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Review Article

The molecular mechanism of mechanotransduction in vascular homeostasis and disease

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Blood vessels are constantly exposed to mechanical stimuli such as shear stress due to flow and pulsatile stretch. The extracellular matrix maintains the structural integrity of the vessel wall and coordinates with a dynamic mechanical environment to provide cues to initiate intracellular signaling pathway(s), thereby changing cellular behaviors and functions. However, the precise role of matrix–cell interactions involved in mechanotransduction during vascular homeostasis and disease development remains to be fully determined. In this review, we introduce hemodynamics forces in blood vessels and the initial sensors of mechanical stimuli, including cell–cell junctional molecules, G-protein-coupled receptors (GPCRs), multiple ion channels, and a variety of small GTPases. We then highlight the molecular mechanotransduction events in the vessel wall triggered by laminar shear stress (LSS) and disturbed shear stress (DSS) on vascular endothelial cells (ECs), and cyclic stretch in ECs and vascular smooth muscle cells (SMCs)—both of which activate several key transcription factors. Finally, we provide a recent overview of matrix–cell interactions and mechanotransduction centered on fibronectin in ECs and thrombospondin-1 in SMCs. The results of this review suggest that abnormal mechanical cues or altered responses to mechanical stimuli in EC and SMCs serve as the molecular basis of vascular diseases such as atherosclerosis, hypertension and aortic aneurysms. Collecting evidence and advancing knowledge on the mechanotransduction in the vessel wall can lead to a new direction of therapeutic interventions for vascular diseases.

Introduction

Deciphering the role of hemodynamic forces in homeostasis and disease development is a fundamental aspect of cardiovascular biology [1–3]. Blood flow generates frictional shear stress (parallel to the vessel wall) and acts on the vascular endothelial cells (hereafter referred to as ECs). Shear stress can be categorized as laminar shear stress (LSS; uniform and smooth flow) and disturbed shear stress (DSS; turbulent or oscillatory flow). Pulsatile pressure originating from cyclic cardiac pumping generates circumferential, axial and radial stresses, and stretches in the vessel wall that acts on ECs and vascular smooth muscle cells (hereafter referred to as SMCs). Hydrostatic pressure is generated by the pressure exerted by the fluid. Notably, this pressure is a critical determinant of fluid distribution across the semi-permeable capillaries and serves a crucial role in microcirculation. Since the present review focuses primarily on hemodynamic forces in large arteries, readers should refer to other reviews for information on hydrostatic pressure and microcirculation [4,5]).

Mechanotransduction is a process in which mechanical stimuli are sensed by cells and converted into biochemical signals to elicit various cellular functions, including changes in cell shape, migration, proliferation and transcriptional regulation (reviewed in [6,7]). The alteration of mechanical stimuli and resultant changes in intracellular signaling in ECs and SMCs are involved in various vascular diseases,

including atherosclerosis, hypertension and aortic aneurysms [2,8]. Arterial stiffness is highly predictive of major adverse events in vascular diseases and modulates mechanotransduction [9,10]. Altering the quantity and quality of the extracellular matrix (ECM) contributes to the overall stiffness of blood vessels and affects the responses of ECs and SMCs to hemodynamics forces. ECs control SMC relaxation and contraction through paracrine mediators such as nitric oxide (NO), calcium ions (Ca^{2+}), oxidative stress, angiotensin II (Ang II) and endothelin under hemodynamic forces [11–13]. SMCs regulate vascular tone as well as matrix synthesis and decomposition, thereby remodeling the vessel wall and maintaining its integrity and elasticity.

The ECM initiates mechanical cues and propagates intracellular signaling by interacting with cells. Recent studies have also proposed that ECM coordinates with hemodynamic forces and initiates signaling to activate mechanosensitive transcription factors [14,15]. Matrix–cell interactions are mediated by integrins and integrin-mediated focal adhesion (FA) molecules such as talin, focal adhesion kinase (FAK), paxillin, and vinculin, which form a mechanotransduction complex connecting the matrix, integrins and actin cytoskeleton that transduces signals to the nucleus (reviewed in [16,17]). Integrins are heterodimeric cell surface receptors composed of α- (18 types) and β- (8 types) subunits that serve as matrix receptors. Integrins can be activated by intracellular signaling that induces talin recruitment to the cytoplasmic domain of β integrin (inside-out signals) or by matrix ligand binding that induces clustering of the activated form of integrins via actin cytoskeleton-generated mechanical forces (outside-in signals) [18] (see articles [19–21] for a more comprehensive review on integrins).

In this review, we first introduce the hemodynamic forces, biomechanics of the vessel wall and arterial stiffness, then highlight molecular events in mechanotransduction triggered by flow-induced shear stress (FSS) and cyclic stretch in ECs and SMCs. We discuss the initial sensors of mechanical stimuli within the vascular cells, including endothelial junctional molecules, G-protein–coupled receptors (GPCRs), multiple ion channels and a variety of signaling molecules such as small GTPases and their effectors. We also present several key transcription factors activated by mechanical stimuli, such as Kruppel-like factor 2 (KLF2), nuclear factor κB (NF-κB) and Yes-associated protein (YAP). A summary of recent developments in 'matrix mechanotransduction' centered on fibronectin in ECs and thrombospondin-1 (Thbs1) in SMCs is also presented, while insights into vascular remodeling and disease development are provided with a focus on atherosclerosis and aortic aneurysms.

Biomechanics in the vessel wall
Hemodynamic forces
In addition to creating FSS that acts on ECs, blood flow also creates circumferential, axial and radial stresses and stretches on both ECs and SMCs in response to changes in transmural pressure (Figure 1A). The FSS can be calculated as follows [22,23] (eqn 1):

$$\tau_w = \frac{4\mu Q}{\pi r^3} \text{ or } \tau_w = \mu \frac{\Delta u}{\Delta y} \tag{1}$$

where τ_w is the wall shear stress, μ is the blood viscosity, Q is the mean volumetric flow rate, π is the ratio of the circumference of a circle to its diameter, r is the radius of the lumen, and $\Delta u/\Delta y$ is the velocity gradient. For example, in the arterial stenosis area, the same blood volume (volumetric flow rate; Q) is pushed through a narrow region, resulting in increased blood velocity. Consequently, the wall shear stress (τ_w) increases inside the stenotic area. Therefore, decreasing vessel diameter significantly influences FSS.

Laminar flow (smooth and steady) is prominent in the straight regions of large arteries, small arteries, small veins and capillaries. In contrast, disturbed flow (turbulent or oscillatory flow) occurs in curves and bifurcation regions or more severe irregularities associated with pathological conditions (Figure 1B). A transition from laminar flow to turbulent flow is characterized by the Reynolds number (R_e) (eqn 2):

$$Re = \frac{\rho u 2r}{\mu} \tag{2}$$

where ρ is the blood density, u is the flow speed, r is the radius of the lumen, and μ is the blood viscosity. A Reynolds number greater than 2000 is defined as turbulent flow.

