ESC European Society of Cardiology

Cardiovascular Research (2023) **119**, 1656–1675
https://doi.org/10.1093/cvr/cvad076

REVIEW

Endothelial mechanobiology in atherosclerosis

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Received 11 September 2022; revised 11 February 2023; accepted 21 February 2023; online publish-ahead-of-print 10 May 2023

Abstract

Cardiovascular disease (CVD) is a serious health challenge, causing more deaths worldwide than cancer. The vascular endothelium, which forms the inner lining of blood vessels, plays a central role in maintaining vascular integrity and homeostasis and is in direct contact with the blood flow. Research over the past century has shown that mechanical perturbations of the vascular wall contribute to the formation and progression of atherosclerosis. While the straight part of the artery is exposed to sustained laminar flow and physiological high shear stress, flow near branch points or in curved vessels can exhibit 'disturbed' flow. Clinical studies as well as carefully controlled *in vitro* analyses have confirmed that these regions of disturbed flow, which can include low shear stress, recirculation, oscillation, or lateral flow, are preferential sites of atherosclerotic lesion formation. Because of their critical role in blood flow homeostasis, vascular endothelial cells (ECs) have mechanosensory mechanisms that allow them to react rapidly to changes in mechanical forces, and to execute context-specific adaptive responses to modulate EC functions. This review summarizes the current understanding of endothelial mechanobiology, which can guide the identification of new therapeutic targets to slow or reverse the progression of atherosclerosis.

Keywords Cardiovascular disease • Shear stress • Endothelial cells • Mechanotransduction • Antiatherogenic drugs

1. Introduction

1.1 Endothelial Mechanobiology

Endothelial cells (ECs) in the lining of blood vessels create a selective barrier for fluid and biomolecule transport. In normal development and adult physiology, ECs activate morphogenic programs to form vascular structures in the embryo, remodel existing vasculature, or create new blood vessels during tissue repair, while endothelial dysfunction leads to pathophysiological states that contribute to vascular disorders such as atherosclerosis and thrombosis.[1]

ECs are exquisitely sensitive to fluid shear stress (FSS), which is a critical determinant of homeostasis but can also be an instigator of disease[2,3] (*Figure 1*). FSS is the tangential component of frictional forces generated at a surface (e.g. the vessel wall) by the flow of a viscous fluid (e.g. blood).[4–6] The blood vascular wall has three mechanical force loadings, i.e. shear stress (SS), normal stress, and circumferential stress. SS used in this article denotes wall SS, which is the component of frictional forces arising from the blood flow and acts parallel to the vessel luminal surface.[5–7] SS is an outcome of fluid viscosity and the velocity gradient between adjacent layers of the flowing blood. Imposed with the pulsatile characteristic of the flow, SS spans a range of spatiotemporal scales. The magnitude of SS is expressed using interchangeable units (1 Pa = 1 N/m^2 = 10 dynes/cm^2), and the value is affected by changes in blood flow velocity, the inner radius of vessel, and the viscosity of blood. Cells face diverse physical, biochemical, and biomechanical environments and possess the ability to respond to these cues to maintain appropriate biological functions. Mechanobiology refers to mechanisms by which cells sense, transduce, and respond to mechanical forces and modulate their functions. Cells achieve this through mechanotransduction—the transmission of mechanical forces through sensing structures, which results in the induction of biochemical signals.[8]

Geometrical properties of arteries and pulsatile flow conditions determine the detailed blood flow patterns. Pulsatile blood flow results in a positive mean flow rate, but the velocity of the fluid oscillates with the frequency of the heartbeat, and the flow exerts pulsatile SS on the endothelium.[9] In straight segments of arteries, blood flows in ordered laminar patterns in a pulsatile fashion dependent on the cardiac cycle. ECs are subjected to pulsatile laminar SS with fluctuations in magnitude that yields a mean positive SS.[8,10,11] Regions of such laminar and physiological levels of SS are generally not prone to formation of atherosclerotic lesions. In the context of physiological laminar flow, ECs assume a quiescent state with characteristics of anti-inflammation, antiproliferation, antiapoptosis, antilipid infiltration, antileucocyte adhesion and migration, antithrombosis, and reduced endothelial to mesenchymal transition (EndMT) and glycolysis.[2,9,12–14] Laminar SS maintains EC quiescence and vascular homeostasis by inducing endothelial production of a number of factors that promote vasodilation [e.g. endothelial nitric oxide synthase (eNOS) and nitric oxide (NO)] and downregulating proatherogenic genes encoding adhesion molecules and chemokines [e.g. vascular cell adhesion molecule-1 (VCAM-1), intracellular cell adhesion molecule-1 (ICAM-1), and monocyte chemotactic protein-1 (MCP-1)], which inhibit the adherence of circulating blood elements.[9,15] Corresponding flow-responsive mechanisms include Kruppel-like factor 2 (KLF2),[16,17] Kruppel-like factor 4 (KLF4),[18,19] nuclear factor (erythroid-derived 2)–like 2 (NRF2),[20,21] and other protective pathways; these pathways synergistically contribute to the atheroprotective effects of laminar flow.[22]

In contrast, atherosclerosis-susceptible regions of arterial branches, bifurcations, and curvatures are characterized by disturbed flow and low

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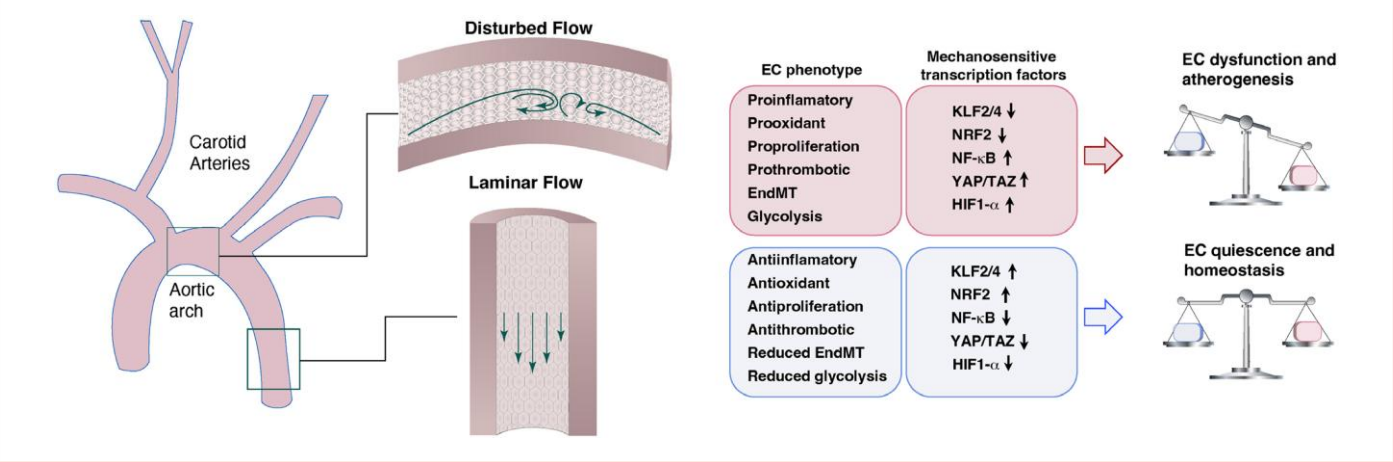

Figure 1 Hemodynamic SS and its role in vessel pathophysiology. The straight part of the artery is exposed to sustained laminar flow and physiological high SS, while flow near branch points or in curved vessels can exhibit disturbed flow. ECs subjected to laminar flow show anti-inflammatory, antioxidant, and anti-proliferative phenotypes accompanied by reduced EndMT and glycolysis, thereby maintaining EC quiescence and vascular homeostasis. Corresponding flow-responsive mechanisms include KLF2, KLF4, NRF2, and other protective pathways. By contrast, ECs in regions of disturbed flow show a proinflammatory, prooxidant, proproliferative response and an enhanced endothelial to EndMT phenotype. As a consequence, disturbed flow leads to EC dysfunction and atherogenesis. The mechanisms responsible for disturbed flow-mediated endothelial dysfunction involves activation of NF-κB, YAP/TAZ, and HIF-1 among other pathways. ↑: upregulation; ↓: downregulation.

time–averaged SS.[8,11,23] Disturbed flow is a complex flow pattern that includes recirculation eddies and changes in direction with space (flow separation and reattachment) and time (reciprocating flow).[24] ECs in these atheroprone sites experience oscillatory and low SS and exhibit low NO production, reduced barrier function, increased proadhesive, procoagulant and proproliferative properties, and an enhanced endothelial to EndMT phenotype.[2,7,25–27] Upon exposure to low and oscillatory SS, activated ECs express atherogenic molecules, including MCP-1, which recruits monocytes into the arterial wall, and platelet-derived growth factors (PDGFs), which stimulate EC proliferation and migration of vascular smooth muscle cells (VSMCs) into the subintimal space.[7,25] These changes in vessel biology are evidently related to inflammatory and tissue repair programs. The mechanisms responsible for disturbed flow-mediated endothelial dysfunction involve activation of nuclear factor κB (NF-κB),[28–30] Yes-associated protein (YAP)/WW domain-containing transcription regulator 1 (TAZ),[31–33] and hypoxia-inducible factor 1α (HIF-1α)[34–36] among other pathways.

Importantly, exercise training is a well-established and potent physiological stimulus which reduces cardiovascular events.[37] Endothelial dysfunction is associated with an impaired dilatory response to increased blood flow-associated SS [flow-mediated dilation (FMD)].[38] Exercise (e.g. handgrip exercise and cycling exercise) can create a sustained, contraction intensity-dependent increase in SS, which potentiates the subsequent brachial artery FMD response to a reactive hyperaemia (RH)–induced increase in SS (RH-FMD; standard test, typically denoted simply as 'FMD').[39–41] The mechanisms responsible for the benefits of exercise training in terms of endothelial function may be related to either direct hemodynamic effects, or secondary effects, mediated through risk factor modification.[42,43] And perturbed flow dynamics are also induced in postsurgical neointimal hyperplasia and predispose to pathophysiologies such as in-stent restenosis, vein bypass graft failure, transplant vasculopathy, and vascular calcification.

1.2 Methodologies for studying the effects of FSS on ECs

To study the mechanisms of EC responses to SS, various models have been developed to mimic *in vivo* flow environments (*Table 1*). *In vitro* models can provide well-controlled conditions for studying endothelial biology over time. However, these models are limited in that they do not fully recapitulate vessel anatomy or mechanical properties, which is significantly more complicated. On the other hand, *in vivo* models are more relevant to disease processes, but fluid dynamics are difficult to accurately measure or control in animal models. Ultimately, a combination of *in vivo* and *in vitro* approaches has led to our current understanding of endothelial mechanobiology and atherosclerosis.

1.2.1 *In vitro* approaches

Early efforts in this area used modified cone and plate viscometer systems to create a well-defined and constant SS[44–47] (*Figure 2A*). In this approach, flow is generated using a cone, which rotates around a central axis that is perpendicular to a fixed plate on which the cells are seeded. Another approach to create SS is the 'shear ring' device, which can provide unidirectional and periodic flow patterns within physiological and pathophysiological ranges using a shaker plate[48] (*Figure 2B*). By restricting the flow pathway within a circular culture dish to the periphery through the placement of an inner ring, the shear ring model can effectively alleviate 'mixed' cellular shear-induced phenotypes.

