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Mechanobiology in vascular remodeling

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ABSTRACT

Vascular remodeling is a common pathological process in cardiovascular diseases and includes changes in cell proliferation, apoptosis and differentiation as well as vascular homeostasis. Mechanical stresses, such as shear stress and cyclic stretch, play an important role in vascular remodeling. Vascular cells can sense the mechanical factors through cell membrane proteins, cytoskeletons and nuclear envelope proteins to initiate mechanotransduction, which involves intercellular signaling, gene expression, and protein expression to result in functional regulations. Non-coding RNAs, including microRNAs and long non-coding RNAs, are involved in the regulation of vascular remodeling processes. Mechanotransduction triggers a cascade reaction process through a complicated signaling network in cells. High-throughput technologies in combination with functional studies targeting some key hubs and bridging nodes of the network can enable the prioritization of potential targets for subsequent investigations of clinical translation. Vascular mechanobiology, as a new frontier field of biomechanics, searches for principles of stress-growth in vasculature to elucidate how mechanical factors induce biological effects that lead to vascular remodeling, with the goal of understanding the mechanical basis of the pathological mechanism of cardiovascular diseases at the cellular and molecular levels. Vascular mechanobiology will play a unique role in solving the key scientific problems of human physiology and disease, as well as generating important theoretical and clinical results.

Keywords: mechanobiology, vascular remodeling, cardiovascular disease, mechanotransduction, endothelial cell, vascular smooth muscle cell, nuclear envelope, microRNA

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INTRODUCTION

Cardiovascular disease is one of the most serious health hazards. Elucidation of the pathogenesis of cardiovascular disease for its prevention is a major field of biomedical research [1]. Cardiovascular disorders, including hypertension, atherosclerosis and stroke, are essentially vascular diseases. They have a common pathogenic mechanism and basic pathological process, i.e., vascular wall remodeling, which includes cardiovascular cell migration, hypertrophy, proliferation and apoptosis, as well as changes in cell phenotype, morphological structure and function [2].

The human body exists in a mechanical environment, which influences the biological processes at every level, including the whole body, organs, tissues, cells and molecules. The cardiovascular system can be considered a mechanical system in which the central position is occupied by the heart, which functions as a mechanical pump. Blood circulation involves the flow of blood, deformation of blood cells and blood vessels, and interaction between blood and vessels, which comprise the rich mechanical mechanisms. Many clinical and experimental studies have demonstrated that biological, chemical, physical, and other factors affect the vascular remodeling *in vivo* and *in vitro*, in which mechanical factors play a direct and important role. We have selected vascular remodeling as a starting point to explore some common modes of pathogenesis for the complex characteristics of multi-gene, multi-pathogenic factors in cardiovascular diseases.

Biomechanics studies the deformation and movement of living entities, through the organic combination of biological and mechanical principles, to recognize the laws of life processes and solve scientific issues in the field of life and health. Y. C. Fung proposed the stress-growth law in his monograph *Biomechanics: Motion, Flow, Stress, and Growth* in 1990, which states that remodeling

of a blood vessel involving growth or resorption of cell and extracellular materials is linked to stress in the vessel [3]. The stress-growth law is a fundamental theory that expounds the intrinsic relationship between the most basic form of matter movement, mechanical motion, and the highest form, life motion, and guides the transformation of biomechanics from mechanics applied to biology to the organic bond of mechanics with biological processes. A qualitative change and the development of biomechanics are observed. Mechanobiology, as a new frontier field of biomechanics, has increased with response to proper timing and conditions. Mechanobiology encompasses several broad research areas and searches for the effects of the mechanical environment in health, disease or injury, mechanosensitive responses and their mechanisms, inter-relations between mechanics and biological processes, such as growth, adaption, remodeling, and repair, and discoveries related to new diagnostic and therapeutic procedures [4,5]. These studies are of great theoretical and practical significance for our understanding of the mechanical mechanisms and natural laws of growth and senility of the human system, expounding pathological mechanisms of diseases, and researching and developing new medicines and technologies for medicine.

Vascular mechanobiology elucidates the principles of stress-growth in the vasculature, as well as how mechanical factors induce biological effects to result in vascular remodeling to elucidate the mechanical basis of blood circulation and the natural laws of growth and senility of the vasculature and to expound the pathological mechanism of cardiovascular diseases on cellular and molecular levels.