Circumferential stress (σ_θ) is due to the wall tension caused by distending blood pressure that changes the circumference cyclically. Axial stress (σ_z) is caused by force in the direction of the long axis, and radial stress (σ_r) is directly related to blood pressure. These stresses can be measured by using Laplace's law formula (eqns 3-5):

$$\sigma\theta = \frac{Pr}{w} \tag{3}$$
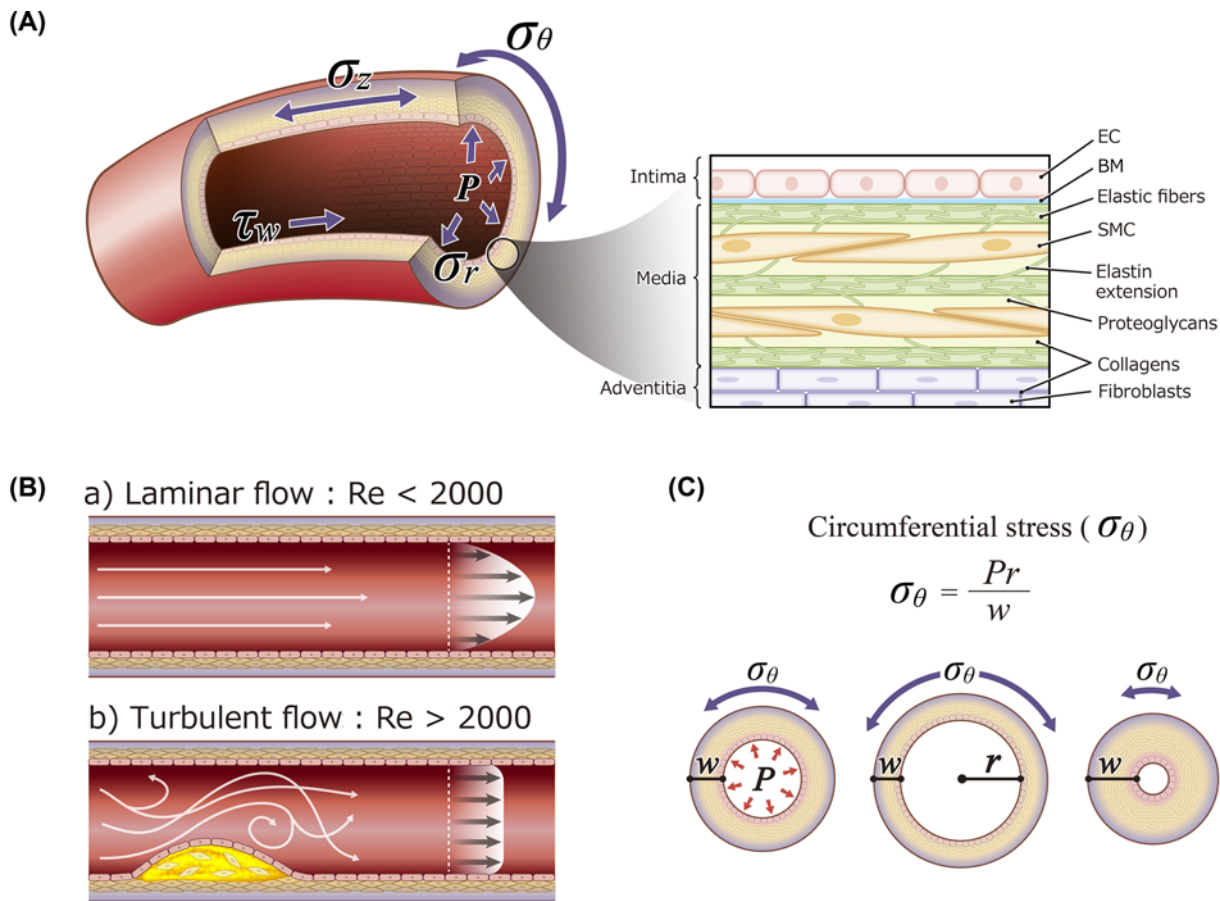
Figure 1. Hemodynamic forces and biomechanics in the vessel wall

(**A**) A section of an artery wall. ECs form the inner layer and align longitudinally, while SMCs form the medial layer and align circumferentially. Pressure (*P*) is perpendicular to the vessel wall, which results in circumferential stretching of the vessel wall. Purple arrows indicate shear stress (τ_w), circumferential stress (σ_θ), axial stress (σ_z) and radial stress (σ_r). A cross-section of the aorta is shown on the right. The aortic wall comprises three layers: (1) the intima includes endothelial cells (ECs) and the basement membrane (BM), (2) the media includes multiple layers of smooth muscle cells (SMCs) and elastic fibers, and (3) the adventitia predominantly includes fibroblasts. (**B**) Schematic figure illustrating the characteristics of flow patterns (white arrows) and blood velocity gradient (black arrow lines): (a) laminar flow is a smooth stream defined by having a Reynolds number (R_e) < 2000, and (b) turbulent flow is defined by R_e > 2000. (**C**) Circumferential stress is directly proportional to blood pressure (*P*) and the inner radius (*r*) and inversely proportional to wall thickness (*w*).

$$\sigma z = \frac{f}{\pi w (2r + w)} \tag{4}$$

$$\sigma r = -\frac{P}{2} \tag{5}$$

where *P* is the transmural pressure, *r* is the radius of the lumen, *w* is the wall thickness and *f* is the axial force. This law states that for a given transmural pressure (*P*), the wall tension (σ_θ) is proportional to the radius of the vessels (*r*). Thus, large thin-walled vessels have high circumferential stress and small thick-walled vessels have low circumferential stress (Figure 1C).

Average wall shear stress (τ_w) in the human aorta varies from 30–60 dynes/cm^2 (1 Pa = 1 N/m^2 = 10 dyne/cm^2) and the mean radial stress (σ_r) is 1.5 Pa. On the other hand, circumferential stress (σ_θ) and axial stress (σ_z) vary from 1 to 2 × 10^6 dynes/cm^2; 100–200 kPa [24,25] (Table 1 [26–28]). Thus, the shear stress (τ_w) and radial stress (σ_r) are smaller in amplitude (1–6 Pa) when compared with axial (σ_z) and circumferential stress (σ_θ) stresses (100 kPa).

Table 1 Flow characteristics in human vessels

Vessel	Diameter (mm)	Average flow velocity (mm/s)	Average shear stress (dynes/cm^2)	Average Reynolds number (R_e)
Ascending aorta	25–30	630	10–60	3600
Descending aorta	20–25	270	30–100	1500
Arteries	2–6	200–500	30–40	800
Capillaries	0.005–0.01	0.5–1	4–8	0.003
Large vein	20	100–150	5–10	900
Small vein	5–10	150–200	1–10	570

*Adapted and approximate normal values from previous reports [26–28].
The $R_e > 2000$ cut off for the transition from laminar to turbulent flow.

However, all four stresses play a crucial role in the activation of mechanotransduction pathways leading to vessel wall remodeling.

Arterial stiffness

Arterial stiffness refers to decreased compliance of blood vessels and is intimately associated with arterial pressure and hemodynamic responses [11]. Clinically, arterial stiffness can be estimated by the pulse wave velocity (PWV), which is determined by the time taken for the pressure pulse wave to travel from the carotid to the femoral artery. The stiffer or less compliant the arteries are, the faster the pressure pulse wave moves along a vessel. PWV is frequently used as a surrogate marker for vascular diseases such as hypertension, aortic stenosis and atherosclerosis, as well as aging-related arterial stiffening [29]. Recently, the arterial stiffness index has been measured by the finger photo-plethysmography and pulse pressure methods, both of which are two independent vascular aging indices that have been shown to predict cardiovascular diseases and mortality outcomes [30].

The association between blood pressure and arterial stiffness generally suggests that hypertension increases pulsatile wall stress and causes elastin degradation, and subsequently progresses arterial stiffness [31]. The postnatal time course study using mutant mice with reduced levels of elastin, however, shows that changes in mechanical properties such as decreased aortic diameter and compliance precedes systolic blood pressure increase [32]. The mutant mice with normal levels of elastin but containing disorganized elastic fibers also show an increase in systolic blood pressure [33]. Recently, the association between short- to mid-term blood pressure, arterial stiffness and the mechanical properties of the blood vessel wall has been examined in a large cross-sectional study. This study showed that greater systolic blood pressure is significantly associated with increased PWV, increased circumferential wall tension and circumferential wall stress despite increased intima-media thickness [34]. This finding suggests that high blood pressure increases arterial stiffness and circumferential stress while also causing maladaptive remodeling of the vessel wall, which may be an underlying factor in increased vascular disease risk.

At its structural basis, the aortic wall comprises a single layer of EC, multiple layers of SMCs in the medial layer, and an adventitial layer with predominantly composed of fibroblasts (Figure 1A). Elastin, which is a major ECM component and secreted by SMCs, assembles into elastic fibers together with microfibrils and provides elasticity and recoiling (reviewed in [35]). Elastic fibers and SMCs alternately align and are connected by elastin extensions that bind with integrins and the actin cytoskeleton to organize the medial layers of the blood vessel [36]. The vessel wall also contains various matrix components, including collagens, glycosaminoglycans and proteoglycans, among which collagens provide structural integrity and tensile strength to the vessel wall [37]. The loss or fragmentation of elastic fibers, deposition of excess collagen or an increase in collagen cross-linking by non-enzymatic glycation results in stiffening of the aorta (reviewed in [38,39]).

