Vascular Smooth Muscle Cells Mechanosensitive Regulators and Vascular Remodeling

Shangmin Liu^{a, b} Zhanyi Lin^{a, c}

^aJi Hua Institute of Biomedical Engineering Technology, Ji Hua Laboratory, Foshan, China; ^bMedical Research Center, Guangdong Academy of Medical Sciences, Guangdong General Hospital, Guangzhou, China; ^cInstitute of Geriatric Medicine, Guangdong Academy of Medical Sciences, Guangdong General Hospital, Guangzhou, China

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Abstract

Blood vessels are subjected to mechanical loads of pressure and flow, inducing smooth muscle circumferential and endothelial shear stresses. The perception and response of vascular tissue and living cells to these stresses and the microenvironment they are exposed to are critical to their function and survival. These mechanical stimuli not only cause morphological changes in cells and vessel walls but also can interfere with biochemical homeostasis, leading to vascular remodeling and dysfunction. However, the mechanisms underlying how these stimuli affect tissue and cellular function, including mechanical stimulation-induced biochemical signaling and mechanical transduction that relies on cytoskeletal integrity, are unclear. This review focuses on signaling pathways that regulate multiple biochemical processes in vascular mesangial smooth muscle cells in response to circumferential stress and are involved in mechanosensitive regulatory molecules in response to mechanotransduction, including ion channels, membrane receptors, integrins, cytoskeletal proteins, nuclear structures, and cascades. Mecha-

noactivation of these signaling pathways is closely associated with vascular remodeling in physiological or pathological states.

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Introduction

The perception and response of living cells and tissues to mechanical forces and the microenvironment they are exposed to are essential for their survival and functional maintenance [1, 2]. Fung et al. [3] put forward the “stress-growth” theory in 1990, pointing out that the remodeling process of living tissue, including cell growth and extracellular matrix (ECM) production and absorption, is closely related to the stress state in the tissue.

Stress regulates almost all aspects of cellular function, including growth, differentiation, migration, gene expression, protein synthesis, and apoptosis, and also directly affects tissue morphology and function [4]. For example, mechanical stress plays a crucial role in regulating the normal development, structure, and function of blood vessels [5]. During the embryonic period, the undifferentiated and immature vascular network (precursor vascular plexus) gradually forms a stratified vascular system

consisting of arteries and veins [6, 7], in which blood pressure can affect the differentiation of mesenchymal cells into vascular smooth muscle cells (VSMCs), resulting in structural differences between arteries and veins [8, 9]. Such structural differences can also have a differential impact on the progression of vascular disease later in life. For example, veins do not usually develop atherosclerotic disease. However, when an autologous superficial saphenous vein is used as a conduit for arterial reconstruction during cardiac or limb bypass surgery, the arterialized vein is subjected to relatively high arterial pressure and is more likely to develop atherosclerotic disease, ultimately leading to vein graft failure [10, 11]. This demonstrates that the mechanical forces to which a blood vessel is subjected are critical to its structure and function. In adulthood, normal blood pressure is also essential for maintaining vascular structure and function, and the pulsatile nature of blood pressure generates radial and tangential forces, mainly in the form of cyclic stretch and shear flow, which have a sustained effect on the vessel wall composition, such that hypertension leads to arterial wall thickening and stiffness increased [12, 13]. Friction between blood and the vessel wall generates fluid shear stresses parallel to the vascular surface, which act mainly on the endothelial cells (ECs) at the blood vessel wall junction [14]; circumferential stresses are perpendicular to the vessel wall and act on all cells in the vessel wall [15, 16].

VSMCs, located in the middle layer of blood vessel, are the most numerous cell types in the arteries. VSMCs have 2 phenotypes: contractile and synthetic. Contractile VSMCs are mature, with a high degree of differentiation, poor proliferation and migration ability, and low synthetic activity. Synthetic VSMCs have few inner myofilaments and have strong synthesis and secretion functions; they mainly synthesize and secrete matrix proteins [17]. During blood vessel formation, the phenotype of VSMCs in the middle wall changes, which reduces ECM protein secretion and increases intracellular sarcoplasm formation. This transition, from synthesis to contraction, maintains the elasticity of the vascular wall and contracts blood vessels [17, 18]. Under pathological conditions, cells in mature blood vessels can undergo a transition from a normal contraction state to a proliferative synthetic state [19, 20]. Under normal circumstances, the morphology, structure, and function of VSMCs change, which causes VSMCs to proliferate and migrate excessively, accompanied by different degrees of aging, apoptosis, and other biological phenomena. This process is called the phenotypic transformation of VSMCs, also known as dedifferentiation [19]. When VSMCs are removed from their native envi-

ronment and placed in vitro, similar changes in the cell phenotype can be observed. This is probably due to the lack of normal physiological signals in the blood vessel walls to maintain and regulate the VSMC phenotype [18]. The VSMC phenotype is a continuum, not a set of discrete phenotypic states [18]. VSMCs play a key role in maintaining vascular homeostasis and regulating pathological vascular remodeling in response to circumferential stress.

To study the effect of circumferential stress stimulation on VSMCs, in vitro mechanical loading systems are often used to circularly stretch the VSMCs to simulate the circumferential stress in vivo, generally with a loading rate of 10%–20% of the mechanical deformation as the overload group and 2%–5% of the mechanical deformation as the normal control group or with 100–200 kPa as the overload group and 50 kPa as the normal control group. In vivo, the hypertension model is used. The model uses blood pressure as a variable to compare the differences in the phenotype and biochemical function of VSMCs under hypertension and normal blood pressure. In an in vitro vascular culture system with independent control of arterial circumferential and shear stresses [21], carotid arteries were cultured under a circumferential stress of 50 or 150 kPa and a shear stress of 0.75 or 2.25 Pa for 3 days. The results showed that matrix synthesis, SMC proliferation, and cell death significantly increased under high circumferential stress, while high shear had no significant effect on cell proliferation and expression of biomarkers of remodeling [21]. Circumferential wall stress is also a mechanism for arteriolar rarefaction and proliferation in a network model [22]. VSMCs can regulate proliferation, apoptosis, and migration, as well as ECM synthesis, degradation, and reorganization, by responding to circumferential stress. This review discusses how VSMCs sense such mechanical stimuli and undergo mechanical signaling to influence gene expression and regulate cellular function, with a focus on the studies of VSMCs in the midvascular layer responding to circumferential circulatory mechanical stimuli and regulating multiple mechanosensitive pathways that are further involved in the development of cardiovascular diseases (CVDs), such as hypertension.

Mechanosensitive Mechanisms and Their Role in VSMCs

The correct regulation of cellular function in vivo requires the integration of numerous biological and mechanical signals generated by the surrounding cells and the ECM, the complex mechanical behavior of tissues and

the ECM, leading to cellular mechanotransduction and affecting cell behavior [23]. VSMCs sense mechanical stimuli and through cell-ECM interactions can affect the protein conformation of the cell membrane, where ion channels, membrane receptors, or transmembrane proteins play a central role in mechanical transduction and trigger downstream signaling cascades leading to force-dependent changes in gene expression [24]. Cells interacting with the ECM in a mechanical manner can also mediate a network of coordinated changes in integrins, connexin, cytoskeleton proteins, and nuclear structure to achieve mechanosignal transduction (Fig. 1); this mechanosignal propagates much faster in the cytoplasm than diffusion-based chemical signals [25, 26].

Mechanosensitive Molecules on Cell Membranes

Mechanosensitive Ion Channels

Mechanosensitive ion channels are a class of cation channels that are affected by mechanical stress and generate excitatory electrical signals. These channels are involved in the mechanical transduction of cells, converting mechanical signals on the cell membrane into bioelectrical signals. VSMCs subjected to mechanical stress exhibit transient increases and depolarization of intracellular cations, affecting their own proliferation and migration, maintaining smooth muscle tone, and participating in cardiovascular mechanobiology, such as vascular development and blood pressure regulation, and related clinical diseases [18, 27].

Piezo Ion Channels. Piezo1 ion channels are mechanically activated nonselective cation channels. Piezo1 was identified in mouse neuroblastoma in 2010 and subsequently detected by sequence homology as the structurally and genetically similar protein Piezo2 [28]. When an external force is applied to the Piezo ion channel, the channel's spatial structure undergoes a reversible flattening effect in response to biological stress, which is important with regard to the sensing of mechanical stimuli [29]. Piezo1 and Piezo2 act as force sensors, pivotal in the determination and maintenance of cardiovascular architecture and function [30]. Piezo1 is expressed in ECs and VSMCs and is activated by shear stress associated with increased local blood flow and by stretching of the cell membrane in response to increased blood pressure [31].

