or mutant overexpression in EC lines results in impaired barrier function and a synthetic cell repertoire suggestive of endothelial to mesenchymal transition. *SMAD6* loss-of-function variants are another cause of bicuspid aortic valve-related TAA.[8] ECs depleted for SMAD6 present with defective barrier function in addition to down-regulation of junction-related genes.[9] A rare variant in *F11R*, coding for the key tight junction component JAM-1, has been identified in a patient with spontaneous coronary artery dissection.[10]

Second, evidence for dysregulation of endothelial nitric oxide (NO) signalling in TAA(D) has emerged. In Marfan mice, Chung *et al.* (2007) demonstrated that impaired endothelium-dependent relaxation was mediated by down-regulation of eNOS/Akt signalling-induced NO production.[11] Additionally, Oller *et al.* (2017) showed that pharmacological inhibition of iNos (Nos2; nitric oxide synthase) rapidly reversed aortic dilation and medial degeneration in the same Marfan mice.[12]

Third, oxidative stress-driven endothelial dysfunction has also been suggested to be involved in TAAD development. Fan *et al.* (2014) showed that reactive oxygen species (ROS)-dependent cyclophilin A (CypA) secretion by endothelial cells determines the susceptibility of the aortic wall to Angiotensin II (Ang II)-mediated aortic dissection.[13] Another group added a specific role for endothelial tetrahydrobiopterin in modulating the haemodynamic and structural changes in Ang II-induced aortic aneurysm formation.[14] Building on these prior observations, Pan *et al.* added further evidence for a role for NO signalling in TAAD development by showing that vascular endothelial barrier dysfunction is counteracted by either selectively inhibiting iNOS activity or denitrosylating the endothelial PLS3 protein (plastin-3).[15] In addition, in Marfan mice, ROS increase aortic wall susceptibility to Ang II-mediated dissection.[16]

Fourth, dysregulation of Ang-II or TGF-β signalling, two pathways with proven involvement in the aetiology of TAA(D), was shown to affect not only VSMCs but also ECs. It should be noted that both the Ang-II and TGF-β signalling pathways intertwine with the NO signalling cascade. For example, death from ruptured TAAD was significantly delayed in Marfan mice with EC-specific but not VSMC-specific At1r inactivation, supporting the notion that losartan (an Ang-II receptor type 1 blocker) mitigates arterial pathology largely by recalibrating the balance of endothelium-derived regulatory signals of vascular tone.[17] Conversely, VSMC-specific disruption of the type II TGF-β receptor gene (*Tgfbr2*) in newborn wild-type mice has been shown to result in endothelial dysfunction associated with NO deficiency and aortic hypercontractility, highlighting the existence of TGF-β-dependent regulatory signals originating from medial VSMCs and instructing intimal EC function.[18]

In this study by Yang *et al*., the initial hypothesis on disturbed tight junction functioning is derived from scRNAseq data of aortic wall samples of four Stanford type A aortic dissection patients (three male, one female; median age 51 years) who underwent total arch replacement and stent implantation. No details are provided on the nature (syndromic or non-syndromic; sporadic or familial) of these TAAD patients. It would be interesting to investigate if the observed tight junction signature is also observed in non-dissected aneurysmal samples, which would shed light on the role of tight junction dysfunction in earlier disease stages.

The authors found that aberrant tight junction expression and function precedes aortic dissection in a beta-aminoproprionitrile (BAPN)-induced TAAD mouse model.[6] Chronic oral (via drinking water) BAPN administration to wild-type mice leads to irreversible inhibition of lysyl oxidase (LOX), impairing cross-linking of lysine residues in collagen and elastin. Extracellular matrix dysfunction is believed to be the primary cause of TAAD in young BAPN-treated mice (~3 weeks old), but not older mice. Although this inducible mouse model is widely used to study TAAD, the immediate first question that arises queries if tight junction dysfunction is also key in other TAA(D) mouse models,

such as the widely used Marfan mouse models, in which TAAD is preceded by prominent aortic aneurysm formation. The current study also provides an interesting, potentially translatable, imaging tool, allowing TAAD prediction. Two novel magnetic resonance imaging (MRI) probes (FITC-dextran-DOTA-Gd and rhodamine B-dextran-DOTA-Gd) were shown to accumulate in the aortic walls of affected BAPN-fed mice and increase of their fluorescent signals (between day 5 and 10) preceded and forecasted TAAD. As no good pre-symptomatic biomarkers for TAAD are currently available, exploration of the application of these probes in the clinical setting is very tempting.

The authors claim that disturbed endothelial tight junction expression/function is not just a marker of TAAD, but a true disease cause. This statement is largely based on two observations (i) administration of AT-1001 (also known as larazotide acetate, a protease-activated receptor-2 (PAR2) inhibitor which seals tight junctions) significantly reduced the BAPN-induced incidence of TAAD and (ii) an EC-specific conditional knockout of ZO-1 (encoded by *Tjp1*) leads to significantly larger aortic diameters and higher TAAD incidence upon BAPN challenge. Although potentially interesting as a potential therapeutic strategy, one should note that the intraperitoneal injection of AT-1001 was started before the BAPN administration. As such, it remains to be proven that its therapeutic efficacy remains when treatment would be started in an already aneurysmatic or in a TAAD-pre-disposed aorta. One could consider testing a higher AT-1001 dose if treatment is initiated in more advanced disease stages. Alternatively, it would be revealing to test this drug in association with other compounds, such as losartan, which has haemodynamic and TGF-β-neutralizing effects. Second, it is remarkable that the incidence of TAAD in BAPN-treated *Tjp1*$^{flox/flox}$ mice is only 4 out of 12 mice after 5 weeks of treatment. It remains unexplained whether that is caused by the somewhat older age at which the BAPN provocation is started and why it took 5 weeks (compared with the 18 days in the initial experiments). Finally, the authors suggest that the endothelial tight junction damage is caused by vascular inflammation, more particularly by infiltration of neutrophils and monocytes. It will be important to determine whether this observation is specific to the BAPN-dissection model or can be extrapolated to other TAA(D) models. So far, the evidence for the driving role of inflammation in human TAA development is quite limited.

Funding

All authors declare no funding for this contribution.

Conflict of interest: None declared.

Data availability

There are no new data associated with this article.

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