The sensing of matrix stiffness by SMCs has been studied *in vitro* using elastomeric substrates with increasing stiffness. Cells sense matrix stiffness through integrins and form focal adhesions to increase actomyosin contractility. This leads to the conformational change of talin and enhances vinculin binding, thereby stabilizing matrix–integrin interactions [40,41]. Recently, collagen-binding receptor discoidin domain receptor-1 (DDR1) has been reported to sense matrix stiffness and lead to RhoA activation and stress fiber formation [42]. Several *in vitro* studies have shown that changes in individual matrix composition and overall matrix stiffness can alter the phenotypes of SMCs. For example, matrix stiffness induces the contractile-to-synthetic phenotype by down-regulating SMC contractile genes and up-regulating matrix genes [43,44]. The DDR1-mediated signaling promotes transdifferentiation to osteochondrocytic lineage [42]. A recent study has identified 3098 stiffness-sensitive genes through transcriptomic analysis, which

includes 157 long non-coding RNAs in aortic and coronary SMCs [45]. Among them, MALAT1 has been shown to positively regulate SMC proliferation and migration, and its expression is down-regulated in response to matrix stiffness.

Arterial stiffness has also been linked to EC dysfunction in human studies. Higher PWV or lower flow-mediated vasodilation (indicative of EC dysfunction) are significantly associated with cardiovascular events [46–48]. Mechanistically, EC dysfunction induced by arterial stiffness is supported by the observation that ECs cultured on a stiff substrate exhibit increased proliferation, increased permeability and leukocyte extravasation, endothelial-to-mesenchymal transition and decreased NO production [49–52]. A vasoconstrictor peptide (Ang II) triggers oxidative stress, matrix degradation and endothelial nitric oxide synthase (eNOS) inhibition and modulates arterial stiffness [53]. The administration of NOS inhibitor, L-NAME, significantly blocked NO production and increased PWV in rats and rabbits [54,55]. Moreover, Ang II receptor antagonist (ARB) and angiotensin-converting enzyme (ACE) inhibitor decrease blood pressure and improve PWV in humans and mice [56]. Furthermore, a recent study noted that a stiff substrate inhibits the expression of glypican—a core protein of cell surface glycocalyx— thereby decreasing the protection of ECs by glycocalyx [57]. Based on the existing literature, it is evident that arterial stiffness triggers and/or modulates responses to mechanical stimuli at the cellular and tissue level and significantly alters mechanotransduction in the vessel wall.

Mechanotransduction of ECs under flow-induced shear stress (FSS)

Etiology of atherosclerosis and flow-induced shear stress response

ECs are directly exposed to flow forces on the apical side and express various mechanosensors, including ion channels, glycocalyx, primary cilia, and cell receptors that sense and transduce mechanosignals to intracellular signaling pathways [58]. As previously described, FSS mediates two types of shear stresses: LSS from the laminar flow and DSS (turbulent or oscillatory shear stress) from the disturbed flow. Notably, these different flow patterns control distinct EC phenotypes [59]. Generally, high shear stress (mediated by LSS) causes anti-inflammatory and antioxidative stress gene expression, which is known as the anti-atherosclerotic phenotype. In contrast, DSS induces proinflammatory and oxidative stress gene expression, which leads to the formation of atherosclerosis. Thus, atherosclerosis is associated with the region of DSS at the lesser curvature of the ascending aorta and arterial branches (Figure 2). Additionally, different flow patterns affect the alignment of ECs, as cells align in the direction of flow under LSS but not in DSS, while the defective alignment of ECs represents a hallmark of atherosclerosis formation [58]. Studies have revealed that ECs induce the binding of subsets of transcription factors to the shear stress-responsive elements (SSREs) in response to LSS and DSS and promote the expression of several downstream genes involved in inflammation and oxidative stress, which serve a central role in the initiation and progression of atherosclerosis [60,61]. This section first introduces mechanosensors located on the cell surface and cell–cell junctions of ECs and describes their pathophysiological roles with a focus on atherosclerosis. Thereafter, the transcriptional regulation of ECs in response to LSS and DSS is highlighted.

Mechanosensors located at the cell surface and cell–cell junctions in ECs

ECs contain tight junctions, adherens junctions and gap junctions at the lateral side (also called cell–cell junctions). The sensing of FSS has been attributed to different mechanosensors and the mechanosensory complex at adherens junction is well studied. This complex comprises platelet and endothelial cell adhesion molecule 1 (PECAM-1), vascular endothelial (VE)-cadherin, vascular endothelial growth factor receptor 2 (VEGFR2) and VEGFR3 [62,63]. PECAM-1 was initially identified as a mechanosensitive homophilic adhesion protein that was phosphorylated by FSS and mediates endothelial cell response to flow [64,65]. VE-cadherin is an EC-specific adhesion molecule that serves as an adaptor protein to assemble VEGFRs to the mechanosensory complex via its transmembrane domain [63,66]. Notably, the function of VE-cadherin in FSS-induced downstream signaling is independent of cell–cell adhesion [62].

Measurements using fluorescence resonance energy transfer (FRET)-based tension sensors for PECAM-1 and VE-cadherin confirmed that LSS rapidly increases the force on PECAM-1 by inducing the association with vimentin in a myosin-dependent manner. A tension force exerted on PECAM-1 activates a Src family kinase and phosphorylates VEGFR2, thereby activating PI3 kinase and integrin [67]. This observation suggests the possible existence of an upstream mechanosensor that initiates vimentin-PECAM-1 binding and transmits forces to PECAM-1 through cytoskeletal rearrangement. The localized tension force placed on PECAM-1 is sufficient to activate a global signaling
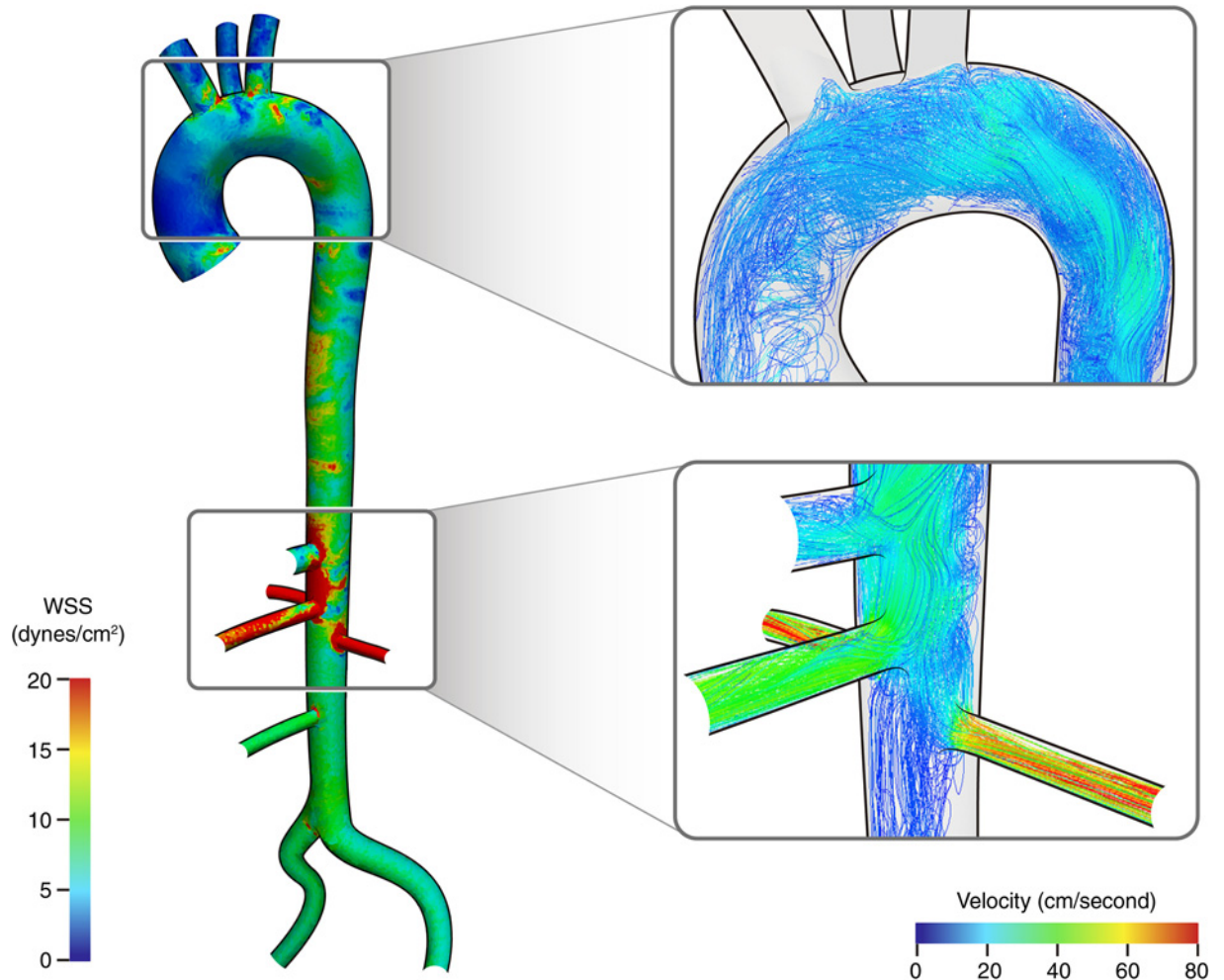
Figure 2. Illustration of disturbed arterial flow
Schematic illustration of calculated flow patterns in an idealized model of the human aorta. Wall shear stress (WSS) immediately after the start of diastole is illustrated on the left. Streamlines in the enlargements indicate the velocity and show the region of disturbed flow at the inner curvature of the aortic arch (top) and the branches of the abdominal aorta (bottom). Images adapted and modified from reference [27] with permission from the Company of Biologists Ltd.