Piezo1 is required for regulating NO formation, vascular tone, and blood pressure [32]. In ECs, Piezo1 can mediate fluid-shear-stress-induced release of adrenomedullin, which increases cyclic adenosine monophosphate (cAMP) levels, promoting endothelial nitric oxide synthase phosphorylation through protein kinase A and regulating vascular tone and blood pressure [33]. Endothelial Piezo1 also mediates flow-induced ATP release and subsequent P2Y₂/Gq/G11-dependent activation of downstream signaling that induces protein kinase B (Akt) and endothelial nitric oxide synthase phosphorylation and activation, thus regulating vascular tone and blood pressure [32, 34]. Shear-stress-induced Piezo1 confers force sensitivity on ADAM10 and Notch1, with downstream consequences for sustained activation of Notch1

Fig. 1. Circumferential stress-induced signaling pathway in VSMCs. Circumferential stress activates transmembrane proteins such as mechanosensitive ion channels (including Piezo1, TRP, K2P, and ENaC), GPCRs, and RTK, integrins, FAK, cell junction (cadherin-11), cytoskeleton, and nuclear protein (lamin and emerin). Multiple downstream signaling pathways would be then activated simultaneously, inducing the expression and repression of multiple genes and leading to many cellular effects. The MAPK, Rho, Hippo signaling pathways, ER stress, and small-molecule gas signaling molecules are involved in these processes. These effects would lead to vascular remodeling, such as hypertension, pulmonary hypertension, and grafted venous atherosclerosis, and the same principle can be applied to the construction of bioengineered blood vessels. ECM, extracellular matrix; Piezo1, pressure-sensitive protein Piezo ion channels; TRP, transient receptor potentials; K2P, 2-pore-domain potassium channel; ENaC, epithelial sodium channel; GPCRs, G protein-coupled receptors; RTK, receptor tyrosine kinases; TGII, transglutaminase II; ATF6, activating transcription factor 6; Klf2, Kruppel-like factor 2; IRE1 α , inositol-requiring enzyme 1 α ; Notch, Notch protein; PTEN, phosphatase and tension homolog; YAP1, Yes-associated protein

1; MEK, mitogen-activated protein kinase; MAPK, mitogen-activated protein kinases; ERK, extracellular regulated protein kinase; JNK, c-Jun N-terminal kinases; P38, P38 MAPK; HIF-1 α , hypoxia-inducible factor-1 α ; G, G protein; PI3K, phosphatidylinositol-3 kinases; Akt, protein kinase B; PKA, protein kinase A; PLC, phospholipase C; PKC, protein kinase C; PIP2, phosphatidylinositol 4,5-bisphosphate; Ras, a family of small GTPase; Raf, RAF kinases; MMPs, matrix metalloproteinases; mTOR, mammalian target of rapamycin; Src, sarcoma; FAK, focal adhesion kinase; Grb2, growth factor receptor-bound protein 2; PyK2, proline-rich tyrosine kinase 2; ILK, integrin-linked kinase; SPRR3, atheroma-enriched protein; Rho, the Ras super family of low molecular weight GTPases; ROCK, Rho-associated coiled coil-forming protein kinase; MLCP, myosin light-chain phosphatase; MHC, myosin heavy chain; iNOS, inducible nitric oxide synthase; NO, nitric oxide; Nox, NADPH oxidase; NF- κ B, nuclear factor kappa-light-chain enhancer of activated B cells; SOCS-1, suppressor of cytokine signaling; TGF- β , transforming growth factor- β ; AP-1, APETALA1; c-FOS, proto-oncogene c-fos gene; TCF, T-cell factor; PAH, pulmonary arterial hypertension; VSMCs, vascular smooth muscle cells.

(For figure see next page.)

target genes [35]. However, under cyclic strain (15% stretch; 60 cycles/min), Notch receptor signaling is inhibited in VSMCs [36]. When the fluid flow along the endothelial surface is high enough, depolarization increases, and Piezo1 in vascular ECs can activate the voltage-gated

Ca²⁺ channels in adjacent VSMCs, causing vasoconstriction, and is thereby involved in hypertension-dependent arterial remodeling [37, 38].

In SMCs, is the Notch signaling pathway responsive to mechanical stimulation associated with the mechanosen-

sitive Piezo ion channel, and does it ultimately affect the biochemical function of SMCs through Piezo ion channel? Studies have confirmed that Piezo1 is indeed involved in the SMCs response to mechanical stimulation in vitro.

Piezo controls Klf2 and Notch activity in the endothelium and Yes-associated protein 1 (YAP1) localization in smooth muscle progenitors [39]. Piezo1 is a determinant of the vascular architecture during early development. Piezo1-deficient embryos are lethal due to disturbed vascular development [40, 41]. Both Piezo1 and Piezo2 are required for normal pressure receptor function, and the use of fiber optic irradiation of the cervical sinus and vagus nerve to activate Piezo expression reduces heart rate and blood pressure, whereas dual failure of Piezo leads to unstable hypertension and blood pressure variability [42]. Piezo1 is also highly expressed in SMCs of small-diameter arteries that are actively involved in the regulation of peripheral resistance, whereas it is more sparsely expressed in conduit arteries [31]. Under hypertensive conditions, opening of Piezo1 ion channels in SMCs induces an increase in vessel diameter and wall thickness, which is reversed by specific expression deficiency and completely inhibits stretch-activated ion channels [43]. Piezo1 ion channel opening increases Ca^{2+} concentrations in the cytoplasm of VSMCs, stimulates transglutaminase II activity, and is involved in cytoskeletal as well as ECM remodeling [43]. Mechanical stimulation of Piezo1 is also dependent on the interaction with ECM proteins, with the presence of some ECM proteins, leading to increased mechanical sensitivity of Piezo1 to membrane tension [44]. These studies illustrate a functional link between the opening of Piezo1 ion channels in VSMCs and clinically relevant arterial remodeling, and involvement in systemic hypertension.

Transient Receptor Potentials. The transient receptor potential (TRP) family of channel proteins consists of 4 TRP subunits in homo- or hetero-tetramers, which can be divided into 7 subclasses based on sequence similarity: TRPC, TRPV, TRPM, TRPA, TRPN, TRPP, and TRPML. Various forms of mechanical stimulation can activate or modulate certain TRP channel proteins, which are important stretch sensors in vascular smooth muscle [45].

TRPs exist in VSMCs and play an important role in the regulation of vasoconstriction. An increase in intraluminal pressure causes a gradual depolarization of VSMCs, which is linked to the opening of nonselective stretch-activated cation channels [46]. In COS-7 cells (an expression system for studying mechanically sensitive ion channels), the TRPP1/TRPP2 ratio regulates stretch-activated

cation channel activity via the filamin A/F-actin cytoskeletal network and modulates the arterial myogenic response to intraluminal pressure [47]. In arterial SMCs, PKD2 (TRPP1) channels regulate systemic blood pressure, and SMC-specific PKD2 knockout can lower both physiological blood pressure and hypertension and prevent pathological arterial remodeling during hypertension [48]. Intravascular pressure (physiological pressure 80 mm Hg) regulates the surface density of SUMO-PKD2-mediated Na currents in arterial SMCs (myocytes) to control arterial contractility [49]. The control group with arterial pressurization at low pressure (10 mm Hg) led to a reduction in the surface SUMO-PKD2 protein to ~42.4% of that in fresh-isolated (0 h) arteries [49].

In VSMCs, enhanced membrane stretch mediates elevated intracellular Ca^{2+} and Na^{+} via activation of TRPC1, TRPC3, and TRPC6 channels, leading to VSMC dysfunction [50]; TRPC6 is required to transduce stress-induced vasoconstriction in the middle cerebral artery (MCA) of mice and is localized in cerebral VSMCs, and the pressure-induced vasoconstriction disappears in the MCA of TRPC6-knockout mice [51, 52]. TRPC6 regulates the phenotype of VSMCs via a cell membrane potential with PTEN-dependent coupling [53]. In pulmonary artery SMCs (PASMCs) from pulmonary arterial hypertension (PAH) patients and animal PAH models, Notch signal enhances TRPC6 store-operated Ca^{2+} channels via direct (nongenomic and stimulatory) and indirect (genomic and upregulating) effects [54]. On the other hand, the activation of Ca^{2+} -sensing receptors evokes Ca^{2+} influx through TRPC6 receptor-operated Ca^{2+} channels, and the athophysiological function of pulmonary aortic VSMCs is mediated by enhanced Ca^{2+} signaling via TRPC6 store-operated Ca^{2+} and receptor-operated Ca^{2+} channels during increased luminal pressure [54].

TRPV2 activation induces nonselective cation channel currents and elevated intracellular Ca^{2+} , inducing myogenic contraction of SMCs [55]; TRPV4 activation induces Ca^{2+} inward flow and endoplasmic reticulum (ER) stress response, which plays an important role in vasoconstriction; TRPV4 is also a mechanosensor in preglomerular VSMCs, contributing to renal myogenic autoregulation, and blocking TRPV4 channels reverses the steady-state myogenic tone (MT) in preglomerular VSMCs and inhibiting pressure-induced membrane depolarization, Ca^{2+} elevation, and distal interlobular artery constriction [56]; TRPV4 channels maintain blood flow-induced vasodilation, preserve vascular function by directly activating Ca^{2+} -dependent K^{+} - Ca^{2+} channels [57], and indirectly induce vasodilation and maintain blood

pressure by releasing NO, also mediating hypoxia-induced increased proliferation and migration of PASMCs involved in the progression of PAH [58].