VASCULAR CELLS RESPOND TO MECHANICAL STRESSES

The blood vascular wall has three mechanical force loadings, i.e., shear stress (SS), normal and circumferential stresses. SS, which acts parallel to the luminal surface of the vessel, is an outcome of fluid viscosity and the velocity gradient between adjacent layers of the flowing blood [1,6,7]. The circumferential stress acts along the vessel wall perimeter to cause stretching, resulting in corresponding deformation of the vessel wall, which is termed the circumferential strain.

Vascular cells respond to mechanical stresses such as SS and stretch, which can be sensed by cells, by regulating the cell signaling pathway, affecting gene expression and influencing cell functions as a result [6–8]. Through various means, the cells transform the exocytic mechanical signal into an intracellular signal, and then trigger the cascade reaction process, which is known as mechanotransduction.

Roles of the cell membrane and cytoskeletons in mechanotransduction

Multiple mechanosensors in the vascular cell membrane have been reported, including integrins [9–18], ion channels [19–28], junctional proteins [29–32], growth factor receptors [29], receptor tyrosine kinases (RTKs) [33,34], G protein-coupled receptors (GPCRs) [35–39], platelet/endothelial cell adhesion molecule-1 (PECAM-1) [40,41], and caveolae [37,38,42], as well as membrane lipids [43,44], glycocalyx [45–50], and primary cilia [51,52].

The above-mentioned mechanosensors are on or in the vascular cell membrane. However, in the endothelium, the interconnected cytoskeletal filaments are also linked to membrane proteins in every part of the cell. The cytoskeleton is made up of actin filaments, microtubules and intermediate filaments, providing elastic stiffness and maintaining the shape and structure of a cell to enable specific cellular functions [53]. Different cytoskeletal networks have interpenetration and interactions, which combined with specific cross-linking, have an effect on the cellular overall mechanical response. In response to shear stress, AMP-activated protein kinase (AMPK) phosphorylation of cortactin, followed by sirtuin 1 (SIRT1)SIRT1 deacetylation, regulates the interaction of cortactin and cortical–actin. This AMPK/SIRT1 co-regulated cortactin–F-actin dynamics is need for a sub-cellular translocation/activation of endothelial nitric oxide synthase (eNOS) and is also atheroprotective [54]. There is ample evidence indicating that cytoskeletal assembly and dynamics respond to different flow patterns. Conceivably, mechanical stimuli acting on the cell surface are transmitted to the cytoplasm via cytoskeletal deformations such as intermediate filament displacement or actin filament deformation. Direct observation of intermediate filament displacement in cells expressing green fluorescent protein has suggested that SS rapidly alters the cytoskeletal mechanics. In addition to its structural roles, the cytoskeleton also regulates gene transcription through nucleocytoplasmic shuttling of mechanosensitive transcriptional activators [55].

Role of the cell nucleus in mechanotransduction

A mechano-vascular proteomic study suggested that the proteins of the nucleus envelope (NE) might