response involving PI3 kinase, integrins, and Rho A, thereby connecting the adherens junctional FSS sensing to cytoskeletal alterations downstream of integrin [68]. In contrast with PECAM-1, LSS decreases tension on VE-cadherin and induces binding between VE-cadherin and VEGFR2 and VEGEFR3, thereby activating shear-mediated signaling [63,67].

A recent study revealed that a small pool of VE-cadherin is phosphorylated on tyrosine Y658 by a Src family kinase and induces the dissociation of p120-catenin, which subsequently binds to the polarity protein LGN and activates NF-κB signaling at the DSS region [69]. PECAM-1 can also be phosphorylated on tyrosine in response to LSS and enhances the association with eNOS. Concordantly, *Pecam-1*-deficient mice show a loss of FSS response and the attenuation of NO production due to the decreased phosphorylation of AKT and eNOS, which may affect the overall response of vessel walls [70]. Interestingly, *Pecam-1*-deficient mice in a hyperlipidemic condition show reduced NF-κB activation and VCAM-1 expression in atheroprone lesions where DSS is dominant [71]. DSS induces the prolonged expression of fibronectin and its increased deposition at the subendothelial layer and activates NF-κB via integrin in a PECAM-1-dependent manner [72]. Thus, the fibronectin-integrin interaction serves a key role in the progression of atheroprone lesions in DSS. These observations highlight the importance of local hemodynamic forces and flow patterns in mechanoresponses downstream of the mechanosensory complex at cell–cell junctions *in vivo*.

EC surface receptors and complexes such as caveolae, glycocalyx, primary cilium, GPCRs, Notch1, Piezo1 and plexins are also identified as mechanosensors that respond to FSS [73–78]. Mechanosensitive ion channels are involved in the earliest cellular event within microseconds [79,80]. Piezo1 was discovered as a mechanically activated cation channel and acts as an FSS sensor [74]. *Piezo1*-deficient mice and EC-specific (under *Tie2Cre*) *Piezo1*-deficient mice exhibit defective vessel remodeling and die at mid-gestation [81,82]. The loss of Piezo1 in ECs leads to impaired calcium (Ca^{2+}) influx and the Ca^{2+}-dependent activation of calpain-2 in response to FSS, resulting in abnormal cell orientation and stress fiber formation. Even in the static condition, the loss of Piezo1 leads to a decrease in the VEGF-dependent phosphorylation of eNOS and impaired EC migration [81,82]. Piezo1 mediates flow-induced ATP release and activates the purinergic receptor $P2Y_2$-$G_{q/11}$ signaling pathway, thereby regulating calcium transients, the phosphorylation of PECAM-1 and VEGFR2, and the activation of eNOS and NO production through the phosphorylation of AKT [83,84].

A more recent study noted that Piezo1 releases adrenomedullin in response to FSS, activates its Gs-coupled receptor and induces the PKA-mediated phosphorylation of eNOS at serine S633, which is distinct from the eNOS phosphorylation mediated by AKT [85]. Interestingly, LSS activates the Piezo1-$G_{q/11}$ pathway and elicits an atheroprotective response, whereas DSS induces a pro-atherogenic response that requires integrin α5 activation and FAK phosphorylation *in vivo* [86]. The ATP-gated P2X4 ion channel (one of seven P2X receptors), also responds to FSS, and increases Ca^{2+} influx, and produces NO [87]. *P2rx4*-deficient mice fail to respond to acute increases in blood flow and induce vessel dilatation [88]. However, it remains unknown whether FSS modulates P2X4 directly or has an upstream mechanosensor.

Among GPCRs, GPR68 has recently been identified as a proton and flow mechanosensor that exhibits FSS-activated calcium transients and is required for FSS responses in peripheral arteries [75]. *Gpr68*-deficient mice exhibit markedly impaired flow-mediated dilation and outward remodeling in mesenteric arteries [75]. Protease-activated receptor 1 (PAR1) has also been identified as a novel mechanosensor that is activated in response to LSS and induces eNOS phosphorylation and the up-regulation of atheroprotective gene expression by regulating Src, adenosine monophosphate-activated protein kinase (AMPK), ERK5, KLF2 and histone deacetylase 5 (HDAC5) [89]. Thus, PAR1-deficient mice exhibit up-regulation of NF-κB activation and subsequent inflammatory gene expression, leading to EC dysfunction and atherosclerosis [89].

Notch1 is a single-pass transmembrane receptor that is activated by FSS to maintain the integrity of cell–cell junctions through canonical and non-canonical pathways [76,90,91]. In the latter case, LSS triggers the delta-like ligand 4 (DLL4)-dependent proteolytic activation of Notch1 and exposes its transmembrane domain, thereby stabilizing the assembly of VE-cadherin and establishing the EC barrier function [91]. EC-specific (under *Cdh5CreER^{T2}*) *Notch1*-deficient mice exhibit the dysregulation of cell alignment to flow direction, enhanced EC proliferation, and compromised cell–cell junctions, which leads to an increase in hypercholesterolemia-induced atherosclerotic lesions in the descending aortas [76]. Plexin D1 (PLXND1), a member of the semaphorin family of cell–surface receptors, has been identified as a direct force sensor that forms a mechanocomplex with neuropilin-1 and VEGFR2, which is necessary for conferring mechanical stimuli to the junctional complex and integrins [92]. The EC-specific (under *Cdh5CreER^{T2}*) deletion of *Plxnd1* in mice results in exhibiting the atheroprotective phenotype and reduces plaque formation in $Apoe^{-/-}$ mice. Apart from cell surface mechanosensors, the long non-coding RNA LASSIE has been recently identified as a new class of FSS sensor that binds to VE-cadherin and PECAM-1 and stabilizes the junctional complex by promoting an association with the cytoskeleton [93]. Similarly, a human-specific LSS-responsive SENCR has been shown to promote junction integrity by binding to cytoskeletal-associated protein 4 (CKAP4) [94].

Collectively, these data indicate that different categories of mechanosensors on the EC surface and cell–cell junctions play a pivotal role in FSS-dependent mechanotransduction (Figure 3). However, it remains to be clarified whether multiple actions of these mechanosensors occur simultaneously or in a coordinated manner, and how the interplay between these mechanosensors takes place in different vessel types, and if the expression of mechanosensors changes during aging.