In human and Sprague-Dawley rat PASMCs with PAH, TRPM7 is downregulated, which activates the mitogen-activated extracellular signal-regulated kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway and decreases cellular Mg^{2+} levels, thus promoting SMC proliferation exacerbating PAH [59]. TRPML1 is crucial for the initiation of Ca^{2+} sparks in SMCs and the regulation of vascular contractility and blood pressure, and TRPML1-knockout mice are spontaneously hypertensive [60].

Two-Pore-Domain Potassium Channel. The 2-pore-domain potassium channel is an important type of cellular potassium channel protein family, which can be classified into 6 isoforms based on gene sequence conservation and channel functional properties: TWIK, TREK, TASK, TALK, THIK, and TRESK [61, 62]. These proteins can be mechanically activated by the membrane stretch in heterologous expression systems or proteoliposomes, and they have a wide range of mechanical force sensing and a low response threshold ($0.5\text{--}12\text{ mN}\cdot\text{m}^{-1}$); the stronger the mechanical force perceived, the higher the likelihood of channel opening [63, 64]. The membrane potential of VSMCs is a key regulator of vascular tone and therefore regulates flow and pressure in the circulation. The negative resting potential of VSMCs is low, between $\pm 40\text{ mV}$ and $\pm 60\text{ mV}$, but they are sensitive to changes in K^+ conductance [65], and 2-pore-domain potassium channels in smooth muscles are the components of myogenic regulation [66].

TREK-1 channel activity is induced by increased membrane stretch and is independent of intracellular Ca^{2+} and ATP, and TREK-1 can respond to membrane stretch in intact cells as well as isolated plaques, demonstrating that the mechanical force can control the channel [67, 68]. In spontaneously hypertensive rats (SHRs), high blood pressure significantly reduces both mRNA and protein expression levels of TASK-1 in aortic SMCs, affecting K^+ conductance and the resting membrane potential and leading to vasoconstriction [69]. The TASK3(KCNK3) gene of the potassium channel is a susceptibility gene for PAH [70, 71], and rats with mutations in the KCNK3 gene exhibit a marked ECM deposition in the distal pulmonary artery. In PASMCs of rats with mutations in this gene, the overall K^+ current is significantly reduced, enhancing vasoconstriction associated with PASMC membrane depolarization; meanwhile, KCNK3 knockdown or blockage can lead to increased hypoxia-inducible factor 1- α expression and excessive proliferation of PASMCs

[72, 73]. In PAH, the reduced expression of TASK5 in VSMCs affects SMC proliferation and migration, also causing pulmonary vasoconstriction [70, 74, 75].

Epithelial Sodium Channel. The epithelial sodium channel (ENaC/DEG) superfamily of proteins are voltage-insensitive sodium-selective channels. ENaC can participate in the regulation of systemic blood pressure [76], and genetic mutations in humans cause gain-of-function/loss-of-function in ENaC, resulting in monogenic forms of hypertension/hypotension [77]. ENaC can respond to mechanical strain to promote cell differentiation [78]. VSMCs have α -, β -, and γ -ENaC isoforms in the plasma membrane, and ENaC proteins are associated with vascular smooth muscle mechanotransduction [79–81].

Changes in extracellular sodium may alter myogenic responses by altering the sensitivity of smooth muscle mechanoreceptors to stretch. Increased extracellular sodium levels decrease MT [82]. β -ENaC is required for normal mechanically gated currents in renal VSMCs, and mice with reduced levels of β -epithelial Na^+ channels (β -ENaC^{m/m}) inhibit the myoconstrictor response in small renal arteries and afferent small arteries in response to myoconstriction [83]. Amiloride and benzamil are relatively high-affinity ENaC inhibitors that selectively inhibit ENaC channels, and low doses inhibit ENaC but do not inhibit other molecules such as the L-type Ca^{2+} channel, Na^+ - H^+ exchanger, Na^+ - Ca^{2+} exchanger, and TRP6, which inhibits pressure-mediated constriction of the MCA, the interlobular artery, and the small renal input artery [84]. Inhibition of ENaC by amiloride (1 mol/L) and the amiloride analog benzamil (30 nmol/L , 1 mol) blocked pressure-mediated myogenic constriction of arteries in an isolated rat MAC model of distraction induction [84]. β - and γ -subunit ENaC are required for vascular mechanotransduction, and VSMCs from rat brain and mouse renal vessels transfected with β - and γ -EnaC short interfering RNA inhibit pressure-induced myogenic contraction [85].

G Protein-Coupled Receptors and G Proteins

G protein-coupled receptors (GPCRs) are 7 transmembrane helix proteins that transmit extracellular signals to cells, usually through conformational rearrangement of their helices and subsequent binding and activation of intracellular heterotrimeric [86, 87]. G proteins are a class of signaling proteins that bind guanosine diphosphate and have guanosine triphosphate (GTP) hydrolase activity. The signal transduction pathway in which G proteins are involved is a conserved transmembrane signal transduction mechanism. When cells transduce extracellular signals, they are first stimulated by dif-

ferent types of GPCRs, which are then activated to further activate heterotrimeric G proteins on the inner side of the plasma membrane; these heterotrimeric G proteins in turn activate various effectors downstream of them to generate intracellular second messengers, thus transmitting the signal down the chain [88, 89].

GPCRs and G proteins play an important role as mechanoreceptors [90], and the GPCR systems in VSMCs play a crucial role in vascular MT [91, 92]. Myogenic vasoconstriction is an autoregulatory function of small arteries, which is essential for maintaining vascular tone, peripheral vascular resistance, and organ perfusion. Vascular MT requires a complex mechanical transduction that translates a physical stimulus (stress) into a biological response (change in vessel diameter) [93–95]. The different involvement of these GPCRs and G proteins in various vascular territories could allow selective targeting of the MT in a specific tissue or organ. Angiotensin II type 1 (AT1Rb) receptor as a type of GPCR [96] is the main sensor of intracellular pressure in the smooth muscle of cerebral arteries. Pressure- or membrane stretch-induced activation of AT1Rb activates TRPM4 channels, leading to inward transient cationic currents that depolarize SMCs and cause vasoconstriction. The use of angiotensin II also activates AT1Ra and AT1Rb to stimulate vasoconstriction in cerebral arteries [97]. Stretch activation of AT1Ra contributes to the myogenic response of mouse mesenteric and renal arteries through the coupling of the AT1Ra with a Gq/11 protein [98]. Membrane stretch leads to agonist-independent activation of G (q/11)-coupled receptors, which subsequently signal to TRPC channels in a G protein- and phospholipase C-dependent manner [91]. Increasing the AT (1) angiotensin II receptor density in mechanically unresponsive rat aortic A7r5 cells resulted in mechanosensitivity [91]. The MT of cerebral and renal arteries is significantly reduced by AT1R blockade; this inhibitory effect is enhanced in blood vessels of mice deficient in the regulator of G protein signaling-2, which is a selective and negative regulator of Gq/11 proteins [99]. These findings suggest that G (q/11)-coupled receptors function as sensors of membrane stretch in VSMCs [91, 100]. The Gq/11 protein-coupled receptor signaling pathway induces vasoconstriction and hypertension, and the Gq/11 inhibitor ligand blocks vasoconstriction induced by multiple Gq-coupled receptors in resistance arterial SMCs [101]. With activation of Gq-11 and G12-13 proteins, Gq-11 cascades through phospholipase C- β (PLC β) and G12-13 through Rho signaling pathway signaling [92, 102].

Another study reported that AT_{1A/1B}^{-/-} arteries exhibit a reduced MT at high intraluminal pressures (>100 mm

Hg) but an unexpected increased MT at low pressures (20–70 mm Hg). At low intraluminal pressures, only cysteinyl leukotriene 1 receptors (CysLT1Rs) were significantly upregulated in AT_{1A/1B}^{-/-} isolated murine mesenteric arteries [103]. The MT is determined by AT1 and CysLT1 receptors acting together as mechanosensors via Gq/11-protein activation [103]. CysLT1Rs can also mediate the contraction of bronchial smooth muscle [104].

Purinergic signaling plays an important role in vascular tone and remodeling, and activation of VSMC receptors promotes vasoconstriction via P2X1 or pyrimidine-sensitive P2Y receptors [105]. Purinergic P2Y6 receptor (P2Y6R), an inflammation-inducible GPCR, promotes Ang-II-induced hypertension in mice. Deletion of P2Y6R attenuates an Ang-II-induced increase in blood pressure, vascular remodeling, and oxidative stress. AT1R and P2Y6R form stable heterodimers, which enhances G protein-dependent vascular hypertrophy but reduces β -arrestin-dependent AT1R internalization [106]. The increased abundance of P2Y6R converts AT1R-stimulated signaling in VSMCs from β -arrestin-dependent proliferation to G protein-dependent hypertrophy [106]. P2Y6-receptor-knockout mice confirmed the involvement of these receptors in aortic-endothelium-dependent relaxation to uridine diphosphate and smooth muscle contraction to uridine diphosphate (and uridine triphosphate) [107]. In arterial SMCs, P2Y6 and P2Y2 receptors contribute to calcium mobilization; P2Y6 activation is mandatory for Ras homolog family member A (RhoA)-GTP binding, myosin light chain (MLC), P42-P44, and c-Jun N-terminal kinase phosphorylation; and P2Y6 receptor blockade blunts the MT in mouse mesenteric resistance arteries and human subcutaneous arteries [108].