To more accurately control fluid exchange over the ECs, parallel plate flow chambers were developed. This apparatus uses a syringe pump or peristaltic pump to pass fluid from a reservoir through the chamber, creating steady unidirectional, pulsatile, or oscillatory flow.[26,49,50] Channels with rectangular cross-section and uniform height along the flow path can be used to study steady or pulsatile laminar flow (*Figure 2C*). Disturbed flow is achieved by introducing a vertical step expansion near the entrance, where the channel height suddenly increases (*Figure 2D*). As fluid passes the step, there is a region of flow separation with low SS where flow recirculation and reattachment occur. Downstream, the disturbed flow transits to laminar flow where the channel has uniform height. Thus, the step flow chamber can be used to examine EC responses to disturbed flow in the vicinity of the reattachment area and laminar flow downstream in the same device.[7,26]

Microfabrication techniques greatly advance the possible complexities of FSS models. Microfabricated channels have been used to mimic vessel branching (*Figure 2E*). Microfluidic devices with bifurcated channels produce regions with SS gradients, allowing detailed studies of the impact of chronic SS spatial gradients on ECs.[51–53] More recently, microfluidic systems allow growth of

Table 1 The pros and cons for *in vitro* and *in vivo* methods modelling SS

***In vitro* models**	**Pros**	**Cons**
Cone-and-plate system	Readily programmable and reproduce different SS waveforms under controlled conditions	Has a relatively low surface area, and hence, a low number of replicate studies can be simultaneously performed
Shear ring model	Effectively limits 'mixed' shear and provide unidirectional and periodic flow patterns within patho/physiological levels	The intrinsic inability to precisely control flow in the shear ring
Parallel plate chamber	The ability to generate steady unidirectional or oscillatory SS in a pulsatile manner in an apparatus	Relatively lengthy and complex setup time, low surface areas, requirements for pumps, and pressurization often requiring sealants and gaskets
Microfluidic bifurcated channel	Model gradient SS regions and changes in pressure or growth factor gradients	Linear rectangular channels that are optically transparent and permeable to gases
Microfluidic cylindrical channel	Incorporate ECM components and more physiologically relevant	Cylindrical, linear channels fail to accurately model the effects of disturbed flow in bifurcating vessels
***In vivo* models**	**Pros**	**Cons**
Perivascular cuff model	Easy to induce vortex shedding, highly turbulent regions, and recirculation zones	Less physiologically relevant due to the acute flow alteration and the dissociation of oscillatory shear from low SS. Cuff placement may evoke intraplaque haemorrhage and plaque rupture with fibrin-positive luminal thrombus
Partial ligation model	More physiologically relevant due to associated chronic flow alteration and colocalization of both low and oscillatory SS. Allows for easy and rapid intimal RNA isolation and provide sufficient quantity of endothelial RNA for genome-wide microarray studies. Short study duration	The presence of thrombus, endothelial denudation, and decreased vessel diameter
AVF model	High clinical relevance of AVF to vascular access in haemodialysis	High cost and technical complexities

ECs in 3D microchannels with dimensions similar to vessels *in vivo* using microfabrication techniques and enable integration of important cells and structural properties such as mural cells, extracellular matrix (ECM), or mechanical deformations (e.g. vessel hoop stress). Microfabricated polydimethylsiloxane (PDMS) hydrogel hybrids have been developed using PDMS scaffolds or channels that contain hydrogels (*Figure 2F*). Cells can then be seeded on the hydrogel, providing a more biologically relevant substrate for studies of EC functions. Such systems have been widely used to investigate capillary sprouting in response to changes in pressure or growth factor gradients.[54–56] Although the flexibility of microfabrication technology helps to recapitulate more realistic vascular features *in vitro*, further modifications are needed to reproduce the correct microanatomy of the vessel wall.

1.2.2 *In vivo* methods

Over the past decades, several animal models have been utilized for studying the effects of atheroprone SS on ECs, including (i) exogenous constriction of a segment of a large vessel, (ii) partial ligation of the carotid artery, and (iii) creation of an arteriovenous fistula (AVF).

One of the most common strategies to induce disturbed flow and the associated low and reciprocating SS *in vivo* is to introduce a local constriction to recapitulate the effects of a stenosis in a segment of a large vessel such as the carotid artery or abdominal aorta (*Figure 3A*, left panel). Mimicking a stenosis (40–60% reduction in diameter) in this way leads to flow separation and low-velocity recirculation in the region immediately downstream from the constriction, and laminar with a relatively higher SS upstream of the constriction and in distal downstream segments.[57,58] Computational fluid dynamics (CFD) studies for the perivascular cuff model further showed that the constrictive cuff results in three distinct regions of SS: relatively lower laminar shear upstream from the stenosis, relatively higher shear within the stenosis, and low oscillatory shear downstream from the stenosis[59] (*Figure 3A*, right panel).

The partial ligation model provides a powerful approach to uncover the pathogenesis of disturbed flow–induced atherosclerosis (*Figure 3B*, left panel). By ligating three out of four branches of the left carotid artery, the partial ligation model causes pathophysiologically relevant disturbed flow with characteristics of low and oscillatory wall SS in the common carotid artery.[60] CFD models have incorporated the geometry of mouse carotid arteries as determined by ultrasound imaging to predict whether partial ligation changes SS levels and direction. Mitra *et al.*[61] further revealed that the SS level is significantly reduced during diastole compared with that of the right common carotid artery (RCA) and becomes negative (because of flow reversal) during diastole in the ligated left common carotid artery (LCA) (*Figure 3B*, right panel). The disturbed flow induces rapid development of atherosclerosis in 2 weeks and advanced lesions by 4 weeks in apolipoprotein E-deficient ($ApoE^{-/-}$) mice.[60,62]

AVF is another approach to obtain an experimental model of perturbed flow *in vivo* (*Figure 3C*, left panel). This is often created by forming a connection between the carotid artery and the jugular vein or between the femoral artery and the femoral vein of rodents, rabbit, or swine. CFD simulations showed that areas of low SS are found along the wall of the anastomotic floor, near the anastomosis heel on the inner wall of the vein and to a lesser extent on the inner wall after the curvature of the vein[63] (*Figure 3C*, right panel). Despite the high flow rates present in AVF after maturation, low and oscillatory SS exists in zones where flow stagnation occurs on the outer wall of the artery and on the inner wall of the juxta-anastomotic site, thereby resulting in vessel stenosis in regions with flow perturbations.[64–66]

Of note, AVF-induced alteration of SS causes acute and chronic structural remodelling in the artery and vein, although the vein has received more attention in AVF research studies. AVF leads to neointima formation and stenosis in the venous region of disturbed flow induced by shunting of arterial blood flow directly into the vein.[67,68] Like arterial endothelium responses to oscillating flow, ECs in such venous segments undergo proinflammatory activation associated with upregulation of MCP-1 and induction of the proinflammatory transcription NF-κB.[67,69] In addition,

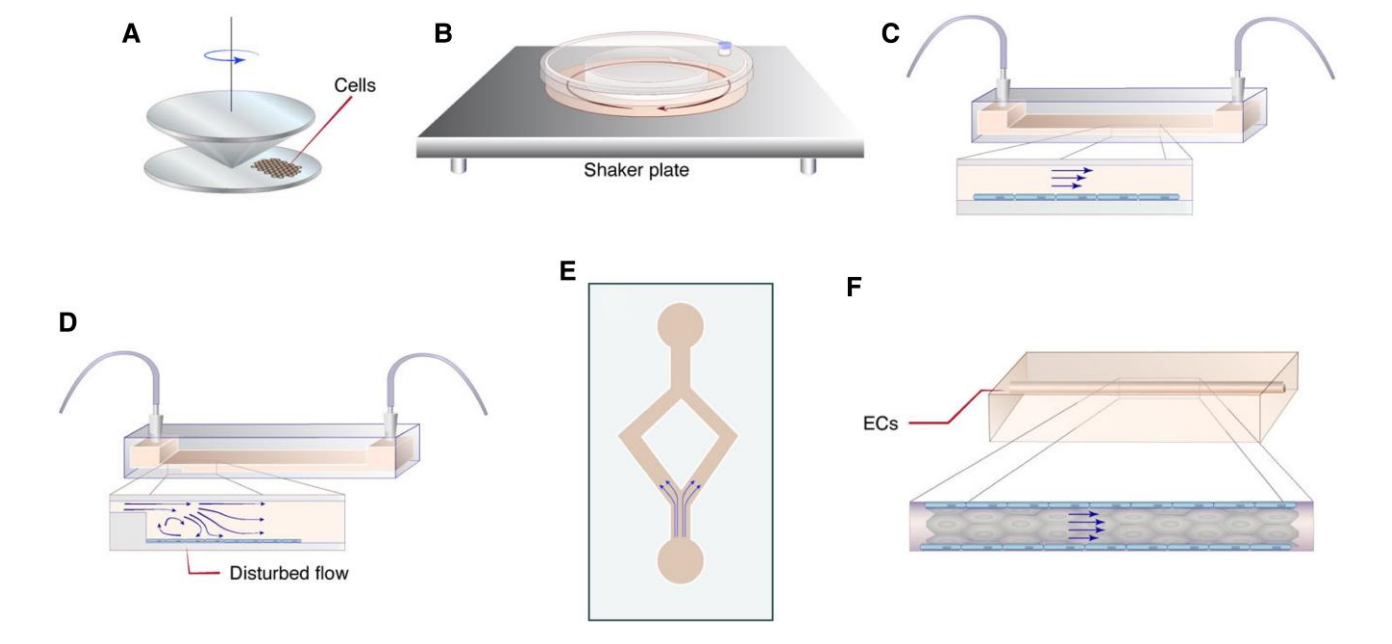

Figure 2 Methods for investigating endothelial SS *in vitro*. (*A*) Modified cone and plate viscometer system for applying spatially uniform SS. (*B*) Shear ring device constructed from petri dishes, with flow driven by placement on a shaker plate. (*C*) Parallel plate flow chamber; flow is controlled by a syringe pump (not shown). (*D*) Step flow chamber. In this modification of the parallel plate flow chamber, a step expansion is introduced to produce localized flow separation/disturbance at the EC surface. (*E*) Bifurcating channel in a microfluidic device. (*F*) Cylindrical channel cast in a hydrogel (e.g. collagen I) and then coated with ECs.

the creation of AVFs induces high flow rates in the arterial segment proximal to the AVF, which leads to arterial remodelling. ECs exposed to high flow conditions show significantly increased cell density and upregulated expression of vascular endothelial growth factor (VEGF), a potent angiogenic factor that stimulates EC proliferation and migration.[70]

In summary, sophisticated methods for modelling shear forces *in vitro* and *in vivo* have greatly advanced our understanding of EC mechanics. The choice of a suitable model to study the effects of FSS on ECs is dependent on the specific endothelial process to be studied. By characterizing the FSS profiles generated by *in vitro* models and pairing them to the FSS profiles identified in atherosclerosis, we can characterize in more detail the contribution of mechanical forces on endothelial health and pathophysiology.