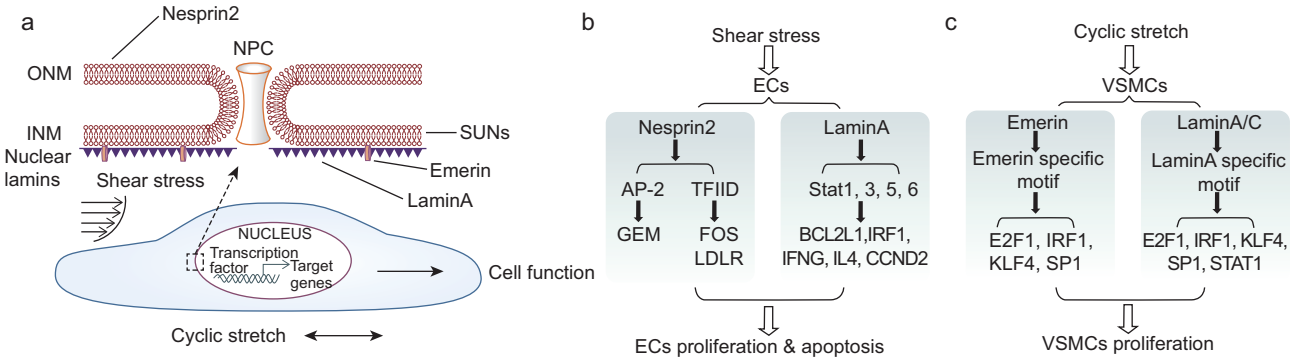


Figure 1. Schematic diagram of the roles of nuclear envelope proteins in vascular mechanotransduction. (a) A schematic diagram of the putative signaling pathways involved in the effects of nuclear envelope proteins on EC or VSMC functions in response to mechanical stimuli. (b) Low SS represses the expressions of nesprin2 and lamin A, which impacts the activation of transcription factors AP-2, TFIID and Stat1, 3, 5, 6, regulates the mRNA levels of their downstream target genes, and then induces the proliferation and apoptosis of ECs. (c) Pathological cyclic stretch suppresses the expression of emerin and lamin A/C, which bind to specific motifs in the DNA segments, and decreases the binding of emerin to the promoter regions of E2F1, IRF1, KLF4 and SP1, and the binding of lamin A/C to the promoter regions of E2F1, IRF1, KLF4, KLF5, SP1 and STAT1, eventually inducing VSMC proliferation.

directly respond to mechanical stimuli and regulate gene expression afterwards [56]. All these molecules are implicated in mechanotransduction of SS and subsequently result in endothelial cell (EC) functional responses, e.g., proliferation, apoptosis, migration and permeability.

The nucleus is the stiffest and largest sub-cellular organelle in most cells, playing an important role in storing and managing genetic information and serving as the site for DNA and RNA synthesis, transcription processing, and coordinating the intricate cellular architecture. Consisting of two lipid bilayers, namely the inner and outer nuclear membranes (INM and ONM), NE is the physical barrier between the cytoplasm and genome, and ONM is an extension of the rough endoplasmic reticulum (ER) and is connected to INM at the nuclear pore complex (NPC). INM and ONM delineate the periplasmic space, which is continuous with the ER lumen. INM proteins interact directly with the nuclear lamina, a specialized meshwork of lamins that constitute the type V intermediate filament family. The INM and ONM are perforated by NPCs that control traffic in and out of the nucleus. NPCs mediate the exchange of different sizes of molecules between the nucleoplasm and cytoplasm, which act as gatekeepers of the nucleus (Fig. 1a).

Compared with the cytoplasm, the role of nuclear mechanotransduction in gene regulation is much less well understood. The cytoskeleton is the major cellular determinant of the physical and mechanical properties, which mediates cellular responses to various environmental cues from the surroundings. Cytoskeletal polymers, including actin filaments, microtubules and intermediate filaments, can connect to the NE through linkers of nucleoskeleton and cytoskeleton (LINC) complexes and transmit mechanical stresses into the nucleus [57]. Recently, the role and function of LINC complexes have gained attention for their involvement in connecting the cytoskeleton to the nucleus to transduce mechanical stimuli throughout the cell.

LINC complexes, conserved from yeast to men, are composed of both ONM and INM proteins that belong to the Klarsicht, Anc-1, and Syne homology (KASH) domain protein families, as well as Sad1 and UNC-84 (SUN) homology domain proteins [58]. The acronym KASH originates from the conservation of the same domain in Klarsicht from *D. melanogaster*, ANC-1 from *C. elegans*, and Syne homology from mammals. Most KASH domain proteins reside in the ONM, and their amino-terminal regions are exposed to the cytoplasm and are associated with the cytoskeleton, such as actin filaments, microtubules, and intermediate filaments. The carboxyl termini of KASH proteins contain the KASH domain, which is a 30-amino-acid peptide typically ending with the conserved motif PPPX or PPPT. The N-terminus of KASH proteins extends into the perinuclear space (PNS) and interacts with the SUN domain of SUN proteins [59]. The SUN domain was first defined as a domain of shared homology between Sad1 in *Schizosaccharomyces pombe* and UNC-84 in *Caenorhabditis elegans*. An amino-terminal nucleoplasmic domain of SUN proteins interacts with nuclear lamina and chromatin-binding proteins. In contrast, a carboxyl-terminal region, containing a conserved SUN domain, protrudes into the PNS. The direct interactions of SUN proteins and KASH proteins across the NE provide a core link between the nucleoskeleton and the cytoskeleton. Thus, it is reasonable to assume that the LINC

complex mediates mechanically induced signals along the NE and then into the nucleus.