Transcriptional regulation of ECs under LSS

A high magnitude of LSS (15–70 dyne/cm^2 in human arteries) occurs in the straight regions of arteries where blood flow is generally smooth and uniform (Figures 1B and 2). Transcription factors, KLF2, KLF4 and nuclear factor erythroid 2-like 2 (NRF2) are highly expressed in regions exposed to LSS via the extracellular signal-regulated kinase 5 (ERK5) signaling pathway [95–99]. Among these transcription factors, KLF2 plays a pivotal role in LSS response in ECs [100]. KLF2 was identified as an endothelial specific gene induced by prolonged FSS *in vitro* [101]. KFL2 regulates downstream target genes, including thrombomodulin (THBD) and eNOS, and also negatively regulates
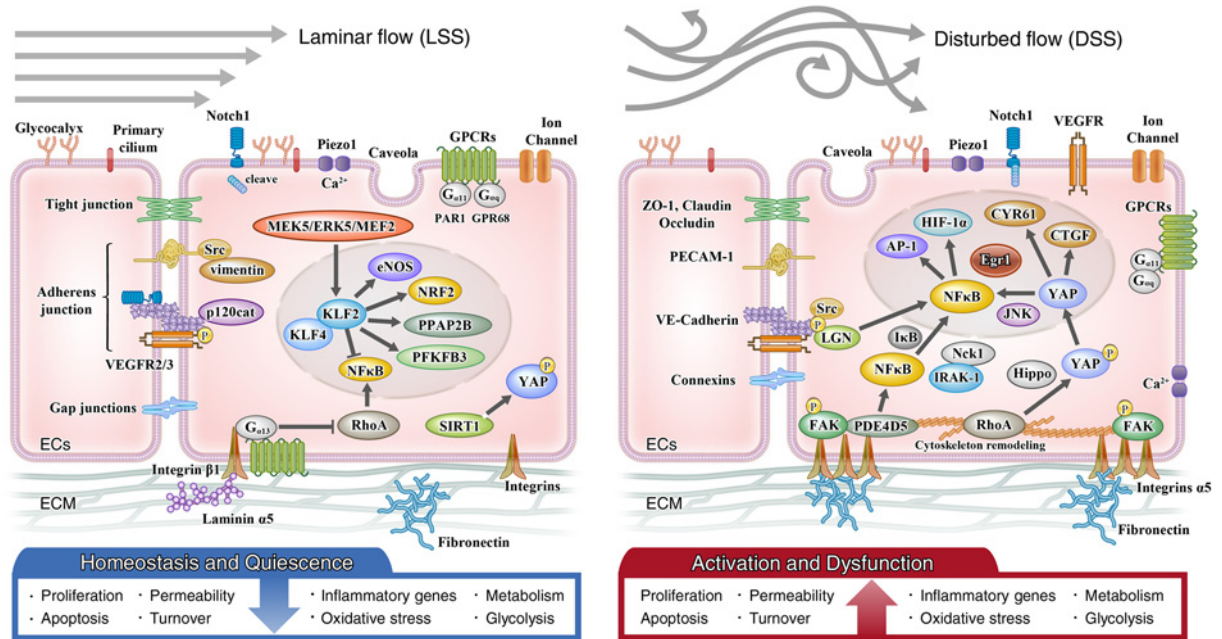
Figure 3. Endothelial mechanotransduction under flow-induced shear stress
Schematic diagram of EC responses to laminar flow (LSS, left) and disturbed flow (DSS, right). LSS induces EC responses that are required for homeostasis and quiescence that up-regulates the expression of anti-inflammatory and antioxidative stress genes. In contrast, DSS induces the activation and dysfunction of ECs, leading to pathological conditions. For information regarding matrix–mediated mechanotransduction, lamininα5 stabilizes VE-cadherin via integrin β1 under LSS, whereas fibronectin binds to integrin α5 and associates with PDE4D5, thereby promoting NFκB-mediated inflammatory gene expression under DSS. GPCRs (G-protein–coupled receptors), PAR1 (protease- activated receptor 1), VEGFR (vascular endothelial growth factor), PECAM-1 (platelet and endothelial cell adhesion molecule 1), VE-cadherin (vascular endothelial cadherin), ERK5 (extracellular signal-regulated kinase 5), JNK (c-Jun N-terminal kinase), SIRT1 (sirtuin 1), KLF (Kruppel-like factor), eNOS (endothelial nitric oxide synthase), NF-κB (nuclear factor κB), Nck1 (NCK adaptor protein 1), IRAK-1 (interleukin-1 type I receptor kinase-1), NRF2 (nuclear factor erythroid 2 like 2), PPAP2B (phosphatidic acid phosphatase type 2B), PFKFB3 (6-phosphofruct 2-kinase/fructose-2, 6-biphosphatase-3), PDE4D5 (phosphodiesterase-4D5), YAP (Yes-associated protein), CTGF (connective tissue growth factor), CYR61 (cysteine-rich angiogenic inducer 61), Egr1 (early growth response 1), HIF-1α (hypoxia-inducible factor 1α), AP-1 (activator protein 1), FAK (focal adhesion kinase), LGN (G protein signaling modulator 2), p120cat (p120 catenin), ECs (endothelial cells), ECM (extracellular matrix).

vasoconstrictive genes and proinflammatory genes such as endothelin 1 (EDN1), vascular cell adhesion molecule (VCAM-1), monocyte chemoattractant protein (MCP1) and plasminogen activator inhibitor-1 (PAI-1) [100,102]. NRF2 is induced by KLF2 after LSS and regulates the expression of genes involved in the antioxidative response, such as NAD(P)H quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HMOX1) [103,104]. KLF2 also regulates phosphatidic acid phosphatase type 2B (PPAP2B) (essential for LSS-induced cell orientation toward the flow and anti-inflammatory effects) and 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3) (contributes to glycolysis and metabolisms in ECs) [105,106]. Furthermore, KLF2 shows protective effects against atherosclerosis formation *in vivo* and *KLF2* deficiency promotes atherosclerosis in LDL receptor-deficient ($Ldlr^{-/-}$) or apolipoprotein E-deficient ($Apoe^{-/-}$) mice [107,108]. Thus, LSS predominantly provides anti-inflammatory and atheroprotective effects through KLF2 and regulates the alignment of ECs, maintains their quiescence, and preserves their homeostasis (Figure 3; left).

Activation of the transcriptional network in ECs under DSS

A low magnitude of DSS is frequently associated with a non-uniform turbulent flow (<12 dyne/cm^2 in human arteries) and generally occurs in areas of arteries that curve sharply or in arteries with stenosis or bifurcation (Figures 1B and 2). DSS enhances the transcriptional activation of NF-κB. NF-κB is a family of transcription factors that is primarily involved in the inflammatory response; notably, five members exist in mammals; p50, p52, p65 (RelA), c-Rel and RelB [109]. The activity of NF-κB is strictly regulated by its phosphorylation and subcellular localization

by the inhibitor of κB (IκB) protein and IκB kinase complex [110,111]. Additionally, it has recently been shown that DSS activates interleukin-1 type I receptor kinase-1 (IRAK-1) by binding to Nck1 (a member of the Nck family of adaptor proteins) in ECs. This binding promotes the nuclear translocalization of NF-κB and the up-regulation of proinflammatory and pro-adhesive genes such as VCAM-1, intracellular adhesion molecule 1 (ICAM-1) and MCP1 [112]. NF-κB is highly expressed in ECs of atherosclerosis regions *in vivo*, and EC-specific (under *Tie2CreER^{T2}*) inhibition of NF-κB reduced atherosclerotic plaque formation in *Apoe$^{-/-}$* mice [113,114].