2. Response of ECs to FSS

2.1 EC morphology

In vitro and *in vivo* studies have clearly identified that ECs in areas of high laminar SS elongate and align in the direction of flow, with the well organized and parallel actin stress fibres in the central region.[11,15,71] Whereas static cultures or cells exposed to disturbed flow are randomly oriented and tend to adopt a more polygonal shape; their actin filaments are short and localized mainly at the cell periphery.[7,11,72,73] It is likely that EC alignment parallel to the flow direction is an adaption response that can reduce flow resistance and induce prosurvival signals in the endothelium.[49,74] Disturbed flow or low SS results in less alignment of the ECs, thus exposing them to higher SS gradients and potentially priming these regions for atherosclerosis.[75] Changes in EC morphology in response to SS are mediated by activation of the Rho family of small GTPases and upregulation of intercellular adhesion molecules, which are associated with structural reorganization of the cytoskeleton, cell-cell junctions, and focal adhesions (FAs).[76]

2.2 EC turnover

Laminar flow leads to reduced EC turnover rate and keeps ECs in a relatively nonproliferative state, which is associated with DNA synthesis inhibition and cell cycle progression suppression with the majority of cells being arrested in the G_0 or G_1 phase.[77] Mechanically, laminar flow enhances the activity of histone deacetylase 1 (HDAC1) and its association with p53 resulting in deacetylation of p53 at Lys 320 and Lys 373. HDAC-deacetylated p53 then activates the growth arrest proteins GADD45 (growth arrest and DNA damage–inducible protein 45) and p21, which cause a decrease in cyclin-dependent kinase activity and then hypophosphorylation of retinoblastoma protein (Rb).[77,78] Rb phosphorylation undergoes periodic oscillations during the cell cycle and predominates in G0/G1 phases. The hypophosphorylated Rb can bind several transcription factors essential for DNA synthesis to prevent their initiation of transcription, thus inhibiting cell proliferation. In contrast, oscillatory/low SS promotes EC turnover with characteristics of increased DNA synthesis and proliferation via G0/G1-S transition by suppressing p21.[7,79] Such accelerated cell turnover would lead to an enhanced macromolecular permeability and contribute to the increases in lipid uptake at regions of disturbed flow.[9,80] Although involved in p53/p21 signalling in EC turnover and senescence, SS—induced cell turnover can be reversed, which differs from cellular senescence, a state of irreversible growth arrest after successive cell division–induced telomere shortening that ultimately triggers DNA damage responses.[81,82]

2.3 EC permeability

Shear force is a major determinant of EC permeability and endothelial barrier function. In the context of physiological high laminar SS, the adjacent ECs are closely connected and restrict the passage of macromolecules such as lipoproteins.[83] Conversely, disturbed flow causes barrier disruption and increases permeability to macromolecules. Perturbed flow results in a large intimal clearance of low-density lipoprotein (LDL) and thus a large mass influx of LDL into

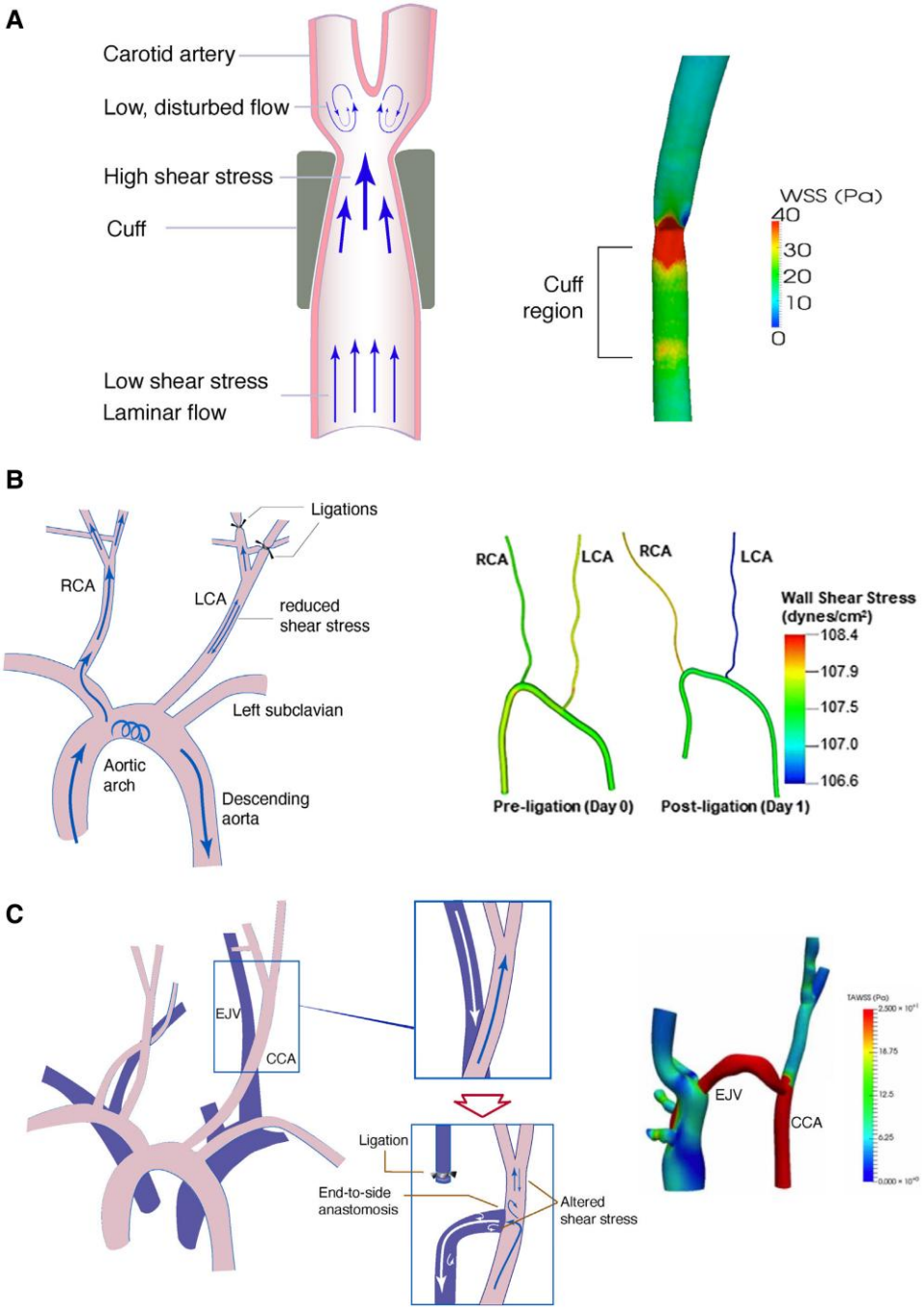

Figure 3 *In vivo* methods for modulating endothelial SS. (*A*) In the SS–modifying cuff model, a restrictive cuff is placed around the right carotid artery (RCA) to modify blood flow. The resulting changes in SS can be estimated using CFD (at right).[59] There is decreased laminar SS upstream and oscillatory SS downstream. (*B*) The partial ligation model. Three branches of the left carotid artery (LCA) are ligated, thus reducing SS in the LCA. (*C*) AVF model. A connection is made between the common carotid artery (CCA) and the external jugular vein (EJV) causing disturbed flow in multiple regions near the anastomosis. Right panel in part A is adapted from Mohri[59]; right panel in part (*B*) is adapted from Mitra[61]; right panel in part (*C*) is adapted from Bai.[63].

the intima.[83] Disruption of the junctional complex proteins such as connexins and VE-cadherins is largely responsible for flow-mediated alterations in permeability.[25,84] Interestingly, state-of-the-art morphological observations of human coronary atheroma reveal microscopic breaches in intimal endothelial continuity and the accumulation of erythrocyte components and polymorphonuclear leucocytes within the region of flow disturbances.[85] The observations suggest that hemodynamically related microscopic lesions are an early contributor to increased permeability and atherogenesis. In addition, the enhanced cell turnover rate, as well as the morphological changes of ECs from elongated to more rounded shape caused by disturbed SS, might also affect cell–cell junctions and contribute to the greater permeability of ECs in atheroprone areas.[26,49,73] Low/oscillatory SS increases the permeability of the internal elastic lamella and elastic lamellae by reducing fenestrae, thus increasing the accumulation of macromolecules in the arterial wall.[86] More

recently, a study by Lu *et al*. established a functional link between the activation of mechanosensitive Ca^{2+} channels, transient receptor potential vanilloid subtype 4 (TRPV4), and endothelial hyperpermeability through the phosphorylation and mitochondrial redistribution of eNOS mediated by PKC (protein kinase C).[87]

Moreover, the shear dependence of macromolecule uptake such as albumin and LDL in the vessel wall has been reported. Compared with the uptake by cells under static conditions, lower SS leads to an increased uptake of LDL into ECs, whereas there is decreased uptake at higher SS.[88] Mechanistically, the endothelial glycocalyx is involved in regulation of shear-dependent uptake of macromolecules into ECs, since neutralization of the glycocalyx induces an increase in albumin uptake by cultured ECs.[89,90] It is thought that the size and steric hindrance as well as electrostatic charges of the glycocalyx and the permeating substance contribute to glycocalyx-dependent permeability.[91]

2.4 EndMT

EndMT, a complex biological process with characteristics of EC transition to mesenchymal phenotype, has been identified to be regulated by mechanical forces. While laminar SS is protective against EndMT, ECs are exposed to disturbed flow undergo EndMT, contributing to atherogenesis.[92] Extensive studies have shown that the multifunctional cytokine transforming growth factor-β (TGF-β) is the main inducer of EndMT,[93] but the processes leading to activation of its signalling remain poorly defined. Analysis of human coronary arteries indicates a strong correlation among reduced fibroblast growth factor receptor 1 (FGFR1) expression, activated TGF-β signalling, the occurrence of EndMT, and the severity of atherosclerosis.[94] Using the parallel plate system and partial carotid ligation model, a recent study has confirmed that ALK5 (TGFβR1) is a receptor responsible for mechano-EndMT and Shc acts as a critical downstream driver of EndMT and atherosclerosis in areas of disturbed SS.[95] The activated TGF-β receptor complex transduces its signal by phosphorylating Smad transcription factors, which subsequently translocate into the nucleus and bind to promoters of EndMT inducing genes. These genes include the key transcription factors GATA4, TWIST1, and Snail.[96,97] In addition to modulating transcription factors, induction of EndMT leads to cytoskeletal remodelling via activation of the Rho GTPase family (RhoA, RAC1, and CDC42) through Smad-dependent and Smad-independent mechanisms.[98] This cytoskeletal reorganization alters the endothelial apicobasal polarity and the cells form spindle shapes with enhanced migration, thus predisposing to the development of atherosclerosis.[99] Moreover, oscillatory SS in a three-dimensional microengineered human coronary-artery-on-a-chip induces transition of ECs into a proinflammatory EndMT phenotype and contributes to atherogenesis, which is mediated by the Notch1/p38 MAPK-NF-κB signalling pathways.[92]

2.5 Anticoagulant activity

Physiological laminar SS promotes anticoagulant characteristics consistent with an atheroprotected phenotype of ECs. Prostacyclin is the first inhibitor of platelet aggregation shown to be released from ECs in response to shear force.[100] Laminar SS–induced release of the vasodilator NO is also responsible for the antiplatelet aggregation properties of ECs.[101] More recently, studies have identified that thrombomodulin (TM), which is a key player in the regulation of coagulation and thrombosis, responds to shear force through interaction with protein C and protein S to inactivate certain clotting factors, thus exerting anticoagulant activity.[102,103] Under laminar flow with high SS, KLF2-mediated upregulation of TM is an important protective antithrombotic and antiatherosclerotic mechanism in large arteries.[102,104] TM is reversibly regulated by mechanical forces in an EC-specific, magnitude-dependent, and hysteresis-free manner.[105] Besides, laminar SS has been shown to stimulate expression of tissue plasminogen activator (tPA) and reduce secretion of plasminogen activator inhibitor type-1.[106–108] Importantly, ECs exposed to turbulent flow fail to show increases in TM and tPA.[109,110]

2.6 Leukocyte adhesion and migration

Adhesion of circulating leukocytes to and subsequent transmigration across the EC monolayer are key events in atherogenesis. The adhesive interactions between leukocytes and ECs are modulated by local blood flow.[111] Circulating leukocytes tether and roll along the vessel wall by establishing transient selectin–mediated interactions with ECs.[112] This initial contact facilitates recognition of chemoattractants (mostly chemokines), immobilized on the apical surface of ECs, by rolling leukocytes. Extensive studies have shown that laminar flow is associated with less adhesion of circulating leukocytes to ECs. This process involves inhibition of expression of adhesion molecules and chemotactic proteins (e.g. VCAM-1 and MCP-1) in ECs. In contrast, disturbed flow with altered SS promotes leukocyte binding and transmigration, thus causing leukocyte accumulation in the arteries and increased propensity for atherogenesis.[73] Increased leukocyte–EC adhesion under disturbed flow may be attributed to (i) the altered expression of adhesive proteins such as ICAM-1, E-selectin, and MCP-1 on EC surfaces and (ii) the enhanced collisions and prolonged contacts between the circulating leukocytes and ECs.[113] Furthermore, accumulating evidence demonstrates that shear forces participate in integrin-mediated leukocyte arrest.[111,114] Ultimately, arrested leukocytes crawl along the endothelium and migrate across ECs (diapedesis or transendothelial migration), either at intercellular junctions (paracellular pathway), by engaging adhesion molecules including platelet–endothelial cell adhesion molecule-1 (PECAM-1), junctional adhesion molecules (JAMs), and molecules of the cluster of differentiation CD99 or through the EC body (transcellular route).[115,116]