The human genome contains six genes encoding KASH proteins. Four of them are nuclear envelope spectrin-repeat proteins (nesprins1–4) [60]. Compared with nesprin 4, nesprins 1–3 are widely distributed and predominantly mediate mechanotransduction to the nucleus in most cells. Cells exposed to a mechanical stimulus show altered cell signaling and cytoskeletal organization leading to changes in the cellular phenotype. The nucleus is also force-responsive, and these mechano responses not only affect nuclear functions, but also subnuclear structures and changes in subnuclear movement [61]. The functions of nesprins have been demonstrated in cellular responses to mechanical force systems. For instance, nesprin1 knockdown increases the number of focal adhesions and substrate traction while decreasing EC migration in response to cyclic strain, resulting in abnormal adhesion and migration [62]. The physical link from the cytoskeleton to NE is decisive for mechanotransduction. The disruption of nesprin–SUN complexes disrupts force transmission from the cytoskeleton to the nucleus, which perturbs the mechanical control of cell differentiation and abrogates their stretch-induced proliferation [63]. A recent study by Han *et al.* showed that nesprin 2 is sensitive to the SS and regulates EC functions. Under low SS, the repressed nesprin 2 is correlated with increased proliferation and apoptosis of ECs [64] (Fig. 1b).

SUN proteins are single-pass transmembrane proteins localized in the INM. Both human and mouse genomes encode at least six SUN proteins. While SUN1 and SUN2 are widely expressed, SUN3 and SPAG4 appear to be limited in several tissue types [65]. SUN proteins can also interact with lamin B to mediate nuclear migration [66]. SUN1 ablation weakens the nucleoskeleton, leading to reduced force transmission to the nucleus [67]. Transmission electron microscope (TEM) analysis has revealed that nesprin 2 or lamin A knockdown results in degradation of the NE phospholipid bilayer, suggesting that nesprin 2 and lamin A regulate NE stability and nuclear structure [64]. Furthermore, the organization of nuclei is disrupted in SUN 1/2 double-knockout mice.

The nuclear lamina, containing A-type lamins (lamins A and C, encoded by the LMNA gene) and B-type lamins (lamins B1 and B2, encoded by the LMNB1 and LMNB2 genes), is linked to chromatin and participates in gene transcription. B-type lamins are broadly expressed. In contrast, A-type lamins, which are expressed in all differentiated cell types, participate in gene expression, cell signaling, high-order chromatin organization, and nuclear architecture. The research on nuclear lamins has focused on their regulation of nuclear architecture. New evidence shows that A-type lamins and their associated NE proteins are key regulators of mechanotransduction. Han *et al.* reported that low SS suppresses the level of lamin A in ECs, and this suppression subsequently leads to EC dysfunction [64]. Down-regulation of A-type lamins in ECs facilitates T cell migration through EC layers, suggesting that the regulation of EC nuclear stiffness by lamin A/C may modulate subendothelial migration of blood-borne immune cells, a key process of atherosclerosis [68]. Brosig *et al.* showed that expression of dominant negative mutants of nesprin and SUN enhances the transcriptional activity of NFκB in C2C12 cells, suggesting that the degradation of nuclear LINC complexes causes conformational changes in chromatin structure and organization that modulate transcription factor binding or transcriptional processes [69]. In response to pathological cyclic stretch, lamin A/C expression is depressed, which ultimately increases the proliferation of vascular smooth muscle cells (VSMCs) [70]. In addition, lamin A is also involved in sensing forces generated from cells within tissue during differentiation. Matrix stiffness directly influences the lamin A level, and lamin A transcription is modulated by the vitamin A/retinoic acid (RA) pathway with broad roles in development [71]. Lamin A in human cell lines from tumor cells to primary mesenchymal stem cells (MSCs) also contributes to migration [72].