Other transcription factors, including activator protein 1 (AP-1), early growth response-1 (EGR1), hypoxia-inducible factor 1α (HIF1α) and YAP are known to be activated by DSS [113,115–118]. AP-1 is a transcription factor belonging to the activating transcription factor (ATF) family and is a heterodimer composed of c-Fos and c-Jun that binds to the 12-O-Tetradecanoylphorbol-13-acetate (TPA) response element (TGA G/C TCA), which is also an SSRE. DSS-induced lipocalin-type prostaglandin D synthase (L-PGDS) in ECs regulates platelet aggregation and arterial relaxation via AP-1 [119]. Additionally, DSS-induced HIF1α expression is regulated by NF-κB and stabilized by reactive oxygen species (ROS) and drives vascular permeability and leukocyte recruitment in ECs [117]. EC-specific (under *VE-cadCreER^{T2}*) *Hif1a*-deficient mice ameliorated DSS-induced atherosclerosis formation in *Apoe$^{-/-}$* mice by decreasing microRNA miR-19a-mediated chemokine CXCL1 expression, thereby leading to a reduction in monocyte adhesion in the atheroplaque regions [120]. These results indicate that NF-κB, AP-1, and HIF1α are prominent transcriptional factors activated under DSS and contribute to the pathogenesis of atherosclerosis.

The mechanical stress response transcription factor EGR1 is also known to be induced by DSS and increases the transcription level of platelet-derived growth factor subunit A (PDGFA) [121]. More recently, transcription cofactor YAP has been identified as a novel mechanotransducer in ECs [115,116,122]. YAP and its cofactor TAZ (a transcriptional coactivator with a PDZ-binding motif) are effectors of the Hippo pathway that bind to the transcriptional enhancer factor domain (TEAD) family of DNA-binding factors and regulate organ size. DSS promotes the nuclear localization of YAP via the suppression of YAP phosphorylation at Ser127, which is followed by the increased expression of YAP target genes such as cysteine-rich angiogenic inducer 61 (CYR61, also known as CCN1) and connective tissue growth factor (CTGF, also known as CCN2) [115]. It has also been shown that DSS inhibits integrin and Gα$_{13}$ interaction and activates RhoA and YAP, thereby activating c-Jun N-terminal kinase (JNK) and up-regulating pro-inflammatory genes [116]. Moreover, the degradation of YAP by autophagy and its nuclear export via sirtuin 1 (SIRT1)-mediated deacetylation of YAP has been described as atheroprotective effects under LSS [123]. EC-specific (under *Tie2Cre*) YAP-deficiency *in vivo* reduces atherosclerosis in *Apoe$^{-/-}$* mice, whereas overexpression of endothelial YAP or the constitutively active form of YAP in *Apoe$^{-/-}$* mice induces atherosclerosis formation [116,124]. DSS exhibits proinflammatory and atherosclerotic effects through the activation and dysfunction of ECs (Figure 3; right). These results indicate that the inhibition of mechanical stress responses in ECs mediated by signaling pathways such as YAP and NF-κB may offer a new target for the prevention and treatment of atherosclerosis.

Mechanotransduction of ECs and SMCs in response to cyclic stretch

The vessel wall rhythmically distends and relaxes, which causes circumferential and axial stresses of approximately 100 kPa (1×10^6 dyne/cm^2) on both ECs and SMCs under physiological conditions. A cyclic stretch system (e.g., FLEXCELL® or STREX® cell stretching system) is an artificial model of mechanical force loading used in *in vitro* that mimics *in vivo* wall distension. A physiological extension is defined as a 5–10% strain, while excessive strain (15–20%) is defined as a harmful pathological mechanical stretch. Mechanical stretch induces the opening of non-selective stretch-activated cation channels (SACs) in vascular cells. Three models have been proposed for the activation of SACs, which include direct activation of the channel due to the tension in the lipid bilayer, the tension produced by the pulling of tethered extracellular matrix proteins and/or cytoskeletal proteins, and indirect activation by the primary mechanical sensors (reviewed in [125,126]). GPCRs such as angiotensin II type 1 receptor can also be activated by mechanical stretch in a ligand-independent manner and induce ERK signaling and phospho-inositides [127]. Excess strain results in the dysregulation of vascular tone and abnormal cellular responses such as over-proliferation, apoptosis and phenotypic switching—all of which are associated with vascular diseases such as hypertension, aortic aneurysms and arterialization [128,129]. Therefore, in this section, we highlight the responses of ECs and SMCs to cyclic stretch and describe the cell behaviors involved in the pathogenesis of vascular diseases under mechanical stretch (Figure 4).
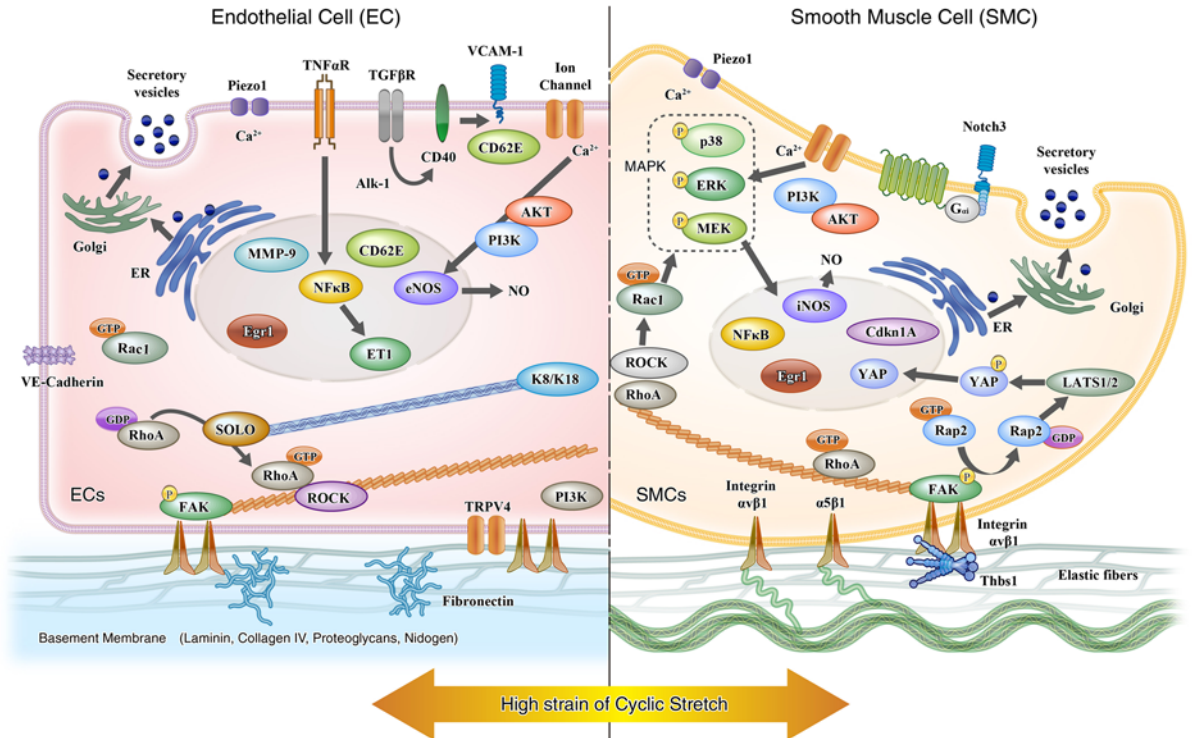
Figure 4. Mechanotransduction in response to cyclic stretch

Schematic diagram of responses to the high strain of (pathological) cyclic stretch in ECs (left) and SMCs (right). A physiological extension is defined as a 5–10% strain, while high strain (15–20%) is defined as a harmful pathological mechanical stretch. GPCRs (G-protein–coupled receptors), TNFαR (tumor necrosis factor alpha receptor), TGFβR (transforming growth factor beta receptor), VCAM-1 (vascular cell adhesion molecule-1), Alk-1 (activin receptor-like kinase 1), CD62E (E-selectin), MMP-9 (matrix metalloproteinase-9), iNOS (inducible nitric oxide synthase), Cdkn1A (cyclin-dependent kinase inhibitor p21), NF-κB (nuclear factor κB), YAP (Yes-associated protein), Egr1 (early growth response 1), TRPV4 (transient receptor potential vanilloid channel 4), FAK (focal adhesion kinase), PI3K (phosphatidylinositol 3-kinase), SOLO (Rho guanine nucleotide exchange factor 40), K8/K18 (keratin 8 / keratin 18), ROCK (Rho-associated kinase), ER (endoplasmic reticulum), ECs (endothelial cells), SMCs (smooth muscle cells).