Additionally, hemodynamic perturbations can activate neutrophils and induce release of neutrophil extracellular traps (NETs), which are comprised of web-liked DNA fibres containing histones and granular proteins.[117] The process of NET formation is called NETosis. During NETosis, neutrophils decondensate and release their nuclear DNA in long chromatin filaments to form NETs.[118] In addition to their known role in the elimination of pathogens, NETs have been newly described as potent inducers of leukocyte extravasation at sites of inflammation.[119,120] NETs can trigger neutrophil rolling, firm adhesion, and emigration in the microvasculature *in vivo*, which can be abrogated by immunoneutralization of P-selectin and its major counter receptor PSGL-1 (P-selectin glycoprotein ligand-1).[120]

2.7 Lipid metabolism

Lipid accumulation in the artery wall is a common clinical manifestation of atherosclerosis and is largely impacted by local hemodynamics.[121] Laminar flow inhibits lipid accumulation in ECs because it decreases LDL permeability as a result of the upregulation of growth arrest genes and the lower lipid uptake and synthesis as a result of the downregulation of sterol regulatory element binding protein 1 (SREBP1). On the contrary, disturbed flow induces sustained activation of SREBP1 and hence leads to an increase in SRE-mediated transcriptional activation of EC genes that promote lipid uptake and lipid synthesis.[26,73] Activated SREBP1 is associated with enhanced expression of genes encoding for the LDL receptor, cholesterol synthase, and fatty acid synthase, thus increasing the intracellular sterol level.[122] The shear-dependent activation of SREBP1 is abolished by blockade of β1 integrin with AIIB2 blocking-type mAb or disruption of actin cytoskeleton with cytochalasin D, suggesting the importance of integrin and actin cytoskeleton in the modulation of EC lipid metabolism in response to SS.[123,124]

2.8 SS and EC interactions with neighbouring cells

Since ECs do not exist alone in the vessel wall, there is crosstalk between ECs and other cells in the vessel wall, and interactions between these cell types are important for proper vascular function. Previous studies suggest that ECs can transmit SS–induced signals to VSMCs, and VSMCs exhibit feedback control to ECs as well.[125,126] The net arterial remodelling under mechanical stimulation is controlled by a dynamic interplay between growth inhibitory signals from ECs and growth stimulatory signals from VSMCs.[9,127] These results highlight the significance of considering cell–

cell communication in studying endothelial mechanobiology and vascular pathologies. Because these interactions are challenging to isolate *in vivo*, they may be more easily studied using *in vitro* systems that have the potential to integrate multiple *in vivo* components including circulating blood cells, ECM, and other vascular cells of the arterial wall (e.g. VSMCs and fibroblasts). For example, artificial vessels surrounded by ECM can be created through the self-assembly of a mixture of ECs and VSMCs. These novel engineered blood vessels provide the correct configuration of lumen, an inner lining of ECs, and outer sheath of VSMCs and exhibit properties such as quiescence, perfusability, and vasoactivity expected for functional vessels.[128] Advances in this field will greatly improve our understanding of the interplay between cell types in the atherogenic vascular wall.

In summary, ECs respond to laminar and disturbed flow by structural and functional adaption which involves perturbations of EC morphology and reprogramming of gene expression to modulate cellular functions (e.g. proliferation, permeability, EndMT, leukocyte extravasation, coagulation, and lipid accumulation) (Figure 4). These responses enable vessel stabilization as well as homeostatic remodelling and are critical determinants of vascular architecture during development.

3. Endothelial mechanosensors

Numerous membrane-associated molecules and microdomains have been identified as mechanosensors in ECs and mediate conversion of mechanical stimuli to intracellular signals, including ion channels, FAs, receptor–tyrosine kinases [e.g. vascular endothelial growth factor receptor (VEGFR)], G-protein-coupled receptors (GPCRs), the glycocalyx, primary cilia, and caveolae (*Figure 5*). Typically, mechanosensors present on the EC membrane directly sense blood flow and transduce the mechanosignal into the cells.[11,129] Altered expression/structure of these mechanosensors leads to altered mechanotransduction, and the occurrence of endothelial dysfunction, evidenced by endothelial injury, leukocyte adhesion to activated endothelium, EndMT, heightened oxidative stress/inflammatory response, senescence, and hyperpermeability.[22,80,130] Dysfunctional status of ECs thus predispose to the development of atherosclerotic plaques and other vascular diseases.

3.1 Ion channels

Activation of mechanosensitive ion channels is one of the most rapid reactions of ECs exposed to flow. Piezo1 and Piezo2 proteins are the most widely investigated flow-responsive cation channels. SS mechanically changes the conformation of Piezo1 and Piezo2, increasing ion transport, which then induces protease activation and cytoskeletal reorganization.[131,132] Disturbed flow, but not laminar flow, triggers Piezo1- and G_q/G_{11}-mediated integrin activation, resulting in focal adhesion kinase (FAK)–dependent NF-κB activation. Mice with endothelium-specific deficiency of Piezo1 or $G\alpha_q/G\alpha_{11}$ exhibit reduced integrin activation, inflammatory signalling, and progression of atherosclerosis in atheroprone areas.[133] Beyond that, TRPV4, a Ca^{2+} entry channel, is also implicated in EC mechanotransduction by forming mechanosensitive heteromeric channels with other transient receptor potential channels such as TRPC1 and TRPP2.[134] Mechanical stimulation induces dynamic microcompartmentation of caveolin-1/TRPV4/K_{Ca} in caveolae of ECs, a process that participates in regulation of flow-induced vasodilation.[135]

To date, the mechanism involved in shear-mediated alteration of ion channel activity remains controversial. Previous studies have mainly focused on direct deformation of the ion channels in response to SS. However, another potential mechanism involves indirect deformation of the channels due to their mechanical coupling to other structures that are strained by fluid forces, including cytoskeletal structures.[136]

3.2 FAs and integrins

FAs serve as linkages between the ECM and cell cytoskeleton and are essential regulators of EC responses to flow. Studies have proposed that SS activates FA signalling pathways mainly in two ways: (i) by triggering the rapid reorganization of FAs and the formation of aggregates of integrins and (ii) by directly activating FA signalling proteins.[137–139] Nuclear mechanics also participate in FA mechanosensing, as disruption of nuclear–cytoskeletal connections in ECs interferes with adaption to SS.[140] This is accompanied by altered cell–cell adhesion, barrier function, cell–matrix adhesion, and FA dynamics.[140]

Integrins, composed of α and β subunits, are major mediators of mechanosensing and mechanotransduction in ECs. By interacting dynamically with ECM proteins, the mechanosensitive integrins activate Rho small GTPase and many signalling events in FAs and the actin-based cytoskeleton to modulate vascular biology.[141] Studies have demonstrated that shear-elicited activation of integrins and the associated RhoA small GTPase can induce ERK activation[142] and stimulate tyrosine phosphorylation of FAK and paxillin,[143,144] thus regulating flow-induced stress fiber formation and actin reorganization.

Additionally, binding of endothelial integrin α5β1 with fibronectin under low/oscillatory SS induced by a parallel plate flow system or partial ligation of $ApoE^{-/-}$ mice causes phosphorylation of the cytosolic nonreceptor protein kinase c-Abl, which then induces tyrosine phosphorylation (at Y^{357}) and nuclear translocation of YAP, leading to proatherogenic gene expression and EC activation.[145] Furthermore, Piezo1-dependent activation of endothelial annexin A2 uniquely binds to integrin α5 subunits and drives integrin movement into lipid rafts under partial ligation-induced oscillatory SS, thus mediating the translocation and activation of integrin α5β1.[146] Once activated, integrin α5β1 stimulates FAK–dependent NF-κB signalling, which resulted in inflammation and formation of atherosclerotic plaques[146]. Integrin $\alpha5^{+/-}$ mice are viable and display significant resistance to disturbed flow-induced EC dysfunction and atherosclerosis.[147]

Currently, most studies of integrin involvement in cardiovascular disease (CVD) have focused on the mechanistic relationship between abluminal integrins and endothelial inflammation under flow conditions. Interestingly, evidence suggested that integrins localized on the apical surface of ECs sense and respond to SS signals as well.[148] Apical β1 integrin is activated by unidirectional SS but not oscillatory SS, potentially contributing to flow direction sensing.[149] The activation of luminal β1 integrins by SS involves caveolae rather than cytoskeleton dynamics.[150] However, the role of apically expressed integrins in mechanotransduction and whether there is coordination between luminal and abluminal integrins require further investigation.

3.3 Cell–cell junctions

High laminar SS promotes the formation of PECAM-1/VE-cadherin/VEGFR2–mechanosignaling complex at cell–cell junctions, which transduces mechanical force into the activation of PI3K (phosphoinositide 3-kinase) and AKT, stimulating NO production from eNOS.[151] VE-cadherin is important for endothelial reorientation and gene expression modulation in response to flow, whereas PECAM-1 serves as a force transducer leading to activation of signalling by VEGFR2 and PI3K.[152] The transmission of mechanical cues through the junctional complex is mediated by phosphorylation of a small pool of VE-cadherin on Y658. Y658 phosphorylation dissociates p120ctn, allowing binding of the polarity protein LGN, which is essential for multiple flow responses *in vitro* and *in vivo*.[153] Additionally, using the *in vitro* flow chamber or cone-and-plate viscometer system and hypercholesterolaemic $ApoE^{-/-}$ mice model, a novel mechanocomplex of plexin D1/neuropilin-1/VEGFR2 has been identified in ECs, which regulates flow-mediated junction signalling, integrin function, and downstream cellular responses, ultimately regulating the site-specific distribution of atherosclerosis.[154]

3.4 GPCRs and G-proteins

GPCRs are sensitive to mechanical stimuli and are linked to a wide range of signalling molecules and effector systems by coupling to G-protein. The $G\alpha_{q/11}$-coupled receptor GPR68 knockdown interferes with the response to mechanical forces, and the mechanosensitivity of GPCRs is attributed to its essential structural motif c-terminal helix 8 (H8).[155,156] Unidirectional flow applied to ECs enhances the phosphorylation of both AKT-1 and