Emerin is another ubiquitous integral membrane protein that is localized in the INM and associates with nesprin 1, 2, SUN1/2, and lamin A/C. Loss of emerin brings about Emery–Dreifuss muscular dystrophy (EDMD), characterized by muscle weakening, and potentially lethal cardiac conduction system defects. Emerin has a LEM-domain and therefore binds to barrier-to-autointegration factor (BAF), a conserved chromatin protein that is essential for cell division. BAF conscribes emerin to chromatin and regulates high-order chromatin structure during nuclear assembly. Most studies on emerin focus on skeletal muscle and myocardium, while little research has been conducted on VSMCs. Recent results indicate that emerin and lamin A/C bind to the respective sequencing-specific motifs of transcription factors to modulate the hyperstretch-induced dysfunction of VSMCs [70] (Fig. 1c). Combined with these results, it has been suggested that nesprin 2, lamin A/C, and emerin modulate the proliferation of ECs and VSMCs in arterial walls in response to cyclic strain and SS associated with hypertension. Other studies have shown that MRTF-A, a cardiomyocyte-related mechanically sensitive transcription factor, plays an important role in

cardiac development. The reduction in lamin A/C and emerin reduces the viability of the nucleus and cytoskeletal microfilaments and results in a decrease in the activity of the transcription factor MRTF-A and suppression of its translocation [73]. This result also shows that lamin A/C and emerin-induced changes in the nuclear structure can directly affect gene regulation. On the other hand, nuclear skeleton elements can also interact directly with chromosomes or multiple transcriptional regulators. For example, lamin A/C binds to pRb, c-Fos and ERK1/2 [74], whereas emerin interacts with β-catenin, BAF and GCL [75]. These results suggest that the structure of the nucleus, plasticity, and mechanical transmission between the nucleus and the skeleton play important roles in the intracellular signal transduction pathways.

In recent years, several new processes associated with nuclear membrane remodeling have been reported, including NE repair after rupture and NE autophagy. Despite major progress made in nuclear mechanotransduction sensors, many other questions remain to be studied, such as whether the DNA binding of NE proteins modulated by mechanical forces is direct or other complexes are involved, whether LINC complex defects could be mainly attributed to changes in the pre-stress state of the cell and how various NE proteins interact with each other. The network of mechanotransduction in the nucleus and the NE proteins involved require further study.