Cell orientation in response to cyclic stretch

ECs in the vessel wall display an elongated spindle morphology with an orientation of the long axis toward the blood flow. Notably, SMCs exhibit a rhomboid shape *in vivo*. The cyclic stretch system induces the reorientation of ECs and SMCs. When these cells are subjected to a uniaxial cyclic stretch, they become elongated and aligned perpendicular to the stretch direction with the remodeling of the actin cytoskeleton [130]. For cells to respond to mechanical stretch and change orientation, integrins serve an integral role in transducing the force to initiate cytoskeletal remodeling. Within FAs, talin adopts the integrin binding site and force-dependent vinculin is recruited on the tip of actin fibers to make a hub that provides a molecular link across integrin to the actin cytoskeleton and leads to maturation of FAs, thereby organizing cell orientation [131,132].

The transient receptor potential vanilloid channel 4 (TRPV4) is abundantly expressed on the plasma membrane of capillary ECs. It is activated by mechanical stretch and involved in the cyclic stretch-induced reorientation of ECs by binding to integrin β1 and the activation of phosphatidylinositol 3-kinase (PI3K) [133,134]. The Rho guanine nucleotide exchange factor (RhoGEF) SOLO (also known as ARHGEF40) was also identified as a key regulator of cyclic stretch-induced cell reorientation in human umbilical vein endothelial cells (HUVECs) [135]. The knockdown of *SOLO* suppressed cyclic stretch-induced RhoA and Rho-associated kinase (ROCK) activation and impaired the proper orientation of HUVECs by reducing their interaction with keratin-8/keratin-18 intermediate filaments [135,136]. In SMCs, p38 mitogen-activated protein kinase (MAPK) pathways and iNOS-mediated NO synthesis are associated with cyclic stretch-induced cellular orientation [137,138]. These results indicate that cyclic stretch contributes to the morphology and orientation of ECs and SMCs.

Modulation of cellular proliferation and apoptosis by cyclic stretch

The abnormal proliferation of ECs and SMCs contributes to the development of vascular diseases such as atherosclerosis and hypertension [139]. There is growing evidence to suggest that cyclic stretch induces the proliferation of SMCs and also induces apoptosis, thereby maintaining the steady status of SMCs [140–142]. A high-magnitude of cyclic stretch (15–25%, 1 Hz) promotes SMC proliferation through the activation of the extracellular signal-regulated kinase (ERK) and AKT signaling pathways [143]. Cyclic stretch also induces apoptosis via the endothelin B receptor and integrin β1-dependent signaling pathway [144,145]. Cyclic stretch under physiological conditions (5–10%, 1 Hz) causes cell cycle arrest in SMCs by inhibiting the G1/S phase transition with the up-regulation of the cyclin-dependent kinase inhibitor p21 (*Cdkn1A*) or suppression of Notch3 in a G_i-and MAPK-dependent manner [146,147]. A recent phosphoproteomics analysis showed that the PKC family (PKCθ and PKCμ), ROCK1, and AKT are phosphorylated under physiological stretch and could be involved in SMC functions [148]. Interestingly, cyclic stretch-induced proliferation is distinctly regulated in SMCs and ECs. SMCs require the activation of RhoA and the subsequent regulation of stress fibers, whereas ECs require the activation of Rac1, rearrangement of lamellipodia and cell–cell contact through VE-cadherin [149]. A high magnitude of cyclic stretch also induces a proinflammatory response and ROS production via Ras/Rac1-p38/MAPK/NF-κB signaling pathways and NAD(P)H oxidase in SMCs [150,151]. In ECs, the expression of CD40 (a co-immunoreceptor) can be induced by a combination of cyclic stretch and co-culture with SMCs through transforming growth factor β1 (TGFβ1) and activin receptor-like kinase-1 (Alk-1) in arterial ECs [152].

Mechanical stress response and aortic diseases
Arterialization

A notable clinical operation involving cyclic stretch is vein graft adaptation to the arterial circulation (also known as arterialization) for patients undergoing coronary artery bypass or peripheral bypass for critical limb ischemia. The autologous-grafted vein must adapt to a new (arterial) hemodynamic environment with high strain from cyclic forces and FSS. Therefore, proliferation and apoptosis must be regulated and well-balanced. However, arterialization often causes excessive proliferation and neointima in the grafted vein due to the structural difference in wall thickness and differential stretch response between arterial SMCs and venous SMCs [153]. The vein remodeling mediated by cysteine and glycine-rich protein 3 (Crp3) has been linked to the adaptation mechanism in arterialization. Cyclic stretch-induced Crp3 upregulation in vein SMCs promotes apoptosis by associating with FAK and inhibiting the signaling downstream of integrin, thereby sensitizing SMCs to apoptosis during arterialization [154]. Consistent with *in vitro* findings, *Crp3*-knockout rats fail to show early apoptosis and develop marked neointima after jugular vein arterialization. The contribution of microRNA-mediated regulation has recently been reported. Using the *in vivo* venous graft model and venous SMCs, it has been shown that mechanical stretch induces micro RNA miR-29a expression and targets DNA demethylase ten-eleven translocation methylcytosine dioxygenase (TET), thereby inducing phenotypic transformation of SMCs [155]. Conversely, grafted veins down-regulate miR-33 expression and increases BMP3 and the phosphorylation of Smad2 and Smad5, which causes the proliferation of venous SMCs [156]. These findings indicate that differential regulation and signaling pathways govern responses to mechanical stress in arterial and venous walls. A more detailed mechanism must be examined for the improvement of arterialization outcomes.

Aortic aneurysms

Aortic aneurysms involve the abnormal dilatation of the aorta, with high mortality in the case of aneurysm rupture and/or dissection. Since elastic fibers in the aortic wall are directly connected to integrins via elastin extensions and indirectly connected to FAs and the actin cytoskeleton of SMCs, this anatomical and functional continuum is called 'elastin contractile units'. Therefore, hemodynamics forces are transmitted to SMCs through the elastin contractile units [36]. Abnormal responses to mechanical stress in ECs and SMCs have been suggested as one of the underlying causes of aortic aneurysms at all stages (i.e., initiation, progression and rupture) [8]. It has been shown that defects in matrix proteins and alterations of the contractile phenotype of SMCs are responsible for developing thoracic aortic aneurysms (TAAs) (reviewed in [157]). SMCs isolated from the aortas of Marfan syndrome mice (in which fibrillin-1 gene is partially deleted) show phenotypic transition to mesenchymal cells, lower traction force-generating capacity, and impaired focal adhesion/actin cytoskeleton organization [158].

Among FA molecules, mice with SMC- or neural crest-specific deletion of integrin-linked kinase (ILK) (under *SM22Cre* and *Wnt1Cre*, respectively) show the aneurysm phenotype with dysregulation of RhoA/ROCK signaling, impairment of actin filaments and round morphology of SMCs [159,160]. The haploinsufficiency of Notch1 ($Notch1^{+/-}$), a mechanosensor identified in ECs, exacerbated the root aneurysms in fibrillin-1 mutant mice ($Fbn1^{C1039G/+}$) [161], suggesting that the proper sensing of FSS can be protective against aneurysm progression. Our

group previously showed that SMC-specific deletion (under *SM22Cre*) of *Efemp2* (*Fbln4^{SMKO}*), the gene that encodes for matrix protein fibulin-4, exhibits ascending aortic aneurysm with disrupted elastin contractile units and the marked dysregulation of actin depolymerization factor, cofilin, and its phosphatase, slingshot-1, in the aneurysmal wall [162,163]. We further found that the matricellular protein thrombospondin-1 (Thbs1) is highly expressed in the aneurysmal wall of *Fbln4^{SMKO}* mice and human TAAs [164]. Additionally, Thbs1 is induced by Ang II stimulation or high strain of cyclic stretch in SMCs in vitro [14,164]. Thbs1 is also induced by disturbed flow in EC *in vivo* [164,165]. Surprisingly, the deletion of Thbs1 in *Fbln4^{SMKO}* mice prevented the formation of ascending aortic aneurysm with restored connections between elastic fibers and SMCs, inactivation of cofilin, and improved actin filaments in SMCs [164]. In human abdominal aortic aneurysms (AAAs), the matrix periostin is markedly up-regulated and rat SMCs subjected to high cyclic stretch induces the periostin-mediated phosphorylation of FAK, ERK, JNK, as well as the up-regulation of MCP1 [166]. Interestingly, ECs subjected to cyclic stretch counteract the TNFα-mediated up-regulation of MMP-9 and inflammatory signals induced by NF-κB, which illustrates a potential differential role of SMCs and ECs in AAAs in response to mechanical stretch [167]. These results suggest that mechanosensor molecules in the vessels and adaptation to circumferential stress serve an important role in preventing aortic aneurysm formation and progression.