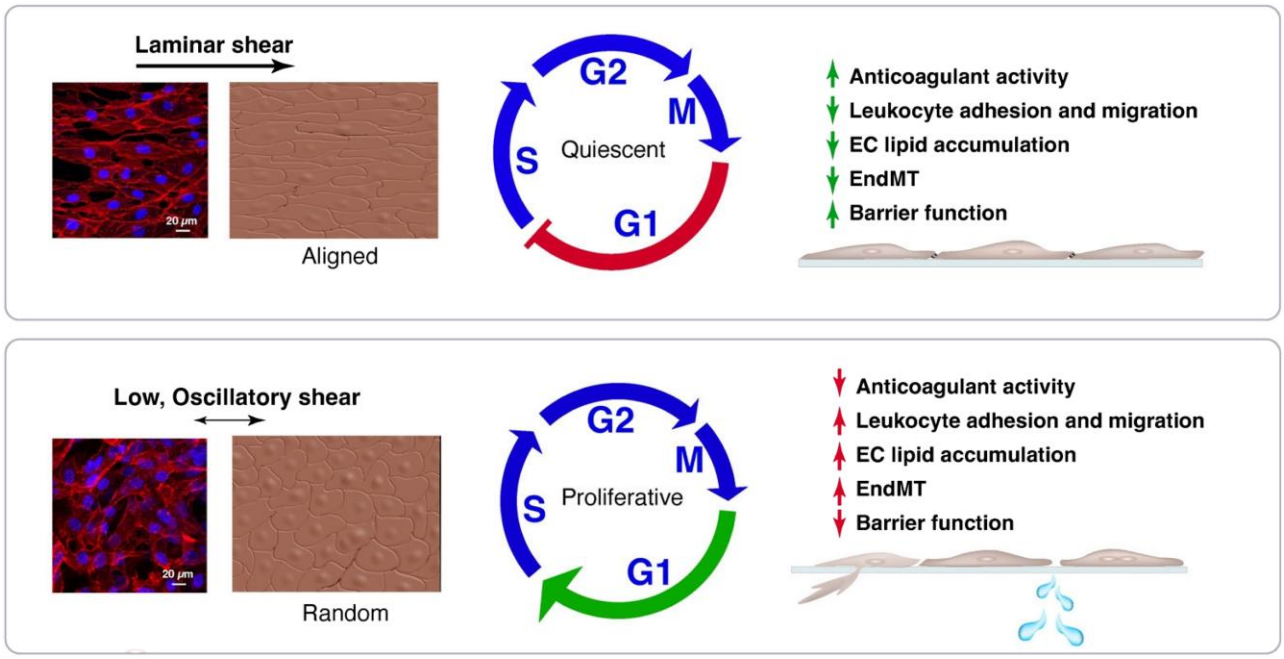

Figure 4 Endothelial response to SS. ECs exposed to laminar flow are spindle shaped, aligned, and elongated parallel to the flow direction, with characteristics of lower turnover rate, increased anticoagulant activity, hypopermeability and less lipid accumulation, reduced leukocyte adhesion and migration, and inhibited EndMT, hence maintaining endothelial barrier function, while disturbed flow leads to a more polygonal morphology of ECs with random orientation and endothelial barrier disruption, which is characterized by accelerated turnover, decreased anticoagulant activity, hyperpermeability and lipid accumulation, enhanced leukocyte adhesion to endothelium, and the occurrence of EndMT.

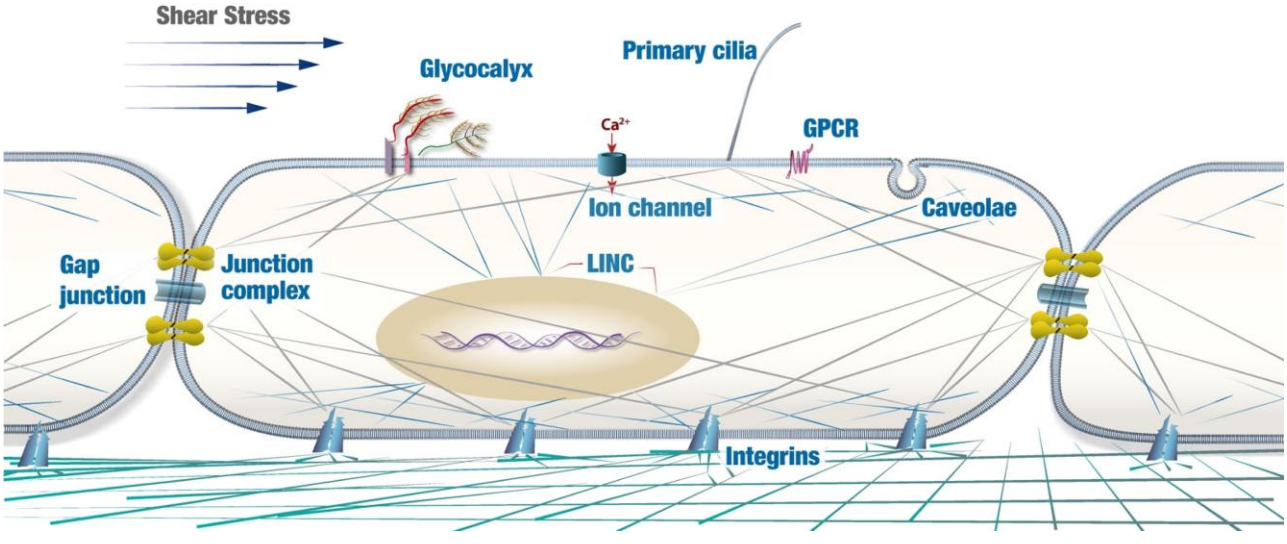

Figure 5 Mechanosensors in the endothelium that sense and convert biomechanical cues into biological signals. The glycocalyx, primary cilia, ion channels, cell–cell and cell–substrate adhesion complexes, G-protein–coupled sensors, caveolae, and NE proteins (e.g. LINC complexes) have been implicated in the transduction of fluid forces.

its downstream effector GSK-3β (glycogen synthase kinase 3β), while silencing of $G\alpha_{q/11}$ completely abrogates this effect.[157] Moreover, $G\alpha_{q/11}$ and PECAM-1 form a mechanosensitive complex at the cell–cell junction under flow condition. The $G\alpha_{q/11}$/PECAM-1 complex experiences a rapid dissociation and reassociation in response to temporal gradients of SS.[158] Another study revealed that PECAM-1 mediates GSK-3β phosphorylation during SS stimulation using an orbital shaker.[159] Collectively, these studies indicated that $G\alpha_{q/11}$ and PECAM-1 participated in regulating flow-induced activation of the AKT-GSK-3β signalling pathway. Additionally, steady SS exerted by a conventional parallel-plate flow chamber induces rapid activation of $G\alpha_{q/11}$ without dephosphorylation of β-arrestin-1 or internalization of $G\alpha_{q/11}$ receptor $S1P_3$, suggesting that $G\alpha_{q/11}$ can also respond to mechanical force, independent of GPCR activation.[160]

3.5 Glycocalyx and primary cilia

The EC surface is covered with a hydrated gel-like structure called the glycocalyx which contributes to the integrity of the endothelial barrier.[161] Endothelial glycocalyx consists of sulfated proteoglycans—transmembrane syndecans and membrane-bound glypicans, with their covalently bound glycosaminoglycans (GAGs)—heparan sulfate (HS) and chondroitin sulfate (CS) and the nonsulfated GAG hyaluronic acid (HA), as well as glycoproteins bearing sialic acids (SA) and plasma proteins.[162] Multiple studies have concluded that the glycocalyx is a key mechanosensor participating in endothelial mechanotransduction mechanisms and itself is physically modulated by the flow.[163] The endothelial glycocalyx is relatively thick and substantially covers the endothelial surface in the common carotid region, where the endothelium is exposed to laminar flow. In contrast, flow perturbances at lesion-prone sites of arterial bifurcations are associated with scarce expression of glycocalyx.[164,165] This renders the endothelium more vulnerable, resulting in disease-like cellular and molecular accumulation in the endothelium or within the blood vessel wall.[166,167]

Changes in blood flow influence conformation of glycocalyx, and the signal is transmitted to cytoskeleton through the intracellular domain of glycocalyx or transduced into biochemical signals through changes in local microenvironment. Disruption of glycocalyx affects flow-mediated actin cytoskeleton reorganization and FA localization.[168] Researchers propose a 'bumper-car' model for the role of glycocalyx, in which the actin cortical web and dense peripheral actin bands (DPABs) are only loosely connected to basal attachment sites, allowing for two distinct cellular signalling pathways reacting to SS: one transmitted by glycocalyx core proteins as a torque that acts on the actin cortical web (ACW) and DPAB, and the other emanating from FAs and stress fibres at the basal and apical membranes of the cell.[168]

Additionally, endothelial glycocalyx is intimately involved in vascular homeostasis, exerting multiple antiatherogenic effects by inhibiting coagulation and leucocyte adhesion, by contributing to the vascular permeability barrier and by mediating SS–induced NO release.[161] These qualities distinguish the glycocalyx as an essential element of a functional endothelium, and glycocalyx can be used to dynamically assess EC barrier function under flow.[169] Studies to date have made great progress in imaging the glycocalyx under flow, its effect on barrier function, and the interplay between blood components (e.g. leucocytes) and the glycocalyx in both static and perfused *in vitro* models. However, ideal *in vitro* models that integrate all of these aspects have yet to be demonstrated.

Primary cilia are mechanical structures that extend from the apical surface of endothelial or epithelial cells. Endothelial primary cilia–mediated SS sensing is coupled to Ca^{2+} signalling and NO production, ciliary length, and cilia distribution.[170] Under conditions of low shear flow, endothelial cilia act together with bone morphogenic protein 9 (BMP9) to keep immature vessels open before the onset of high SS–mediated remodeling.[171] However, exposure of ECs to high laminar SS in a flow chamber leads to disassembly of primary cilia, suggesting that cilia cannot tolerate extreme levels of SS.[172] Additionally, work conducted on zebrafish embryos indicates that endothelial cilia actively recruit vascular mural cells to control the development and maturation of the vertebrate vascular system through a mechanism that involves ciliary regulation of Notch activation and transcription factor foxc1b expression.[173]

3.6 Caveolae

Caveolae, cell membrane invaginations that are rich in cholesterol, sphingolipids, and a variety of signalling molecules, are mechanotransducers that reportedly respond to changes in blood flow.[174–177] Signalling molecules that have been implicated in rapid EC response to flow include Src family tyrosine kinases, eNOS, Ras, and select heterotrimeric G proteins that are enriched in EC caveolae.[178,179] The caveolae coat protein caveolin-1 knockout mice have impaired flow-dependent arterial remodelling, defects in flow-induced vasodilation, and blunted eNOS activation, which can be rescued with the reconstitution of endothelial specific caveolin-1.[175] Compared to static conditions, exposure of ECs to atheroprotective shear leads to increased caveolae number, achieved by translocation of caveolin-1 from the Golgi to the luminal plasma membrane, while also increasing caveolae/caveolin-1 polarization to the portion of ECs that lies upstream of the flow direction.[180,181] However, it remains obscure how caveolae mediate endothelial mechanotransduction, but these structures may be functionally linked to other mechanosensitive molecules. SS–induced integrinβ1 activation results in Src-family kinase–dependent phosphorylation of caveolin-1, suggesting that caveolae and integrins are functionally linked.[150,182] Other evidence supports that both junctional complexes (VE-cadherin and PECAM-1) and FAK most likely collaborate with resident caveolar proteins (caveolin-1, VEGFR2, and eNOS) to integrate flow-dependent responses *in vivo*.[183,184] Moreover, it is reported that the adaptation of caveolae/caveolin-1 to protective or atherogenic flow may involve endothelial glycocalyx and possibly other elements.[164,185]

3.7 The nucleus in mechanotransduction

The cell nucleus is the storage house of genetic information and also integrates epigenetic mechanisms that participate in regulation of transcription. This epigenetic regulation is central to mechanobiology[186,187] and involves DNA methylation, histone modifications, and RNA-based mechanisms, which result in an altered chromatin structure and gene expression and play an important role in the pathogenesis of atherosclerosis.[188–190] In the field of shear force transmission in ECs, a particularly exciting concept is the emerging role of nuclear envelope (NE) proteins in vascular mechanotransduction.[191,192] LINC (linker of nucleoskeleton and cytoskeleton) complexes are the nuclear membrane proteins which cross the nuclear membrane and physically connect with both the cytoskeleton and the nuclear lamina which forms the scaffold beneath the inner nuclear membrane.[193] Recent studies reported that the LINC complexes and lamina of NE may act as direct force-responsive structures and participate in multiple cell functions, including chromatin organization, DNA replication, and gene transcription.[194,195] Specifically, nesprins, which are KASH (Klarsicht–ANC-1–SYNE homology) domain proteins located on the outer nuclear membrane, are LINC components connected to actin filaments as well as to microtubules and intermediate filaments. The nesprin proteins are also connected directly to LINC proteins at the inner nuclear membrane—the SUN (Sad1p–UNC-84) domain proteins—thereby forming a physical bridge between the outer and inner nuclear membranes. The SUN domain proteins are in turn connected to the nuclear lamina and to chromatin through a number of adaptor proteins, thereby providing a direct mode of physical signal transmission from the cell membrane into the nucleus.[186,196–198] Low SS reduces the expressions of nesprin2 and lamin A, which affects the activation of transcription factors AP-2, TFIID, and Stat1, 3, 5, 6. In this way, SS regulates the mRNA levels of downstream target genes to modulate EC proliferation and apoptosis.[194,199] Studies of NE mechanotransduction have revealed that different components sense the mechanical force directly from extracellular environment or via the cytoskeleton.[194,196,199] However, this is an emerging area of study, and the details of the structure of the NE, its involvement in mechanotransduction, and the implications for vascular biology remain to be elucidated.