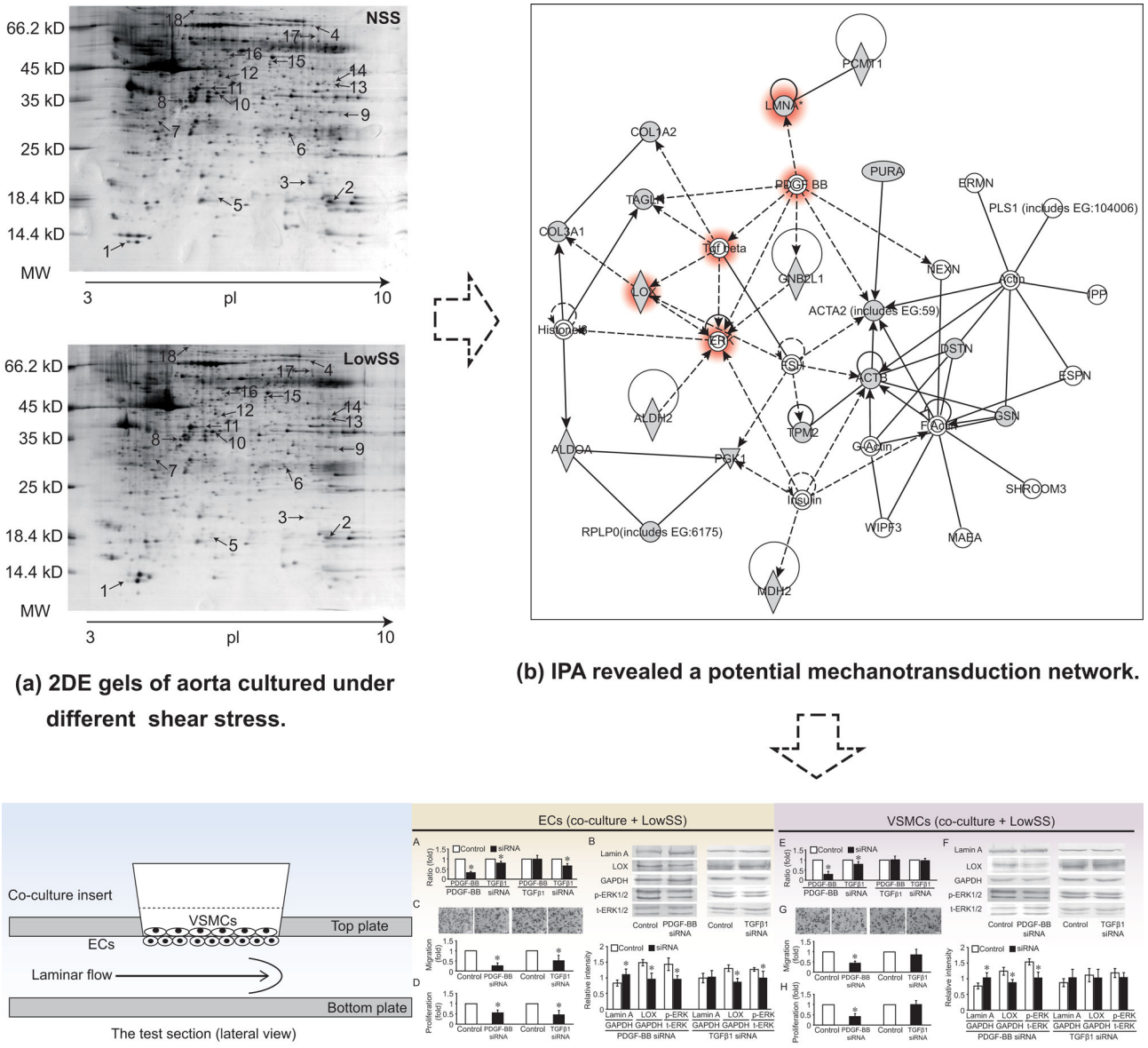
MECHANOTRANSDUCTION NETWORK BASED ON HIGH-THROUGHPUT BIOTECHNOLOGY

Mechanotransduction initiates the cascade reaction process through a complicated signaling network in cells. High-throughput biotechnology, such as proteomics, phosphoproteomics, genomics, and transcriptomics, among others, can provide enormous amount of data for bioinformatics and/or system biology analyses to reveal key genes or proteins in the regulatory network. These key hubs and bridging nodes of the network can enable the prioritization of potential targets for subsequent validation experiments for clinical translation [56,76,77].

ECs and VSMCs are the major cellular constituents of the vessel wall. The interactions, crosstalk and synergy between VSMCs and ECs play a critical role in vascular biology in health and disease. ECs in the intima of the arterial wall are exposed to SS constantly, and then transduce the mechanical stimuli to intracellular signals [6,10]. ECs induce gene expressions of PDGF-AA, PDGF-BB and TGFβ in co-cultured VSMCs [78], and SS regulates the migration, apoptosis, proliferation and gene expressions of VSMCs in an EC-dependent manner [79–81]. SS modulates the EC phenotype, with subsequent alterations in the release of pro-inflammatory cytokines, as well as VSMC proliferation, apoptosis and gene expressions [82,83].

With the use of a systemic biological approach encompassing high-throughput screening, bioinformatics analysis and biological validation, a vascular cell mechanotransduction network has been established [56]. Using proteomic analysis, the protein profiles of rat aorta cultured under low shear stress (LSS, 5 dyn/cm^2) and normal shear stress (NSS, 15 dyn/cm^2) were compared (Fig. 2a). The differential expressed proteins were analyzed by Ingenuity Pathway Analysis (https://analysis.ingenuity.com/pa/installer/select). A signaling network that is highly associated with mechanotransduction exerted by LSS, involving platelet-derived growth factor BB (PDGF-BB), transforming growth factor beta1 (TGFβ1), lamin A, lysyl oxidase (LOX), and extracellular signal-regulated kinases 1/2 (ERK 1/2), was revealed (Fig. 2b). The network mediating LSS-induced migration and proliferation of ECs and VSMCs and the cross-talk between these two cell types was investigated in a co-cultured system in a parallel-plate flow chamber (Fig. 2c). In comparison to NSS, LSS upregulates migration and proliferation of ECs and VSMCs, and increases the production of PDGF-BB and TGFβ1. Additionally, PDGF-BB recombinant protein shows an effect similar to LSS on ECs and VSMCs. In contrast, TGFβ1 recombinant protein has a similar effect on ECs to PDGF-BB, but not on VSMCs. When PDGF-BB expression is 'knocked down' in ECs, the effects of LSS are mitigated or abolished, and this effect is also blocked by pre-incubation of VSMCs with PDGF-BB neutralized antibody. TGFβ1 'knockdown' in ECs and neutralizing antibody pre-incubation with VSMCs mitigates the EC responses to LSS but has no effect on VSMCs. These results suggest that ECs respond to LSS stimuli by upregulating PDGF-BB and TGFβ1. However, these two growth factors play different roles in LSS-induced vascular remodeling. While PDGF-BB is involved in the paracrine control between ECs and VSMCs, TGFβ1 takes part in the feedback control from VSMCs to ECs [56].