Mechanical stress activates AR and downstream signaling pathways, such as ERKs, and increases VSMC proliferation [109]. Protease-activated receptors (PARs) are also a class of GPCRs that are activated by specific serine proteases through protein hydrolysis of their NH₂ termini, and mechanical stress upregulates PAR-1 expression in VSMCs [110, 111]. PARs are involved in the development of atherosclerosis, and atherosclerosis improves in PAR2-deficient mice [112, 113]. In a mouse model of hypertension, GPR75 knockdown prevented elevated blood pressure, smooth muscle contraction, and vascular remodeling [114]. GPR68 senses flow and is essential for vascular physiology, and GPR68-deficient mice show marked acute flow-mediated dilation (FMD) impairment and chronic flow-mediated outward remodeling in small mesenteric arteries [115]. The mechanical stimulus mentioned in this paper is flow shear stress, which mainly acts on ECs instead

of directly on VSMCs, and MT is a property of SMCs and flow-mediated dilation a property of ECs. Some studies have characterized GPR68/OGR1 activation in VSMCs. In human aortic SMCs, GPR68 is a major receptor involved in the extracellular acid-induced stimulation of PGI₂ production and cAMP accumulation, which has been shown to be inhibitory for proliferation and migration [116]. Thus, in these acidic circumstances, GPR68 activation may be considered to counteract vasoconstriction and inflammation. In airway SMCs, GPR68 can activate multiple signaling pathways, involving p42/p44, PKB, PKA, and calcium mobilization, which is the direct target and mediates airway smooth muscle contraction [117]. Extracellular acidification stimulates interleukin-6 production and Ca²⁺ mobilization through proton-sensing OGR1 receptors/Gq-11 proteins in human airway SMCs [118], which induces connective tissue growth factor production, thereby inducing ECM protein formation and airway remodeling. In these studies, GPR68 was regarded as a proton-sensing receptor in SMCs, responding to the extracellular acidic environment and activating response signaling pathways to affect the proliferation and contraction of SMCs. However, whether GPR68 can directly respond to mechanical stimuli and conduct mechanotransduction in VSMCs remains to be further investigated. These results indicate that VSMCs can respond to mechanical signals via GPCRs and G proteins on the cell membrane and perform a cascade response.

Receptor Tyrosine Kinases

Receptor tyrosine kinases (RTKs) are the largest class of enzyme-linked receptors, acting as both receptors and enzymes [119], and transmembrane proteins with intracellular kinase domain sites and extracellular domain sites [120], capable of binding to ligands and acting as receptors for a variety of growth factors, including epidermal growth factor (EGF), nerve growth factor, platelet-derived growth factor (PDGF), vascular endothelial growth factor, fibroblast growth factor, insulin, and insulin-like growth factor (IGF) [121, 122]. Mechanical stress can nonspecifically activate RTKs, and the intracellular C-terminal kinase structural domain of activated RTKs phosphorylates its own tyrosine residues, resulting in binding sites for the Src homolog 2 (SH2) and phosphotyrosine-binding structural domains. Proteins containing SH2 or phosphotyrosine-binding structural domains bind to RTKs, so they are activated and signal to downstream factors [123–126].

Mechanical stretch increases matrix metalloproteinase-2 (MMP-2) production in VSMCs by activating the

PDGF receptor (PDGFR)- β /Akt signaling pathway [127]. The stretch of VSMCs induces rapid PDGFR α phosphorylation and the effect depends on the stretch amplitude [128, 129]. Cyclic strain activates PDGFR β , thus inducing mouse embryonic stem cell differentiation into VSMCs [130]. Mechanical stretch enhances insulin-induced VSMC proliferation by upregulating IGF-1 receptor and downstream Src/EGF receptor-mediated activation of ERK and Akt [131], and also stimulates and maintains tyrosine phosphorylation of IGF-1R and insulin receptor substrate 1 (IRS-1), leading to increased VSMC proliferation [132]. Mechanical stretch (15%, 1 Hz) significantly upregulates the protein expression of EGFR and mitogen-activated protein kinase (MAPK) phosphatase-1 in SHR, promoting VSMC proliferation [133]. Cyclic stretch (20%, 1 Hz) also induces EGFR phosphorylation in rat VSMCs and interacts with the bridging protein (Shc/Grb2), leading to rapid phosphorylation activation of ERK1/2 and stimulation of SMCs growth [134]. These results suggest that RTKs on the VSMC surface play a role in the mechanosensing of stretch.

As described above, ion channels, membrane receptors, or transmembrane proteins on the cell membrane play a central role in mechanical signaling in SMCs and trigger downstream signaling cascades that lead to force-dependent changes in gene expression, affecting SMC proliferation, migration, phenotypic transition, and ECM synthesis.

Integrins

Integrins are a family of cell membrane surface protein receptors, heterodimers consisting of α - and β -subunits, that not only mediate the interconnection between cells and cells, the ECM, and the cytoskeleton but also act as signal receptors and are considered important transmitters of the conversion of mechanical forces into chemical signals [135, 136].

In response to mechanical stimulation, integrins can interact with the ECM and cytoskeleton and other signaling molecules [137, 138]. Integrins accumulate in focal adhesion, which can interact with a variety of proteins (Src, FAK, Talin, vinculin, and paxillin) to form focal adhesion complexes [139], and integrins activate signaling pathways, such as Rho GTPase and the MAPK pathway, which in turn produce a series of effects that convert mechanical signals into biochemical cascades [140, 141].

Integrin expression and activity are also affected by mechanical stimulation. In rat aortic SMCs, the expression of integrin $\alpha_v\beta_3$ and the generation of thrombin on the surface of VSMCs increased by 30% without inducing

apoptosis by cyclic stretch (10%, 1 Hz) during 60 and 360 min [142]; cyclic stretch also stimulated Src phosphorylation, talin cleavage, and prothrombin binding to VSMCs, whereas the integrin $\alpha_v\beta_3$ antagonist peptide (cRGDPV) blocked the cyclic-stretch-induced $\alpha_v\beta_3$ expression, Src phosphorylation, and thrombin generation [142, 143]. In both human and mouse atherosclerotic lesion VSMCs, mechanical cyclic stretch (15%–20%, 1 Hz) activates integrin $\alpha_1\beta_1$, as well as upregulating SPRR3 expression, and integrin $\alpha_1\beta_1$ plays an essential role in cyclic stretch-mediated SPRR3 protein expression [144]. When VSMCs are exposed to progressively high stress, integrins convert mechanical stimuli into intracellular Ca^{2+} signaling events to mediate myogenic contractile responses in small arteries, promoting vasoconstriction [145].

Integrins are activated when the vascular mechanics microenvironment is altered, and enhanced mechanical force applied to integrin adhesion promotes smooth muscle proliferation. The integrin $\alpha_v\beta_3$ /PI3-K/Akt pathway mediates the proliferative and apoptotic responses of VSMCs [142, 146]. Integrin $\alpha_5\beta_1$ affects integrin-linked kinase and focal adhesion kinase (FAK) mRNA expression, mediating the proliferation and migration of human aortic SMCs [147]. The integrin receptor/integrin-related kinase/Rac-1 pathway mediates vascular smooth muscle phenotypic transformation [148]. VSMCs can coordinate type I collagen assembly through integrin $\alpha_2\beta_1$, RhoA, and fibronectin (FN) polymerization [149]. In summary, VSMCs respond to changes in the mechanical environment by mediating their proliferation, migration, and phenotypic transition, and ECM synthesis through the mechanotransduction of integrins.

Focal Adhesion Complexes

Focal adhesion (FAs) complexes are integrin-containing protein complexes that are regulated by a network of protein-protein interactions [150]. They are formed in a spatiotemporal manner following activation of integrin transmembrane receptors [151], which are key factors in triggering cell adhesion and many other cellular processes, including cell migration, spreading, and proliferation. After activation in response to mechanical stimuli, integrins further activate FAs to complete mechanical signaling [152].

The integrin signaling pathway has a profound effect on proliferation and phenotype in porcine coronary artery SMCs, when cells adhere to FN, the integrin signaling pathway can be activated, which synergizes with the growth factor signaling pathway, leading to FAK activation and cell proliferation [153]. FAK and proline-rich tyrosine kinase 2 (Pyk2) activation by $\alpha_v\beta_3$ integrin pro-

mote proliferation of PSMCs in PAH [154]. The FAK-PIK3 signaling pathway interacts with integrins β_1 and β_3 and also affects VSMC proliferation and migration [155]. The $\alpha_v\beta_3$ /FAK-dependent YAP pathway is activated in human aortic SMCs after percutaneous coronary intervention, and integrin $\alpha_v\beta_3$ mediates FAK phosphorylation and actin cytoskeleton reorganization, leading to dephosphorylation of YAP in hASMCs, which is involved in promoting hASMC proliferation and migration, leading to in-stent restenosis, and knockdown or inhibition of YAP reverses this phenomenon [156]. Integrin $\alpha_v\beta_3$ binds to osteoprotegerin, a secreted protein upregulated in PAH patients, which in turn leads to FAK activation [157]. Integrin $\alpha_5\beta_1$ mediates FAK phosphorylation and is involved in VSMC migration [158]. The reduced interaction between integrin β_3 and FAs, the altered cytoskeleton, and downregulation of glycogen synthase kinase 3 β /NF κ B signaling can inhibit VSMC migration [159].