In short, mechanical cues perceived by ECs can be relayed from the cell membrane to nucleus via multiple flow-responsive molecular elements.

The cytoskeleton links the luminal surface to cell–matrix adhesion sites, intercellular junctions, and the nuclear membrane, providing a structural framework for EC mechanotransduction.[200] Importantly, different mechanosensing elements are interconnected in the initiation of the mechanotransduction process, e.g. the links between integrins, GPCRs, and nucleus,[201–203] as well as synergistic effects between Piezo1 and TRPV4 in response to shearing.[204] Hence, cellular responses to external forces are complex, and dynamic changes in cell phenotype are determined by integration of multiple mechanotransduction axes.

4. Mechanisms of mechanotransduction in ECs

Endothelial mechanosensors discussed above sense and respond to mechanical stresses either directly through conformational changes that alter protein activity or ion transport, or by initiating a complex network of signal transduction pathways that affect gene transcription, cell cycle, and other programs relevant to cardiovascular pathophysiology (*Figure 6*).

4.1 KLF2/4

Flow-responsive transcription factors KLF2/4 play an essential role in maintaining EC quiescence. Atheroprotective laminar flow, but not disturbed flow, upregulates expression of KLF2 and KLF4. KLF2/4 activate anti-inflammatory mediators (e.g. eNOS and TM) and repress NF-κB activity, which decreases expression of adhesion (e.g. ICAM-1 and VCAM-1) and prothrombotic [e.g. plasminogen activator inhibitor 1 (PAI-1)] molecules, thus conferring antiatherosclerotic effects.[205] Mechanically, laminar flow increases KLF2 expression via the MEK5/ERK5/MEF2 signalling pathway, while oscillatory flow downregulates KLF2 expression through SMAD1/5 activation to mediate cell proliferation and cell cycle progression.[206,207] Laminar SS modulates endothelial hyaluronan (HA) production through increased KLF2 and subsequent membrane localization of hyaluronan synthase 2 (HAS2) and UDP-sugar availability.[165] Epigenetic modulation is also involved, as laminar flow stimulates HDAC5 phosphorylation and nuclear export in ECs, thus dissociating MEF2 to activate KLF2 and eNOS expression.[208] Moreover, disturbed flow in atherosusceptible regions reduces Foxp (forkhead box P) transcription factor 1 (Foxp1) expression, which activates the NLRP3 inflammasome and promotes interleukin-1β (IL-1β) secretion, thereby impairing endothelial function and promoting monocyte adhesion and infiltration into the vessel wall to become proinflammatory foam cells and to facilitate atherosclerotic lesion formation.[209]

Studies to date suggest that there is significant overlap between the endothelial targets and functional effects of KLF2 and KLF4. Future studies should further evaluate whether KLF4 and KLF2 have competitive or cooperative effects on the promoters they regulate and how this regulation changes under conditions of SS.

4.2 NRF2

Antioxidant transcription factor NRF2 is a downstream target of KLF2 and functions synergistically with KLF2 to induce quiescence of ECs by the reduction of inflammatory responses.[210] Laminar SS induces binding of NRF2 to the antioxidant response element (ARE) present in the promoters of many antioxidant and phase II detoxifying genes, such as glutathione-S-transferase, heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase (NQO1), peroxiredoxin 1, glutamate-cysteine ligase modifier subunit (GCLM), and glutamate-cysteine ligase catalytic subunit (GCLC).[211] Using a parallel-plate flow chamber, a study found that flow-mediated NRF2 nucleus translocation and inflammation suppression involves inhibition of the p38 MAPK-VCAM-1 signalling axis and ERK5 activation.[212] In contrast, disturbed flow significantly diminishes the binding of NRF2 to the NQO1 ARE, which involves deacetylation of NRF2 by class I HDACs.[213] However, the role of NRF2 in atherogenesis seems to be genotype dependent. Myeloid deletion of NRF2 in LDL receptor–deficient mice ($Ldlr^{-/-}$) leads to increased proinflammatory gene expression and atherosclerosis.[214] While global NRF2 knockout in $ApoE^{-/-}$ mice unexpectedly decreased atherosclerotic lesion formation compared with wild-type $ApoE^{-/-}$ mice.[215,216] Consistently, the absence of NRF2 in the bone marrow-derived cells decreases the lesion size, exerting a protective effect at the advanced stage of atherosclerosis.[217] Additional work is needed to fully elucidate the complex role of NRF2 in these various models.

4.3 YAP/TAZ

Disturbed flow in a rat abdominal aorta crossclamping model induces YAP/TAZ activation to promote inflammation and atherogenesis by enhancing JNK (c-Jun N-terminal kinase) activity, whereas unidirectional SS inhibits YAP/TAZ activity by modulating the integrin–Gα13–RhoA pathway.[203] Oscillatory SS by partial ligation of the carotid artery activates integrin α5β1 and its downstream kinase c-Abl in ECs to induce phosphorylation of YAP at Y^{357} and strong, continuous YAP nuclear translocation, thereby promoting the proatherogenic responses.[145] Interestingly, a study using zebrafish embryos and an *in vitro* parallel plate–type apparatus found that short-term unidirectional laminar flow (15 dyne/cm^2 for 10 min) increases the nuclear localization of YAP in a LATS1/2-independent but an angiomotin-regulated manner.[32] Furthermore, mechanoregulated YAP/TAZ transcriptional activity is inhibited by interaction of the ARID1A-containing SWI/SNF complex and YAP/TAZ inside the nucleus, while nuclear F-actin competitively binds to SWI/SNF to interfere with the formation of the ARID1A–SWI/SNF–YAP complex and facilitates association of YAP/TAZ with TEAD (TEA domain transcription factor (TEAD).[218] The identification of the transcription factors YAP and TAZ as EC mechanotransducers has helped to clarify how mechanical cues are sensed and transduced at the molecular level to regulate gene expression in atherosclerosis. However, the crosstalk of YAP/TAZ activation with endothelial apoptosis and enhanced lipid uptake in the vessel remains poorly understood.

4.4 BMPs

Laminar SS inhibits expression of BMP4 in ECs to exert antiatherogenic effects, while oscillatory SS activates BMP4 in atherosclerotic lesions, associating with NF-κB–mediated increase of ICAM-1 expression and monocyte adhesion.[219,220] In the experimentally stenosed rat abdominal aorta and a parallel-plate flow chamber, atheroprone flow induces sustained activation of bone morphogenetic protein receptor (BMPR)–specific SMAD1/5 in ECs through activation of mammalian target of rapamycin and p70S6 kinase, leading to upregulation of cyclin A and downregulation of p21CIP1 and p27KIP1 and, hence, EC cycle progression.[221] Low SS activates SMAD2/3 to regulate inward remodelling in atherosclerotic vessels through the type I TGF-β family receptor ALK5 and the transmembrane protein neuropilin-1, which together increase sensitivity to circulating BMP9.[222] Interestingly, disturbed flow by rotation of a Teflon cone stimulates ECs to coexpress BMP antagonists and BMP4, the balance of which controls inflammation in atherosclerosis.[223]

4.5 Notch

Generally, laminar SS activates endothelial Notch1 and leads to polarization and Notch1 intracellular domain (NICD) translocation, thereby promoting stabilization of endothelial functions.[224] Independent of canonical Notch signalling, high SS induces the cleavage of Notch1 and formation of a mechanosensory junctional complex consisting of Notch 1 transmembrane domain (TMD), the transmembrane protein tyrosine phosphatase LAR, and VE-cadherin, which further recruits the RAC1 guanidine exchange factor TRIO and RAC1 to control downstream cortical actin, thus resulting in barrier reinforcement.[225] Recent work examining both postnatal retina neovascularization and cultured ECs in a parallel plate flow chambers describes a novel force-responsive pathway, in which arterial shear activates Notch signalling and causes upregulation of GJA4 (commonly known as connexin37) and the downstream cell cycle inhibitor CDKN1B (p27), promoting EC cycle arrest to enable arterial gene expression.[226] However, evidence also showed that low SS by cast-induced stenosis of common carotid artery activates the Notch1 signalling pathway via

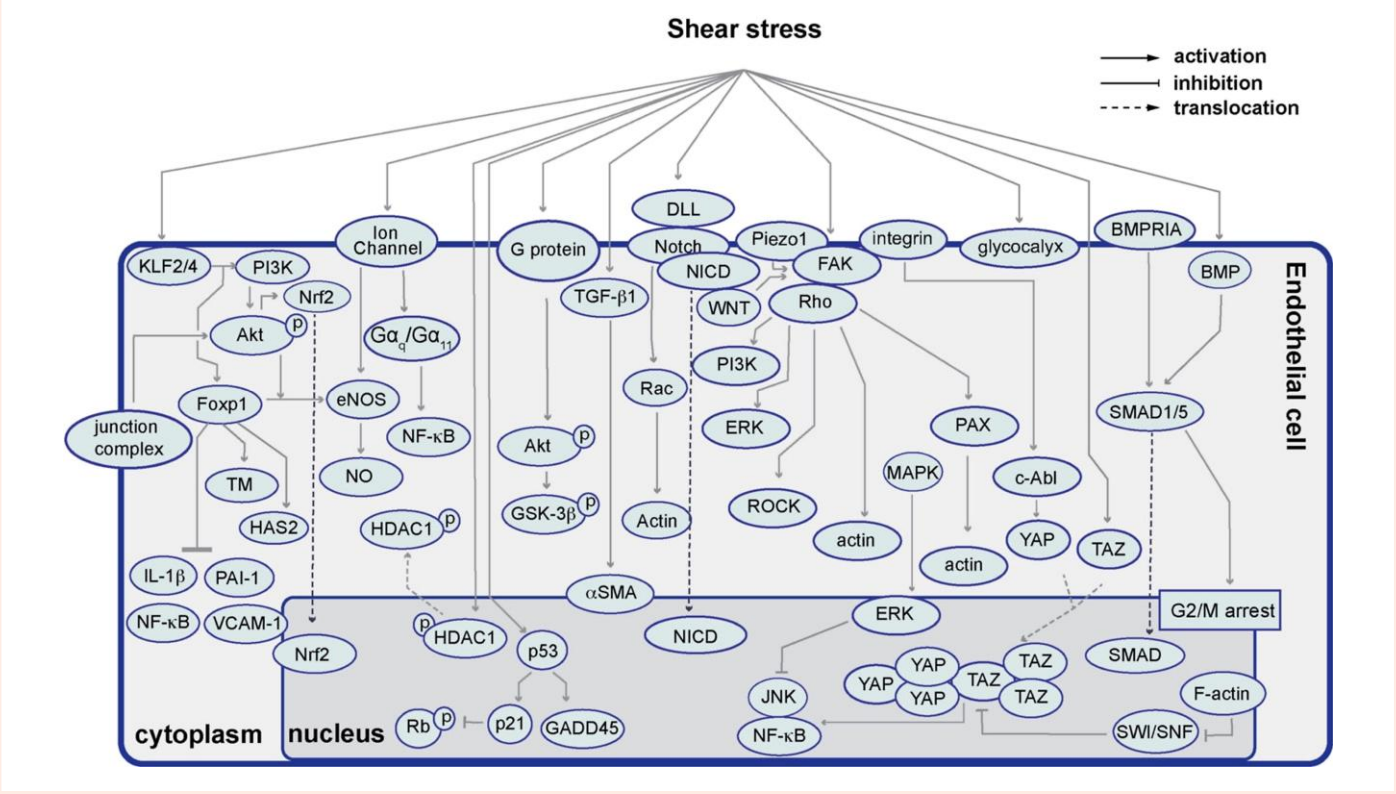

Figure 6 EC mechanotransduction pathways involved in FSS–mediated endothelial function.

caveolin-1 and that inhibition of Notch1 significantly alleviates low SS–induced plaque formation and inflammatory response through NF-κB.[227] Together, these observations suggest that the effects of flow-induced Notch1 activation on EC physiology are highly context dependent.