Matrix-mediated mechanotransduction involved in the pathogenesis of vascular diseases

The extracellular matrix initiates mechanical cues and activates intracellular signaling through matrix–cell interactions. In addition to physical properties provided by assembled extracellular matrix such as elasticity or stiffness, consideration of the biochemical properties of each matrix is crucial for understanding matrix-mediated mechanotransduction. This section includes a discussion on the contribution of each matrix in mechanotransduction, how they influence cellular functions and the behavior in ECs or SMCs, and how the altered matrix-mediated signaling leads to the pathogenesis of vascular diseases.

Matrix mechanotransduction in ECs

ECs align on the basement membranes that are typically consisted of laminin, collagen IV, proteoglycans and nidogen. These matrix membranes serve roles in the selective barrier function and maintenance of the EC phenotype under LSS. DSS-induced inflammatory signals in ECs are regulated by fibronectin, but not by collagen IV or laminin [168,169]. *In vivo*, fibronectin is deposited at atheroprone regions of arteries in *Apoe$^{-/-}$* mice, and its receptors integrin $\alpha5\beta1$ and $\alpha v\beta3$ induce the activation of NF-κB, thereby promoting inflammatory signals [168,170–172]. Mechanistically, fibronectin binds to integrin $\alpha5$ on ECs, and $\alpha5$ subsequently associates with phosphodiesterase-4D5 (PDE4D5) and suppresses cyclic adenosine monophosphate (cAMP)—an anti-inflammatory signal—by promoting the phosphodiesterase (PDE) activity of PDE4D5 via protein phosphatase 2A [173]. Blocking the fibronectin-mediated integrin $\alpha5$ pathway by changing the cytoplasmic tail of $\alpha5$ to that of $\alpha2$ attenuates atherosclerosis plaque formation in *Apoe$^{-/-}$* mice [174]. Additionally, the basement membrane component, laminin 511 (laminin $\alpha5$; *Lama5*), is involved in FSS response in small arteries [175]. Laminin $\alpha5$ stabilizes VE-cadherin via integrin $\beta1$ under FSS. *Lama5*-deficient mice show an abnormal response to LSS and the EC-specific (under *Tie2Cre*) deletion of *Lama5* reduces the junctional tension of ECs *in vivo*.

Matrix mechanotransduction in SMCs

SMCs are tethered to elastic fibers via elastin extensions that primarily consist of fibrillin-1, fibronectin, and integrins $\alpha5\beta1$ and $\alpha v\beta3$ [176]. These extensions are necessary for the proper mechanosensing of SMCs as described in the previous section. The disruption of elastin extensions results in the up-regulation of mechanosensitive molecules such as ACE, Egr1 and Thbs1 in SMCs and the formation of thoracic aortic aneurysms [163,164,177]. Although the deletion of either integrin $\alpha5$ or αv alone in mice shows no vascular defects, both integrin deletion exhibit interrupted aortic arch and large brachiocephalic artery aneurysm [178]. These results suggest that connections between SMCs and elastic fibers (presumably via integrin $\alpha5\beta1$ and $\alpha v\beta3$) are crucial for the maintenance of SMC signaling and vascular development.

We have recently reported that Thbs1 is an extracellular mediator of mechanotransduction that promotes the nuclear shuttling of YAP (activation of YAP) in response to the high strain of cyclic stretch [14]. Thbs1 is one of 87 proteins secreted in response to cyclic stretch in SMCs, some of which include lumican, fibrillin-1, collagens and fibronectin, which are involved in blood vessel development and matrix–cell adhesions. We further showed that secreted-Thbs1 binds to integrin $\alpha v\beta1$ and regulates maturation of the FA complex that controls cell stiffness, thereby

promoting nuclear shuttling of YAP in a small GTPase Rap2- and Hippo signaling-dependent manner. Interestingly, although cyclic stretch induces upregulation of Thbs1 in HUVECs, the translocation of Thbs1 to FA is not observed and the nuclear shuttling of YAP does not occur, which indicates that the Thbs1-mediated activation of YAP is dependent on integrins αvβ1—which are expressed in SMCs but not in ECs. The *in vivo* significance of the Thbs1-mediated nuclear translocation of YAP is further supported by the observation that *Thbs1* deletion in mice resulted in the maladaptive remodeling of the ascending aorta in response to pressure overload by transverse aortic constriction (TAC). Surprisingly however, *Thbs1* deletion resulted in the inhibition of neointima formation upon carotid artery ligation. These context-dependent actions of YAP mediated by Thbs1 indicate that the matrix plays pivotal roles in mechanotransduction by connecting the extracellular environment to intracellular signaling during vascular remodeling *in vivo*.

Future perspectives

Hemodynamic forces initiate vessel adaptation and remodeling to maintain vessel homeostasis. The present study described the remarkable abilities of ECs and SMCs to continually sense and respond to multiple stimuli to regulate dynamic changes of intracellular signaling pathways in the vessel wall. The present review further discussed how mechanotransduction plays an essential role in the initiation and progression of vascular diseases, focusing on atherosclerosis and aortic aneurysms. In atherosclerosis, DSS can trigger an inflammatory signaling pathway predominantly mediated by NF-κB in ECs via DSS mechanosensors; this results in alteration in matrix composition (such as fibronectin) and amplification of inflammatory responses. In aortic aneurysms, specifically in thoracic aortic aneurysms, defects in the elastin-contractile units in SMCs impair mechanotransduction and reduce actomyosin-mediated force generation. This leads to the activation of cytokines and secretion of matrix proteins (such as thrombospondin), thereby activating the intracellular signaling.

Elucidating the molecular mechanisms of mechanotransduction in each vascular cell type and identifying molecular players that respond to hemodynamic forces represent important directions for this field of research. However, it remains unclear how FSS-induced signals are transmitted from ECs to SMCs and how cyclic stretch-induced secreted factors from SMCs affect the functions and behaviors of ECs, especially near the EC-SMC boundary. Importantly, it remains unknown whether a specialized mode of communication exists or whether cells communicate with each other via mechanical stress-induced signals over multiple layers of cells or direct cell–cell contact. Also, the identification of the key downstream molecules(s) in the mechanotransduction pathway and the enhancing or blocking of its function(s) without correcting an abnormal environment (i.e., DSS or stiffness) may offer a therapeutic rationale. Understanding the interplay between mechanosensors and matrix-mediated transduction pathways, and how they are regulated, may shed light on an advanced therapeutic strategy for vascular diseases.

Abbreviations
Ang II, angiotensin II; AP-1, activator protein 1; DSS, disturbed shear stress; ECM, extracellular matrix; ECs, endothelial cells; EGR1, early growth response1; FA, focal adhesion; FSS, flow-induced shear stress; GPCR, G protein–coupled receptor; HIF1α,

hypoxia-inducible factor 1a; KLF, kruppel-like factor; LSS, laminar shear stress; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NOS, nitric oxide synthase; PECAM, platelet and endothelial cell adhesion molecule 1; PWV, pulse wave velocity; ROCK, rho-associated protein kinase; SMC, smooth muscle cell; Thbs1, thrombospondin-1; VEGFR, vascular endothelial growth factor receptor; YAP, yes-associated protein.

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