Zyxin is also an adhesion patch protein, a mechanosensor for integrin-mediated responses to mechanical forces. Atomic force microscopy (AFM) was used to apply localized pulling forces to VSMCs through an FN focal adhesion induced by an FN-coated bead on the cell surface. The application of force with the AFM induced an increase of zyxin accumulation at the site of the FN-bead FA that accompanied the VSMC contractile response, and inhibition of zyxin expression using zyxin-short hairpin RNA (shRNA) also eliminated the contractile response of VSMC to AFM tension [160]. Mechanical stretch inhibits SOCS-1 mRNA and protein expression in human VSMCs. Colocalized with FAK, stretch disrupts FAK polyubiquitination and proteasomal degradation, resulting in FAK upregulation in stretched VSMCs [161]. Integrin-linked kinase regulates VSMC proliferation and migration by acting as a molecular scaffold in FA and by regulating the stability of cell-cell contact proteins and β -catenin signaling [162]. FAs interact with integrins for mechanosignaling, promoting VSMC proliferation, migration, and phenotypic transition involved in associated pathological diseases.

Cell Junction

Cell-cell junctions, particularly adherens junctions, can withstand large forces between neighboring cells and transmit the forces to the cytoskeleton [163]. Adherens junctions consist of cadherin molecules that bind homologically to cadherin on adjacent cells and, like integrin-mediated adhesions, connect intracellularly to the actin cytoskeleton via numerous scaffolding proteins [164].

Cell junctions via calnexin can play an important role in several aspects of cell behaviors, including prolifera-

tion, differentiation, apoptosis, and polarity. Abnormal mechanical forces can trigger abnormal proliferation of SMCs, and cell junctions are involved in the proliferative response of SMCs induced by mechanical stretch. SMCs, unlike ECs, respond to stretch without coming into contact with neighboring cells. In SMCs, stretch activates RhoA, whereas in ECs, stretch activates Rac1 [165]. Cadherin-11 is a regulator of ECM synthesis and tissue mechanics [166] and regulates the expression of the key transcription factor serum response factor, which is important for the transition of mesenchymal stem cells into SMCs [167]. Furthermore, cadherin-11 is necessary for the development of contractility in smooth muscle-containing tissues, such as arteries, where cadherin-11 is involved through homophilic interactions, leading to rapid activation of the transforming growth factor- β and Rho kinase (ROCK) pathways and phosphorylation of downstream effectors. Subsequent activation of the key transcription factors myocardia-related transcription factor A (MRTF-A; also known as MKL1) and MYOCD leads to significant upregulation of collagen and elastin genes [167]; tissues containing smooth muscle or myofibroblasts, such as aorta, appear significantly impaired in mechanical strength and have significantly reduced collagen and elastin content in cadherin-11 gene deficient mice (CDH11^{-/-}) compared with wild-type (WT) mice, indicating that cell junctions are regulators of ECM production, and this mechanism may regulate important biomechanical processes in novel ways.

Cytoskeleton

The structural and functional integrity of the vascular wall is due in part to the interaction between the collagen- and elastin-rich ECM and VSMCs. In response to mechanical force stimuli, VSMCs interact with the ECM to mediate the perception of changes in external mechanical forces by ion channels, membrane receptors, and integrin receptors on the cell membrane, which are transmitted intracellularly to myosin contractile units and induce cellular adaptation to external stress. Cells are embedded in the support network of ECM components. The ECM not only constitutes a structural scaffold but also affects cell proliferation, migration, and differentiation [168], which has a profound impact on cell behavior.

Emilin, an ECM glycoprotein, is one of the components of elastin fibers and is mainly localized at the elastin-microprotein interface [169], which has a C-terminus gC1q domain typical of the gC1q/TNF superfamily members containing an N-terminus unique cysteine-rich EMI domain. These glycoproteins are homotrimeric and as-

semble into high molecular weight multimers [19, 170]. Emilin1 gC1q is an adhesive substrate and provides anchorage and/or homeostatic control of cell proliferation, suggesting that it might also contribute to the cell number and the size of VSMCs in arterial walls [171]. Emilin1 is implicated in elastogenesis and in maintenance of vascular cell morphology [172]. Targeted inactivation of the Emilin1 gene in mice induces multiple phenotypes characterized by a decreased diameter of arterial vessels and systemic hypertension. Vascular smooth muscle Emilin1 is a regulator of arteriolar myogenic response and blood pressure [173]. Vascular smooth muscle Emilin1 is a regulator of arteriolar myogenic response and blood pressure [174]. In mice with selective SMC ablation of Emilin1, TGF β expression significantly increases, which stimulates heparin binding, EGF, and subsequent transactivation of EGFR, and these signals can recruit TRPC6 and TRPM4 [175]. In resistance arteries from hypertensive patients, Emilin1 decreases and the MT is enhanced, which depends on increased TGF β -EGFR signaling [175]. The dynamic rearrangement of the actin cytoskeleton is necessary to redistribute the physical forces required for cell contraction, allowing the cells to adapt to the extracellular microenvironment. Mutations in ACTA2, which encodes smooth muscle alpha-actin, result in impaired VSMCs function, which leads to a reduced ability of cells to sense matrix-mediated mechanical stimuli [176]. Transmural pressures in the aortic rings of ACTA2^{-/-} mice are lower than in WT mice at any given level of stretch. ACTA2^{-/-} cells are unable to generate external forces to remodel the matrix due to reduced contractility and interaction with the matrix [176].

In a mouse model of pressure overload induced by transverse aortic constriction surgery, biomechanical stress after p47 (phox) KO upregulates N-calmodulin and β -catenin, but disrupts the actin filament cytoskeleton and reduces phosphorylation of FAK, leading to vasoconstriction dysfunction after surgery, suggesting an important role for the cytoskeleton in mechanotransduction [177].

Intermediate filaments are involved in stress-related cellular mechanical properties and plasticity by regulating FA and the actin network. Vimentin is one of the proteins of intermediate filaments, and vimentin knockout (Vim^{-/-}) mice have increased the expression of laminin, FN, perlecan, type IV collagen, and VE cadherin in the subendothelial basement membrane and von Willebrand factor deposition; smooth muscle myosin heavy chain, α -SM actin, and smoothelin expression were decreased. Electron microscopy revealed enhanced smooth muscle cell-matrix interactions [178]. In Vim^{-/-} mice, expression

of integrin α_v , talin, and vinculin increases in FAs; in addition, the expression of phosphorylated FAK and its targets Src and ERK1/2 increases. $\text{Vim}^{-/-}$ (but not synemin knockout) results in increased carotid artery stiffness and contractility as well as endothelial dysfunction [178]. RhoA/ROCK signaling regulates smooth muscle phenotypic modulation and vascular remodeling via the c-Jun N-terminal kinase (JNK) pathway and vimentin cytoskeleton [179]. Vimentin also plays an important role in mechanotransduction as a cytoskeleton.

Nuclear Membrane Protein

The nucleus is not only a repository of genetic information but also actively involved in sensing changes in mechanical load. The nucleus contains actin stress fibers to maintain nuclear morphology and changes in nucleus morphology [180–182], due to mechanical forces or substrate stiffness can also lead to changes in gene expression [183, 184]. Mechanical forces are transmitted to the nuclear membrane via the actin cytoskeleton or microtubules, leading to changes in gene expression [185, 186].

The main intermediate filament that makes up the nuclear membrane is lamin A. Mutations in lamin cause a disease called laminopathies, and abnormalities in lamin A can lead to premature atherosclerosis or myotonic dystrophy, which suggests that lamin plays an important role as a load-bearing structure necessary for structural integrity and normal cell nuclear mechanics [187].

In hypertensive rats, VSMC proliferation was significantly increased and emerin and lamin A/C expression was inhibited in somatic common carotid arteries compared to normotensive controls; in an in vitro cyclic stretch model, it was consistently concluded that VSMCs under high (15%) cyclic stretch had significantly increased proliferation and decreased expression of emerin and lamin A/C compared to normal (5%) cyclic stretch control group [188]. Using targeted short interfering RNAs to mimic the inhibitory effect of 15% retraction on emerin or lamin A/C expression in VSMCs, the proliferation was found to be significantly enhanced under static and 5% retraction conditions. Overexpression of emerin or lamin A/C reversed the 15% stretch-induced VSMCs proliferation. Thus, emerin and lamin A/C play a key role as mechanosensitive factors in inhibiting VSMC overproliferation induced by hyperextension [188].