4.6 WNT

Loss of WNT5a/WNT11 enhances endothelial shear sensitivity, resulting in axial polarization and migration against flow under low shear conditions.[228] Canonical WNT signalling is implicated in oscillatory SS-induced angiopoietin-2 expression, which controls vascular development and repair.[229] Consequently, inhibition of canonical WNT signalling suppresses systemic inflammation and atherosclerosis progression in angiotensin II–infused ApoE$^{-/-}$ mice.[230] In addition, atheroprone flow in ApoE$^{-/-}$ mice and by a cone-and-plate system constitutively induces endothelial β-catenin nuclear localization, T-cell-specific transcription factor (TCF) activation and downstream expression of the proinflammatory molecule fibronectin to precede atherosclerosis lesion development, acting through the PECAM-1/GSK-3β pathway.[231] As might be expected, a recent study involving multiple, single laboratory microarray analyses of shear force-responsive endothelial gene expression suggests that there is cross talk among WNT, TGF-β, and Notch signalling in ECs.[232]

4.7 NF-κB

The transcription factor NF-κB family, consisting of RelA (p65), RelB, c-Rel, and the precursor proteins NF-κB1 (p105) and NF-κB2 (p100), is central to the cell's response to disturbed flow and its activity regulates plaque progression.[233] In a flow-altering, constrictive cuff model, atheroprone flow induces NF-κB activation, which increases the expression of proinflammatory molecules (e.g. ICAM-1, VCAM-1 and MCP-1). This is in part controlled by JNK-ATF2 signalling, which positively regulates the expression of the RelA NF-κB subunit in response to low/oscillatory SS and initiates focal arterial inflammation at atherosusceptible sites.[234] Moreover, oscillatory SS, but not atheroprotective physiological SS, upregulates miR-34a expression to promote endothelial inflammation, which depends on increased acetylation of RelA at residue Lys310.[235] Additionally, in the partially ligated ApoE$^{-/-}$ mice model and *in vitro* orbital shaking or a parallel plate flow system, low SS–mediated NF-κB activation leads to HIF-1α accumulation, which promotes lesion initiation by enhancing inflammation and inducing excessive EC proliferation.[35]

4.8 MAPKs

MAPK pathways are involved in converting mechanical cues into a wide range of cellular responses. Using a parallel-plate flow chamber, short-term laminar SS exerted on ECs (30 min) triggers an eightfold increase of JNK activity compared to cells in static culture, resulting in actin remodelling and cell alignment.[236] However, other studies showed that laminar flow inhibits the activation of JNK induced by inflammatory cytokines (e.g. TNF-α and IL-1) through the MEK5–BMK1/ERK5 pathway, which inhibits the MAP kinase kinase kinase (MAPKKK) and apoptosis signal–regulating kinase (ASK1) by inducing thioredoxin-interacting protein.[237–239] In-depth research provided an explanation for this discrepancy in the response of JNK to laminar SS generated in a parallel plate flow chamber. Hahn *et al.* showed that JNK activation in response to laminar and long-term oscillatory flow is matrix specific, preferentially occurring at high-fibronectin deposition sites, which are regulated by the new binding of integrin with fibronectin as well as the downstream MAPK kinases MKK4 and p21-activated kinase.[240]

4.9 PI3K/AKT

Laminar flow in a cone–plate viscometer induces eNOS phosphorylation and AKT activation via a PI3K-dependent pathway and promotes NO production and an antiatherogenic phenotype in ECs.[241] There is an initial

rapid Src kinase-dependent and VEGFR2-dependent tyrosine phosphorylation of Grb2-associated binder 1 (Gab1) in response to laminar flow (12 dynes/cm^2). Subsequently, Gab1 interacts with PI3K subunit p85 to activate PI3K/AKT/eNOS signalling.[242] Under conditions of perturbed flow in a parallel-plate flow chamber, pretreating ECs with PI3K inhibitors LY294002 suppresses the association of HDAC-1/2/3 with NRF2 and HDAC-3/5/7 with MEF2 and its deacetylation, thereby upregulating NQO1 and KLF2 expression in ECs.[213]

In addition, PI3K has been implicated in SS–induced integrin activation in ECs. Using a monoclonal antibody Fab fragment (WOW-1) that binds selectively to high-affinity αv integrins, studies revealed that treatment with PI3K inhibitors LY294002 and wortmannin completely blocks integrin activation.[152,243] Interestingly, the PI3K/AKT pathway can converge with the same integrins that the MAPKs interact with and can lead to activation of eNOS.[123,141]

Collectively, studies to date have revealed a complex signalling network by which different hemodynamic forces impact on EC functions and atherogenesis. Although informative, a long list of mechanosensors has accumulated, and the mechanisms for some of these remain to be elucidated. In addition, different mechanosensing elements across the cell are integrated and form molecular circuits that coordinate the cellular responses to mechanical cues. More work is needed to deconvolve the signal transduction networks to identify better targets for the treatment of vascular diseases.

5. Pharmacological targeting of mechanotransduction pathways in atherosclerosis

As described above, many mechanotransduction pathways are involved in endothelial responses to flow and SS–induced atherosclerosis. In this section, we overview the current antiatherosclerotic agents that can alter mechanotransduction in ECs (*Table 2*).

5.1 Pharmacological targets for the KLF2 pathway

Because KLF2 is a master regulator of SS–induced EC homeostasis, it represents a promising pharmacological target for atherosclerosis treatment. Statins are the most widely used agents for atherosclerotic vascular diseases and act by decreasing circulating lipids and inflammatory factors. This involves KLF2 pathways, as genetic silencing of KLF2 reduces the beneficial effects of statins, including eNOS and TM production.[244] Statins also affect ERK5 and MEF2-dependent signalling.[245,246] Likewise, resveratrol and tannic acid promote endothelial vasoprotection in part by increasing KLF2 expression in a MEK5/MEF2-dependent, ERK5-independent pathway.[279–281] Laquinimod, an experimental immunomodulator, has a clinical potential application as an antiatherosclerotic agent. The mechanism of action of laquinimod is to reduce the expression of adhesion molecules (VCAM-1 and E-selectin) and central inflammatory cytokines and chemokines (IL-6 and MCP-1) via upregulating KLF2 expression in an ERK5-dependent manner.[257]

Additionally, several other well-studied drugs exert cardioprotective effects by KLF2 induction. For example, suberanilohydroxamic acid (SAHA, also known as vorinostat or MK0683), an approved anticancer drug, has been shown to activate KLF2, reduce monocyte adhesion, and limit vascular inflammation and atherosclerosis in a MEF2–dependent manner.[249] Montelukast—another drug commonly used for treatment of inflammatory diseases in the airway—can activate KLF2 in an ERK5–dependent manner, leading to reduced monocyte adhesion and suppression of adhesion molecules (VCAM-1 and E-selectin) in the early stages of atherosclerosis.[250,251] Similarly, liraglutide, a clinically approved glucagon-like peptide 1 (GLP-1) receptor agonist, can reduce endothelial inflammation via ERK5-dependent KLF2 activation.[252,253] Metformin pretreatment can alleviate endothelial inflammatory responses, partially ascribed to AMP–activated protein kinase (AMPK) activation–mediated HDAC5 phosphorylation and KLF2 upregulation.[255] Other cellular programs may also be involved, as metformin-induced upregulation of KLF2 can inhibit foam cell formation and alleviate atherosclerosis in ApoE$^{-/-}$ mice by enhancing autophagy.[256] However, their therapeutic effectiveness for atherosclerosis and other vascular diseases warrants further assessment of clinical efficacy.

5.2 Pharmacological targets for the NRF2 pathway

Despite the controversial role of NRF2 in atherogenesis in preclinical animal models, pharmacological activation of NRF2 appears to be generally related to atheroprotection. Statins activate the NRF2 pathway and induce a significant increase of cytoprotective genes such as HO-1.[247] Resveratrol enhances NRF2 activity in a dose-dependent manner and significantly upregulates its target genes NQO1, GCLC, and HO-1, thereby conferring endothelial protective effects.[275] Sulforaphane can repress atherosclerosis progression and improve endothelial dysfunction.[212,282–284] The atheroprotective mechanism involves NRF2–mediated inhibition of p38–VCAM-1 signalling in ECs.[212] In addition, dimethyl fumarate, a drug commonly used in multiple sclerosis treatment, can significantly reduce the plaque area through the NRF2/ARE pathway.[258]

5.3 Pharmacological targets for the YAP/TAZ/TEAD pathway

Pharmacological inhibition of YAP/TAZ signalling can prevent atherosclerosis, and statins are among the most potent YAP inhibitors of the 640 clinically applied drugs.[287] Statins suppress YAP/TAZ transactivation through inhibition of RhoA, an upstream activator of YAP/TAZ, thus reducing EC proliferation and inflammation caused by disturbed flow.[203] Moreover, constitutive activation of YAP/TAZ in ECs can partially antagonize the anti-inflammatory effect of simvastatin, further supporting that YAP/TAZ inhibition contributes to antiatherogenic effects of statins.[31] Bosutinib, a tyrosine kinase inhibitor, profoundly reduces YAP^{Y357} phosphorylation through blunting oscillatory SS– or fibronectin-mediated activation of integrin α5β1 and c-Abl, hence, completely inhibiting lesion formation in aortic arch but not thoracic aorta in ApoE$^{-/-}$ mice.[145] Verteporfin, a second-generation photosensitizer, is the first identified pharmacological inhibitor of YAP that acts by disrupting the interaction between YAP and TEAD.[259] Intra-arterial administration of verteporfin concurrent with photoactivation can lead to rapid and high accumulation of verteporfin in the plaque, which then inhibits atherosclerosis.[260]

In addition, methotrexate, a chemotherapeutic and anti-inflammatory drug used to treat rheumatoid arthritis, exhibits atheroprotective effects by interfering with endothelial YAP/TAZ activation under disturbed flow, reducing expression of YAP/TAZ–associated inflammatory genes, and decreasing monocyte adhesion.[33] Apart from these drugs, other compounds such as niacin,[203,261] apelin,[263] and ApoA-I Milano (a mutational variant of ApoA1)[264,265] have shown promise in preclinical and clinical studies, acting through YAP/TAZ to reduce inflammatory effects. However, the role of Apelin in atherosclerosis is controversial. Other studies have found that Apelin could induce adhesion of monocytes–ECs by inducing the expression of adhesion molecules and chemokines through activation of the PI3K and NF-κB/JNK signal pathway.[288,289]

5.4 Pharmacological targets for the NF-κB pathway

The NF-κB pathway is a representative mechanism that is enhanced by disturbed flow. Hence, endothelium-restricted inhibition of NF-κB is also a potential therapeutic strategy against atherosclerosis. Statins can prevent TNF-α–induced NF-κB activation in ECs through a mechanism independent of the classical IKK cascade; the observed suppression of NF-κB depends on impaired transport of the p65 subunit of NF-κB into the nucleus, induced by decreased endothelial AKT phosphorylation.[248] Similarly, resveratrol effectively interferes with proinflammatory