In conclusion, mechanical forces can be transmitted by interacting with the ECM, ion channels, membrane receptors, or transmembrane proteins in the cell membrane, playing a central role in mechanical transduction and triggering downstream signaling cascades, leading to

force-dependent changes in gene expression. Cells interacting with the ECM in a mechanical manner can also mediate a network of integrins, cytokines, cytoskeletal proteins, and coordinated changes in the nuclear structure to achieve mechanical signaling, translating physical forces into biochemical responses.

Signaling Pathways

Rho

ROCK is an effector of Rho GTPase, which increases vascular smooth muscle contractility by phosphorylating MLC and inactivating MLC phosphatase. ROCK plays a key role in the composition, differentiation, and function of the actin cytoskeleton of VSMCs and is involved in the pathogenesis of CVD [189, 190]. A variety of extracellular stimuli activate RhoA via GPCRs.

In VSMCs, inhibition of Rho/ROCK signaling disrupts the actin cytoskeleton, which affects iNOS expression, release of NO, and consequently vascular diastolic function [191]. The VSMC glycocalyx mediates shear stress-induced contractile responses via the ROCK-myosin light-chain phosphatase pathway [192], and in VSMCs, Rho activation regulates vasoconstriction [193]. ROCK activation and ER stress can be considered cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) vasculopathy, and CADASIL vessels show impaired vasodilation, sluggish vasoconstriction, and hypertrophic remodeling. In CADASIL, with Nox5 upregulation, and increased Notch3 and ER stress target, ROCK activity, superoxide production, and cytoskeleton-associated protein phosphorylation all increase. The use of inhibitors of Notch (γ -secretase inhibitor), Nox5 (melatonin), ER stress (4-phenylbutyric acid), and ROCK (fasudil) ameliorates the abnormal vascular response and the Notch3-Nox5/ER stress/ROCK signaling pathway in CADASIL [194]; RhoA/ROCK alters fetuin A uptake and regulates calcification in bovine VSMCs, and the RhoA/ROCK signaling pathway can act as a negative regulator of vascular calcification [195].

The Rho/ROCK pathway is involved in differential migration of human coronary artery bypass VSMCs at different sites. VSMCs' PDGFR β expression decreased and PDGFR α increased in the internal mammary artery (MA) group compared with the saphenous vein (SV) group. In the MA group, PDGF-BB-induced lower RhoA activity and less MA-VSMC stress fiber formation than in the SV group. Dominant negative RhoA mutants inhibited stress fiber formation induced by PDGF-BB, while active mutants resulted in maximal stress fiber formation in MA and SV groups [196]. Rho is involved in the pro-

liferation, migration, and contractile function of VSMCs in response to differences in the mechanical microenvironment in which the blood vessel is exposed.

Mitogen-Activated Protein Kinase

Protein kinases are signal transduction enzymes that catalyze the phosphorylation of specific residues in target protein substrates, which in turn exert corresponding biological effects. MAPK is a highly conserved class of serine/threonine protein kinases in cells, whose activation depends on the dual phosphorylation of threonine and tyrosine in the conserved region between the VII and VIII subdomains of the protein kinase [197]. At the molecular level, activation of the MAPK family is mediated by a specific 3-layer kinase cascade, whereby MAP3Ks/MEKKs activate MAP2Ks/MKKs and MAP2Ks in turn activate MAPK [198]. The MAPK signaling pathway has 3 subfamilies: ERK1/2, JNK/stress-activated protein kinase (SAPK), or P38 MAPK.

In acute hypertension, both JNK and ERK are activated in the vascular wall [199]. JNK is activated in the arterial wall after balloon injury or angioplasty in animals [200]. This suggests that mechanical stress is associated with JNK activation. Calcitonin-gene-related peptides partially protect cultured SMCs from oxidative stress-induced apoptosis by activating ERK1/2 MAPK [201]. In SHR, exercise promotes a phenotypic conversion of VSMCs via Akt and MAPK, and VSMC phenotypic markers are reversed when p-Akt inhibitors, p-ERK inhibitors, and p-P38 MAPK inhibitors are administered [202].

Mechanical stretch increases VSMC proliferation and apoptosis by activating the protein disulfide bond isomerase (PDI) redox system, thereby accelerating the formation of atherosclerotic lesions in grafted veins [203]. The use of PDI inhibitors inhibits the PDI-ER stress system, thereby attenuating the concomitant increase in VSMC proliferation and apoptosis in response to mechanical stretch. However, MAPK inhibitors PD98059, SP600125, and SB202190 significantly reduce the activation of the ER stress signaling cascade, and the ER stress inhibitor reduces PDI activation and ER stress signaling [203], suggesting that small beriberi inhibits mechanical stretch-induced vascular smooth muscle cell proliferation and apoptosis. In summary, the MAPK signaling pathway can be used as a mechanical signaling pathway in VSMCs in response to hypertension and mechanical stretch *in vitro*.

YAP-TAZ

The Hippo-YAP signaling pathway plays a critical role in mechanotransduction events. Dupont et al. [204] re-

ported in 2011 that YAP/transcriptional coactivators with PDZ-binding motifs (TAZ) can act as a mechanistic signaling transcription factor, and ECM elasticity, cell shape, and cytoskeletal forces have closely correlated with YAP/TAZ activity and expression levels. YAP/TAZ expression can govern cellular behavior, and based on this mechanism of action, it is possible to elucidate how physical forces shape tissue morphogenesis and homeostasis *in vivo* (e.g., constant remodeling when the mechanical environment of the tissue changes). YAP is considered as a core component of mechanotransduction signals sensing changes in the extracellular mechanical microenvironment, such as ECM stiffness, intercellular or cell-matrix adhesion, and different cell density and cell morphology [205, 206]. YAP transduction is associated with the tissue properties of the organ itself, such as tissue topology, mechanical properties, and organ size, and these key tissue properties determine the cell-to-cell-ECM and cell-to-cell environmental responses, which further trigger individual cytoskeletal remodeling and changes in cellular traction, thereby altering the fate of individual cells [207–209]. YAP/TAZ integrates mechanical cues with the response to soluble signals and metabolic pathways to control multiple aspects of cell behavior, including proliferation, cell plasticity, and stemness essential for tissue regeneration [210–212]. Mechanoregulation and pathology of YAP/TAZ can via Hippo and non-Hippo mechanisms [213].

Mechanical stimuli can cause changes in the structure and dynamics of the actin cytoskeleton [214]. YAP plays a key role in actin-cytoskeleton-mediated mechanical signaling [215]. Increasing F-actin levels promotes YAP/TAZ nuclear localization, and loss of F-actin causes YAP/TAZ to accumulate in the cytoplasm. The sensitivity of YAP/TAZ to the status of the F-actin cytoskeleton has been harnessed not just for mechanical regulation but also for GPCR signaling, which modulates Hippo signaling by acting through Rho GTPase to increase F-actin levels [216–218]. Pressure promotes F-actin depolymerization, RhoA downregulation, and LPAR1 (Gα12/13-coupled receptor) inactivation, which leads to YAP cytoplasmic translocation and decreased clustering [206].

The YAP/TAZ signaling pathway has been reported to be involved in vascular remodeling and CVD [219], such as atherosclerosis [220–222]. In ECs, YAP/TAZ responds to shear stress [223]. In mice and human ECs, laminar flow decreases the level of nuclear YAP and YAP target gene expression (connective tissue growth factor and cysteine-rich protein 61) by promoting Hippo kinase LATS1/2-dependent YAP (serine 127) phosphorylation [221]. Disturbed flow (DF, without a clear direction), not

laminar flow (with a clear direction), activates YAP/TAZ to promote atheroprone phenotypes (proliferation and inflammation) [220]. DF also promotes YAP activation and endothelial vascular cell adhesion molecule 1 (VCAM1)-dependent inflammatory responses by increasing YAP phosphorylation (at tyrosine 357) dependent on tyrosine protein kinase activity [224]. YAP/TAZ activation in the endothelium induces JNK-mediated anti-inflammatory gene expression to promote arteriogenesis in response to DF. YAP/TAZ inhibition suppresses JNK signaling and downregulates pro-inflammatory gene expression, thereby reducing monocyte attachment and infiltration [221]. YAP/TAZ inhibition could contribute to the anti-inflammatory effect of simvastatin, and the integrin- α 13-RhoA-YAP pathway holds promise as a novel drug target against atherosclerosis [221].

The YAP/TAZ pathway is involved in PAH [225]. ECM remodeling and pulmonary vascular stiffness activate YAP/TAZ, which induces the microRNA (miR)-130/301 family expression in pulmonary artery ECs and SMCs. In turn, the miR-130/301 series inhibits miR-130/301 by activating YAP and TAZ through a feedback mechanical transport cycle, which improves ECM remodeling and PAH [226]. YAP/TAZ contributes to pulmonary fibrosis and alveolar regeneration via mechanotransduction, G protein-coupled receptor signaling, and a conventional Hippo/YAP signaling pathway, and selectively inhibiting YAP/TAZ in lung fibroblasts may inhibit fibroblasts' proliferation and ECM deposition [227]. During stiffness and flow sensing, YAP/TAZ tightly controls cell-ECM interactions [212]. The FA protein DLC1 is a direct transcriptional target of YAP/TAZ and is crucial for YAP-driven collective cell migration and sprouting angiogenesis by ECs [228, 229]. YAP/TAZ is also involved in the mechanical transformation of coronary vein graft disease [230]. YAP plays an important role in 3D organogenesis, which can regulate actomyosin activity, thus participating in the regulation of 3D tissue morphology and alignment [231]. In hydrogels and artificial substrates, YAP/TAZ plays a critical role in stem cell proliferation and differentiation via mechanical signals [232].