Table 2 Pharmacological treatments based on mechanotransduction pathways

Drug	Target	Pathway	Status	Ref
Statins	(+) KLF2 (+) NRF2 (–) YAP/TAZ (–) NF-κB	(+) MEK5/ERK5/MEF2 (+) NRF2/HO-1 (–) RhoA/YAP/TAZ (–) PI3K/AKT	Approved	203,244–248
Suberanilohydroxamic acid (SAHA, vorinostat)	(+) KLF2	(+) MEF2	Approved for T cell lymphoma Preclinical trials	249
Montelukast (singular)	(+) KLF2	(+) ERK5	Approved for asthma and allergies Clinical trials (NCT00351364)	250,251
Liraglutide (Victoza, Saxenda)	(+) KLF2 (–) NF-κB	(+) ERK5 (–) NF-κB	Approved for diabetes and weight management Clinical trials (NCT04881110; NCT04146155)	252–254
Metformin	(+) KLF2	(+) AMPK/HDAC5 (+) Autophagy	Approved for diabetes Clinical trials (NCT03962686)	255,256
Laquinimod	(+) KLF2	(+) ERK5	Clinical trials (NCT01047319)	257
Dimethyl fumarate (Tecfidera, Fumaderm)	(+) NRF2	(+) NRF2/ARE	Approved for psoriasis, multiple sclerosis Preclinical trials	258
Bosutinib	(–) YAP	(–) Integrin α5β1/c-Abl/YAP	Approved for chronic myeloid leukaemia Preclinical trials	145
Verteporfin	(–) YAP/TAZ	(–) YAP/TEAD	Approved for macular degeneration Preclinical trials	259,260
Methotrexate	(–) YAP/TAZ	(–) YAP/TAZ	Approved for psoriasis Clinical trials (NCT02312219; NCT02576067)	33
Niacin	(–) YAP/TAZ (–) NF-κB	(–) YAP/TAZ (–) Notch1/NF-κB p65	Approved	203,261,262
Apelin agonist (BMS-986224)	(–) YAP/TAZ	(–) YAP/TAZ	Clinical trials (NCT03281122)	263
ApoA-I Milano (MDCO-216)	(–) YAP/TAZ	(–) YAP/TAZ	Clinical trials (NCT02678923)	264,265
Aspirin	(–) NF-κB	(–) NF-κB/CX3CL1	Approved	266–268
Canakinumab	(–) IL-1β	(–) Nck1/IRAK-1/NF-κB	Approved for cryopyrin-associated periodic syndromes and Still's disease Clinical trials (NCT00995930; NCT01327846)	269,270
Anakinra	(–) IL-1β	(–) p38 MAPK/NF-κB	Approved for rheumatoid arthritis Clinical trials (NCT02126280)	269,271

Investigational naturally occurring compounds	Target	Pathway	Status	Ref
Vinpocetine	(–) NF-κB	(–) IKK activity (+) AKT dephosphorylation	Clinical trials (NCT02878772)	272–274
Resveratrol	(+) KLF2 (+) NRF2 (–) NF-κB	(+) AMPK/ERK5/MEF2 (+) SIRT1/MEK5/MEF2 (+) NRF2/ARE (+) SIRT1/RelA/p65 deacetylation	Approved	275–280
Tannic acid	(+) KLF2	(+) MEK5/MEF2	Preclinical trials	281
Sulforaphane	(+) NRF2	(–) p38/VCAM-1	Clinical trials (NCT01114399)	212,282–284
Colchicine	(–) NF-κB	(–) NF-κB/IκB	Clinical trials (NCT02162303; NCT05347316)	285
Diosgenin	(–) NF-κB	(–) IKKβ/NF-KB	Preclinical trials	286

(+), activation; (–), inhibition; KLF2/4, Krüppel-like factor 2/4; NRF2, nuclear factor erythroid 2-related factor 2; NF-κB, nuclear factor-κB; YAP/TAZ, Yes-associated protein/transcriptional coactivator with PDZ-binding motif; TEAD: TEA-domain transcription factor; MEK5, MAP/ERK kinase 5; ERK5, extracellular signal-regulated kinases 5; MEF2, myocyte enhancer factor 2; HO-1, heme oxygenase-1; PI3K, phosphoinositide 3-kinases; AKT, serine/threonine protein kinase B; AMPK, AMP-activated protein kinase; HDAC5, histone deacetylase 5; IRAK-1, interleukin-1 receptor–associated kinase 1; ARE, antioxidant response element; c-Abl, cytosolic nonreceptor protein kinase; IKK, IκB kinase; SIRT1, sirtuin1; VCAM-1, vascular cell adhesion protein-1; IKKβ, nuclear factor kappa B kinase beta subunit.

cytokine–induced NF-κB activation through the blockade of p65 subunit phosphorylation and consequent prevention of NF-κB nuclear translocation.[276,277] Moreover, resveratrol regulates the transcriptional activity of NF-κB via activation of sirtuin1 (SIRT1), which is a nicotinamide adenosine dinucleotide-dependent HDAC.[278] SIRT1 physically interacts with the RelA/p65 subunit of the NF-κB complex, leading to RelA/p65 deacetylation at lysine 310 and NF-κB transcriptional inhibition.[290]

Colchicine, an anti-inflammatory compound showing potential benefits for CVDs, can decrease the expression of NF-κB in ECs or NF-κB in nuclear fraction and thereby regulates the expression of inflammatory cytokines.[285] Aspirin and diosgenin, antithrombotic agents that can reduce platelet activation, exert antiatherogenic effects through NF-κB inhibition.[267,268,286] Low-dose aspirin administration inhibits NF-κB activation and subsequent expression of fractalkine (CX3CL1), another NF-κB target gene important in atherosclerosis. This reduces atherosclerotic plaque size in hyperlipidaemic mice.[266] However, high-dose aspirin usage is associated with gastrointestinal bleeding. GLP-1 hormone is involved in immunomodulation of atherosclerosis.[291] Liraglutide promotes eNOS expression and suppresses endothelin-1 (ET-1) expression by inhibiting NF-κB phosphorylation and its translocation from the cytoplasm to the nucleus.[254] Canakinumab and anakinra, immunomodulators that act through IL-1β suppression, reduced cardiovascular events in a high-risk patient cohort. The protective effects were ascribed to suppressed activation of NF-κB and decreased production of proinflammatory cytokines triggered by the Toll/interleukin-1 receptor homology domain and the adaptor protein myeloid differentiation primary response 88 (MyD88).[269–271]

Additionally, niacin, a water-soluble vitamin that acts as a broad-spectrum lipid-regulating medication, has been shown to inhibit inflammation by suppressing NF-κB p65 and Notch1 expression in ECs.[262] Vinpocetine can attenuate high-fat diet–induced atherosclerosis in ApoE$^{-/-}$ mice.[272,273] Vinpocetine inhibits NF-κB–mediated inflammatory responses by directly affecting IKK activity, independent of its well-known action on cyclic nucleotide phosphodiesterase 1 (PDE-1).[274] AKT dephosphorylation in part contributes to the inhibitory effects of vinpocetine on the NF-κB cascade.[273]

6. Conclusions

Interactions between flowing blood and ECs play important roles in the regulation of vascular integrity and homeostasis. Generally, ECs maintain quiescence in laminar, pulsatile flow but respond to disturbed flow by initiating programs for structural and functional adaption. Specifically, sustained SS alternation leads to EC dysfunction in a series of changes that involve the morphology, cell turnover rate (proliferation and apoptosis), macromolecular permeability, and inflammation.[7]

CVDs and their clinical sequelae remain the major cause of morbidity and mortality worldwide. The significance of mechanical stimuli in the physiopathology of CVDs such as atherosclerosis has catalyzed much research in the past decades. However, despite our increased understanding of the effects of blood flow on endothelial biology, numerous questions in this field remain unanswered. Dysregulation of mechanosensing mechanisms in ECs is implicated in a wide range of vascular diseases, especially in atherogenesis. Many recent studies discussed above indicate that drugs targeting key mechanosensitive transcription factors such as KLF2/4, NRF2, YAP/TAZ/TEAD, NF-κB, and others can produce antiatherosclerosis effects. Nevertheless, it remains challenging to translate findings from mechanobiological studies into interventions that actively target dysregulated mechanosensing mechanisms to treat diseased vasculature. This may be ascribed to the existence of multiple mechanotransduction mechanisms and complex interactions between distinct mechanisms. Thus, the interplay of redundant or complementary mechanotransduction pathways has to be viewed in a 'systems biology' context.

The vast majority of cellular response studies have been performed on cultured cells *in vitro*. In general, *in vitro* systems for studying endothelial biology are simplified, do not fully recapitulate the mechanics in biological systems, and require rigorous validation using *in vivo* methods. Novel experimental techniques such as microfabrication technology have greatly facilitated the study of cell mechanics and mechanobiology *in vitro* but still have limitations. For example, many of these studies use ECs adhered to artificial substrates such as PDMS, so vessel mechanics are not accurately reproduced. Other aspects of vessel mechanics are also generally lacking; ECs are exposed to not only FSS but also cyclic stretch, so techniques that incorporate appropriate tissue mechanics and fluid forces would improve physiological relevance. There are additional limitations related to the ability to integrate multidimensional *in vivo* components such as the ECM, mechanical stimulus, and chemical signals. Efforts to recapitulate the vessel wall structure *in vitro*, including the anatomy of the smooth muscle layers and pericytes, are promising, but still in the development stage.[128] Finally, while the adhesion of circulating leukocytes, including monocytes, has been extensively studied using *in vitro* technologies, additional examination of the subsequent effects on the generation of foam cells and atherosclerosis is needed.

For *in vivo* models, there are exciting advances that allow control of flow in the microvasculature of live animals and the monitoring of specific biological responses including microvascular permeability and intracellular calcium.[292,293] It is also possible to study the endothelial glycocalyx *in vivo* by using a variety of imaging methods.[294] Such studies open the door for future investigations of flow-responsive events *in vivo*. However, access to the microvasculature is considerably easier than that to the medium and large arteries in which atherosclerosis develops; therefore, new tools need to be developed in order to provide the capability of monitoring flow-induced EC responses in larger arteries *in vivo*.

Multiple mechanosignalling pathways have been implicated in the regulation of EC function in physio/pathological conditions. Although informative, these hypothesis-based studies on the mechanisms that control focal lesion formation have only partially revealed the complexity of flow-induced pathways. Furthermore, there is mounting evidence that different mechanosensing mechanisms can interact with one another to orchestrate mechanotransduction signalling and cell function. For example, the interactions of KLF2 with NRF2,[210,295,296] and NF-κB with HIF-1α,[34,35] as well as KLF2/4 with NF-κB[34] have been described recently. The current challenge is to determine how these systems work together, and to identify specific mechanosensitive pathways that can be targeted to alleviate or reverse vascular pathologies. A useful approach could be to determine how mechanotransduction occurs as an intracellular 'systems' response for each physiological or pathological context of interest, with appreciation of the potential redundancy of elements within the system. By discovering how ECs coordinate and integrate their response to mechanical signals, we can identify novel therapeutic targets to slow or reverse the progression of atherosclerosis.

The complexities of endothelial mechanobiology present both opportunities and challenges in the battle against CVDs. On one hand, new mechanosensitive pathways are being discovered and implicated in endothelial dysfunction during atherogenesis. These pathways may reveal new targets for preventing or reversing CVDs. On the other hand, the complexity of the system requires new experimental tools and conceptual advances to advance our understanding of how endothelial mechanobiology contributes to homeostasis in atheroprotective environments, but disease progression in atheroprone regions. This distinction is subtle, but key to the development of robust therapeutics with minimal toxicity.

Author contributions

X.W. (conceptualization: equal; writing—original draft: lead; writing—review & editing: equal), Y.S. (writing—review & editing: equal), M.S. (writing—review & editing: equal), X.L. (conceptualization: supporting; writing—review & editing: equal), L.L.M. (conceptualization: equal; writing—review & editing: lead).

Conflict of interest: Dr. Munn has received equity from Bayer AG.

Funding

The authors are funded by the National Natural Science Foundation of China (11932014, 31971239, 32071312 and 31870939; X.L. and Y.S.)

and the National Institutes of Health (R21EB031982 and U01CA261842; L.L.M.)

Data availability

No new data were generated or analyzed in support of this manuscript.

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