This review focuses on how VSMCs undergo mechanical transduction. In human umbilical arterial SMCs (HUASMCs), cycle stretch (0.5 Hz, elongation 13%, 24 h) leads to PI3K and phosphatidylinositol-dependent kinase 1 activation. Phosphatidylinositol-dependent kinase 1 activation triggers the dissociation of Hippo core kinases from the scaffolding protein SAV1/WW45, leading to Lats inactivation, dephosphorylation, and YAP nuclear translocation [233]. In addition, YAP/TAZ knockdown

significantly attenuates mechanic-induced proliferation and pro-inflammatory phenotypes in HUASMCs [233]. In the VSMCs of aortic walls of patients with ascending aortic aneurysms, the expression of YAP was significantly reduced, which is associated with ECM disorders of the media in ascending aortic aneurysms [234]. VSMCs secrete significantly more Thbs1, which binds via integrin $\alpha_v\beta_1$ and establishes mature FAs, facilitating YAP nuclear shuttle in a small GTPase RAP2- and Hippo-dependent manner, thereby leading to Thbs1/integrin/YAP-mediated matrix mechanotransduction in vascular remodeling [235]. The thromboxane A₂ receptor (TP) signaling activates YAP/TAZ and promotes DNA synthesis and cell migration in VSMCs to facilitate VSMC proliferation and migration, and participates in the restenosis process after vascular injury. Knockdown of TP or knockdown of the downstream G protein α 12/13 blocks YAP activation; in addition, the Rho inhibitor of the actin cytoskeleton disruption also prevents YAP/TAZ activation [236]. cAMP-induced actin cytoskeleton remodeling suppresses YAP/TAZ-TEAD-dependent mitogenic gene expression in VSMCs. Exerting an anti-promitogenic effect inhibits excessive proliferation of VSMCs and prevents postvascular restenosis and vein graft failure [237]. The YAP signaling pathway is also involved in the phenotypic transformation of VSMCs, in vitro and in vivo arterial injury models, YAP expresses the increase in ions, which promotes SMCs migration and proliferation, while attenuating smooth muscle contractile gene expression and neointima formation [238].

SMCs Are Involved in ECM Secretion and Remodeling in Response to Mechanical Stimulation

The ECM is the structural entity that surrounds and supports tissues and cells. The ECM consists of many tissue-specific proteins, such as collagen, proteoglycans, elastin, glycoproteins, MMPs, and growth factors [239]. Mechanical stimuli are delivered to cells via the ECM, which provides an adhesive surface for cells and tissue structures. In addition, cells can interact dynamically with the ECM and synthesize, degrade, and rearrange it [240, 241]. VSMCs are located in the middle layer of the vascular system and are primarily subjected to cyclic tensile force caused by blood pressure and are responsible for vascular contraction and remodeling. When the lumen is under high pressure or when cyclic stretch is exerted in vitro, VSMCs sense changes in the mechanical stress to which they are subjected and regulate the expression and

synthesis of ECM molecules to adapt to changes in the external mechanical environment through ion channels, transmembrane proteins, integrins, signaling cascades, and mechanosensitive pathways described before.

In hypertensive patients, the mechanical tension of arteries increases by ~15%, with a corresponding increase in collagen [242]. The expression profile of ECM genes was examined in VSMCs from chronic overloaded PAH by real-time PCR, and stress-induced ECM protein expression was significantly elevated [243].

In an *in vitro* stretch model, collagen synthesis in SMCs increased with increasing circumferential strain. When cyclic stretch (20%, 1 Hz) was performed for 20 h, there were 87 significant differences in the expression of secreted proteins in rat VSMCs (2-fold differences). Gene ontology analysis revealed that these differential genes are involved in ECM secretion and reorganization, such as a significant increase in *Thbs1* secretion by VSMCs, which is involved in vascular remodeling via integrin/YAP signaling [235]. After 5 days of cyclic stretch (25%, 0.05 Hz), total protein and collagen synthesis significantly increased [244]. Long-term (6 days) circumferential strain upregulated both collagen type III and elastin gene expression [245]. In cyclically strained human aortic SMCs, thrombomodulin and MMP-1 expression reduced and ECM synthesis increased [246]. Cyclic stretch stimulation induced a switch from a contractile phenotype to a synthetic phenotype in VSMCs, and mechanical strain induced increased AT1 receptor protein expression, increased collagen $\alpha 1$ and FN synthesis, and increased ECM synthesis compared to static cells [247].

In *in vitro* 3D tissue-engineered matrix vascular cultures, collagen content increased under pulsatile conditions (5%, 2.75 Hz) compared to static cells when SMCs and ECs were grown together on polyglycolic acid scaffolds [248]; SMCs on collagen scaffolds under cyclic stretch (2.5%–10%, 0.5 Hz) showed no change in collagen synthesis, but elastin protein synthesis significantly increased [249]; HUASMCs were added to a 3D collagen matrix and cultured for 7 days under cyclic stretch (5%, 0.1 Hz–Hz), which showed increased collagen, elastin, and MMP-2 expression and increased matrix remodeling over time at increasing cyclic mechanical strain frequencies [250]. Similar conclusions were obtained in previous studies by our group. HUASMCs were grown on a polyglycolic acid scaffold with a pneumatic left heart circulation assist pump as the driving device to provide a pulsatile tensile stress environment (120 mm Hg, 1 Hz) and cultured continuously for 4 weeks. During this period, the tissue-engineered blood vessels (TEBVs) were measured by optical coherence tomography to de-

tect changes in its thickness to predict vascular remodeling. Results showed a significant increase in TEBV thickness and a denser tissue structure in the pulsed group compared to the nonpulsed group, as well as a significant increase in collagen expression [251, 252].

Cyclic stretching of aortic SMCs in 2D culture resulted in a 2-fold increase in the expression of hyaluronic acid and chondroitin 6-sulfate in SMCs compared to the unstretched group [253]. Mechanical stretch also resulted in the shedding of syndecan-4 from the cell surface, which facilitated cell motility by reducing local adhesions [254]. MMP synthesis in SMCs was also regulated by cyclic stretch, and in *in vitro* mechanical stretch model, mechanical stretch promoted the expression of MMP7 and MMP11 in rat VSMCs [255]. In mechanically stretched venous and arterial SMCs, the MMP-2 and MMP-9 expression increased significantly [256–258].

These studies suggest that mechanical stress plays an important role in tissue remodeling and tissue engineering applications, and that *in vitro* mechanical stimulation by cyclic stretch almost consistently increases ECM synthesis in SMCs and performs remodeling. This suggests that cellular remodeling can be directed to improve arterial regeneration and remodeling through the use of cyclic mechanical stimulation.

Mechanosensitive Pathways in SMCs Are Involved in Vascular Remodeling

Blood pressure plays an important role in regulating vascular structure and function. ECs in the vasculature are dynamic regulators of blood flow, inflammation, and coagulation. VSMCs are the main cells of the arterial cell mass and are important in maintaining the dynamic changes in the arterial structure and optimizing arterial mechanics [259].

Under normal physiological conditions, mechanical stimulation induces ECs to release and balance the effects of vasodilators and vasoconstrictors, thereby maintaining normal vascular tone. However, under pathophysiological conditions, the persistent abnormal increase in mechanical stress induced by hypertension leads to alterations in vascular structure and function. VSMCs play a key role in vascular remodeling by responding to the increased pressure on the blood vessel wall and affecting important biological functions such as cell proliferation, cell migration, and ECM synthesis [9].

In animal models of hypertension, with increased proliferation of VSMCs, for example, induced by Ang II in-

jection in mice, Piezo1 ion channel opening increases the cytoplasmic Ca^{2+} concentration in the VSMCs, and specific knockdown of the Piezo1 gene leads to reduced activity of triglycerides [43], which play an important role in arterial SMC proliferation and PAH [260]. Piezo1 gene deletion leads to a significant reduction in the arterial diameter, wall thickness, and cross-sectional area [43]. In an SHR disease model, multiple isoforms of TRP channels are activated and activated TRP mediates elevated intracellular Ca^{2+} and Na^+ [50]. In PAH, TRPM7 is down-regulated to activate the MEK/ERK pathway and intracellular Mg^{2+} levels are reduced [59]; in addition, mechanical stretch potentiating the Ang II type 1/EGFR/ERK-dependent signaling pathway directly regulates VSMC proliferation induced in SHR [133]. Activation of tyrosine kinase phosphorylation in SMCs in hypertension [125] and activation of the MAPK signaling pathway both promote VSMC proliferation and migration [203]. In addition, the cytoskeletal proteins emerin and lamin A/C, which are involved in regulating cellular function in response to cyclic stretch, can also induce VSMC proliferation [188].

In hypertension, there is increased production of reactive oxygen species (ROS), which can crosstalk with intracellular free Ca^{2+} signaling, with the involvement of TRPM channels [60, 261]; reactive oxygen species can contribute to the conversion of VSMCs from a contractile to a synthetic phenotype [262, 263]; activation of the MAPK signaling pathway can also mediate SMC phenotypic conversion [202, 264]. Effectors of the Hippo pathway, YAP-TAZ, are involved in stretch-induced phenotypic conversion of human umbilical vein VSMCs to a synthetic state and also regulate the proliferation and survival of pulmonary artery VSMCs and pulmonary arterial remodeling [233].

These studies suggest that mechanosensitive regulators of VSMCs responses to mechanistic stimuli can be activated in hypertension-related diseases, and that various signaling pathways can interact and cross talk to some extent, affecting cell proliferation, cell migration, and ECM synthesis, further participating in the development of vascular remodeling.

Mechanosensitive Pathways in VSMCs Are Involved in Tissue-Engineered Blood Vessels

CVD is a serious disease threatening human health. Ischemic diseases, such as atherosclerotic, remain one of the leading causes of mortality and morbidity worldwide.

These diseases have led to a continuous need for vascular grafts to solve problems such as reconstruction or vascular occlusion and aneurysms [265, 266]. With the complications of vascular transplantation surgery, the frequent shortage of autologous vascular grafts, and the immune rejection of large-animal-derived vessels [267–269], artificial vascular grafts were first introduced in the 1950s to replace occluded arterial vessels [270]. However, despite advances in all aspects of pharmacology, materials science, and equipment manufacturing, the implantation of these synthetic vascular grafts did not significantly reduce overall mortality and morbidity, and synthetic small-diameter vascular grafts have disadvantages such as low vascular compliance and patency [271], the need for repeated interventions in the pediatric population, and susceptibility to infection.

A TEBV is prepared, constructed, or regenerated by mimicking normal blood vessel wall cells and ECM components and their combinatorial arrangement. The evaluation criteria include high vessel compliance, strong mechanical properties, low thrombosis and regenerative remodeling, and, ultimately, successful fusion after implantation [272, 273]. To obtain a good-quality TEBV, it is important to consider the structure of the blood vessel as well as the microenvironment in which it resides. The mechanical microenvironment in the body has a significant impact on the mechanical properties of blood vessels. The tension and shear stress of blood vessels caused by vascular pulsation and blood flow in a physiological state stimulate the proliferation and migration of ECs and VSMCs and the secretion of corresponding growth factors, which regulate the barrier function of blood vessels and the formation of collagen and elastic fibers. Therefore, without considering biomechanical stimuli, the constructed blood vessels will lack sufficient mechanical strength to obtain a tissue structure and mechanical properties close to those of natural blood vessels. At present, the 2 main challenges facing TEBV development are that it lacks sufficient elastic mid-membrane support under static conditions *in vitro* and it is not mechanically strong with similar mechanical properties as natural blood vessels. After implantation, autologous cells have difficulty growing, particularly ECs, resulting in low long-term patency rates. Therefore, it is of great significance to find a method of constructing a TEBV that mimics the cardiac pulsatile vasomechanical microenvironment *in vivo*.

Laura E. Niklason's team has developed a TEBV based on an *in vitro* bioreactor that induces ECM secretion from target cells inoculated on a biodegradable scaffold by applying appropriate mechanical stimuli, after ECM

remodeling, resulting in a TEBV with good biochemical and mechanical properties [274]. The TEBV based on this method is the first artificial blood vessel to enter phase III clinical trials for use in the hemodialysis pipeline for patients with end-stage renal disease. After decellularization and implantation, recruitment of host myofibroblasts, ECs, and progenitor cells transforms the previously acellular TEBV into a functional, multilayered living tissue that maintains blood transport and exhibits self-repair after cannulation injury, effectively making it like the patient's own vessels [275]. To make a biological engineered artery to function successfully, it needs to meet the requirements of stringent design criteria, including ECM of sufficient quality to provide suitable tensile, suture retention, and rupture strength properties [273]. It is necessary to ensure that the artificial artery can assume the natural physiological function and that, when implanted in the body, it functions immediately without any maturation period to be fully competent in terms of mechanical strength or inflammatory properties [273]. The mechanical stimulus is a crucial factor, and it is therefore essential to obtain a TEBV with good mechanical properties (appropriate tensile strength, suture retention, and fracture strength properties) by mimicking as closely as possible the specific mechanical microenvironment in which the *in vivo* blood vessel is located during TEBV culture, allowing it to secrete and remodel the ECM.

TEBV *in vitro* by using pulsatile perfusion bioreactors and elastic PLCL scaffolds enhances tissue development and the retention of the differentiated cell phenotype, and the mechanical stimulation (1 Hz, 5% radial distention of the initial radius) induces cellular alignment and contractile phenotype of SMCs in bioengineered tissues [276]. The incremental addition of pulsatile radial stress at 110–120 beats per minute (bpm) significantly heightens the mechanical properties of the human-induced pluripotent stem cell-SMCs-TEBV [277]. In a bioreactor system, cyclic mechanical strain (1 Hz, 10% radial distention) was applied to engineered vascular constructs made of isolated SMCs in a 3-dimensional (3D) collagen type 1 matrix, which causes a characteristic increase in gel compaction and cell proliferation, and expression of the contractile protein smooth muscle α -actin [278].

In previous studies, we constructed bioreactor-based 3D culture systems with adjustable mechanical parameters, successfully cultured a TEBV with well-arranged collagen fibers and good mechanical properties [251, 252], and established a quantitative system for quantifying the mechanical stimuli applied to the TEBV [279]. The composition, metrics, and mechanical properties of

the cultured TEBV differed significantly between the different intensities of circumferential stress stimulated over time. Therefore, it is important to clarify how circumferential stress stimulation mediates SMC behavior and thus affects TEBV construction and to investigate its molecular mechanisms in order to lay a foundation for targeted improvement of the conditions for TEBV construction. Different types of mechanical stretch can elicit different molecular and cellular responses [280]. Circumferential stress regulates VSMC proliferation, apoptosis, and migration, as well as collagen synthesis, degradation, and reorganization [281], and the unique mechanical properties of blood vessels are closely related to the distribution and arrangement of collagen fibers. Whether the mechanosensitive molecules and signaling pathways in SMCs mentioned before are involved in the construction of a TEBV and how much they contribute to the formation of the mechanical properties of a TEBV remain to be further studied.

Perspectives

Circumferential stress induced by pulsatile blood pressure activates intracellular signaling networks through a variety of mechanosensory mechanisms that regulate VSMCs function, which in turn regulates transcription factor/gene expression and cellular function. With increasing research on the regulation of mechanical stress and its effects on cell structure and function in recent years, the mechanisms by which VSMCs and ECs respond to adaptive stress and mechanical transduction are gradually being understood. However, there are still many unknown details in this field, such as the effects of mechanical form of loading, the pressure waveform of mechanical loading on cells, the effects of the size and frequency of mechanical stimulation on the biochemical function of VSMCs and ECs, and the effects of mechanical stimulation on the formation, repair, and reconstruction of vascular tissue and TEBVs *in vitro*, as well as the mechanisms of mechanical signal transduction and regulation of gene expression in VSMCs and ECs during this process.

Therefore, further exploration of mechanosensitive signaling in vascular cells under stress and the mechanobiology of mechanics affecting vascular cellular activity will help to understand the role of mechanics in the pathogenesis of certain diseases (e.g., vascular diseases) and in the repair process after damage, allowing for targeted countermeasure strategies. It is also of great signif-

icance in the field of tissue engineering and regenerative medicine. Currently, there is a great clinical need for engineered arterial replacement for the treatment of atherosclerotic diseases, infections, and traumatic injury diseases. However, the equally enormous simultaneous challenges of immune acceptance, requisite tissue mechanics, low thrombogenicity, and immediate availability have made the broad clinical application of engineered arteries quite difficult to achieve [273]. Understanding the effects of mechanical factors on vascular cells and the underlying mechanisms is of great importance to optimize engineered blood vessel construction.

In this review, we described the molecules involved in the VSMCs response to circumferential stress/cyclic stretch, including mechanosensitive ion channels, membrane receptors or transmembrane proteins, integrins, cytoskeletal proteins, and nuclear structures, which translate mechanical stimuli into biochemical events and ultimately lead to some basic mechanisms of biological responses. The understanding of more such proteins/molecules and mechanosensitive signaling pathways will help us identify the intracellular mechanisms of key signaling pathways and elucidate more clearly CVD pathogenesis, as well as helping to address a major challenge currently faced by TEBVs—the lack of sufficient mechanical properties. Selecting appropriate mechanical stimulation conditions, as well as genome-editing techniques to intervene with defined mechanically sensitive regulatory molecules in the future, to direct the biological behavior of VSMCs and to secrete and reconstitute the ECM, will guide the development of more effective clinical therapies.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.L. conceived, wrote, and revised the manuscript. Z.L. conceived, revised the manuscript, and approved the final version of the manuscript.

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Data sharing is not applicable to this review article as no datasets were generated or analyzed during the current study.

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