Over at least the last two decades, molecular and cell biology approaches have been used to research the roles and involvement of multiple molecules in mechanotransduction. Most, if not all, of these studies focus on the level of the single molecule and/or pathway. To date, information regarding mechanotransduction in cardiovascular cells at the systemic level is largely lacking, resulting in difficulties



(a) 2DE gels of aorta cultured under different shear stress.

(b) IPA revealed a potential mechanotransduction network.

(c) Verifying the network by the parallel-plante flow chamber for the co-culture model of ECs and VSMCs in vivo.

Figure 2. Schematic drawing outlining the vascular cell mechanotransduction network based on mechano-vascular proteomics. (a) 2D electrophoresis (2DE) gels of aorta cultured under different shear stresses. The protein profiles of rat aorta cultured under NSS (15 dyn/cm^2) and LSS (5 dyn/cm^2) are compared by using comparative proteomic techniques, 2DE and MALDI-TOF mass spectrometry. (b) IPA reveals a potential mechanotransduction network. Differentially expressed proteins are analyzed by IPA and a signaling network that is highly correlated with mechanotransduction of LSS, involving PDGF-BB, TGFβ1, lamin A, LOX and ERK 1/2. (c) Validation of the network by the parallel-plate flow chamber (left panel) for the co-culture model of ECs and VSMCs *in vivo*. In the EC/VSMC co-culture parallel-plate flow chamber, ECs and VSMCs are grown on opposite sides of a 10-μm-thick polyethylene terephthalate (PET) membrane, and the ECs are subjected to SS. The interactions of ECs and VSMCs are able to occur through 0.4-μm diameter PET membrane pores. Using this system, the expressions of molecules involved in the networks, namely, PDGF-BB, TGFβ1, lamin A, LOX and phospho-ERK1/2, and the migration and proliferation of ECs and VSMCs separately under two levels of shear stress at 5 and 15 dyn/cm^2 are studied.

elucidating the complex regulatory mechanisms of cells in response to stresses in a comprehensive manner. Recent advancement in high-throughput technology, such as 'omics' experiments, have facilitated comprehensive, systematic, dynamic and networked approaches for a wide variety of biomedical research. It is expected that such high-throughput technology will also be used in the near future to explore life phenomena, reveal the pathogenesis of diseases and search for drug targets.

Although based on the concept of proteomics and networks, scholars have conducted a large amount of research investigating vascular tissue/cell regulatory mechanisms during vascular remodeling, which is involved in a variety of proteins and a very complex and dynamic regular network, but the available research still does not describe the cell mechanotransduction network synthetically. Research examining modification-related proteomics after translation is in its infancy. Dynamic and quantitative analysis of the full spectrum and large-scale, high-flux studies examining protein modification after translation remains a problem that must be solved in the future. Furthermore, the verification and functional analysis of mechanotransduction in the context of vascular biology as well as the intracellular mechanical stress signal transduction network established by existing data are far from complete. A more efficient and accurate new theory, algorithm and software based on high-throughput technological data remain to be established. Research on the above issues seems likely to be an important frontier in the study of vascular mechanobiology.

MECHANOREGULATION OF NON-CODING RNAS IN VASCULAR REMODELING

Non-coding RNAs (ncRNAs) are functional RNAs that are not translated into proteins. This new class of RNAs is functionally involved in the epigenetic regulations of gene expression, and ncRNAs are ubiquitously present in animals and plants as well as fungi [84,85]. Increasing evidence has revealed that ncRNAs participate in the regulation of various biological processes, e.g., metabolism, development, cell differentiation, proliferation and apoptosis, cell, oncogenesis, and vascular homeostasis [84–87].

Non-coding RNAs include microRNAs (miRNAs), long non-coding RNAs (long ncRNAs, lncRNAs), small interfering RNAs (siRNAs), piwi-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs) [85,88]. Little is known about the roles of siRNAs, piRNAs and snoRNAs in the cardiovascular system, let alone their engagement in mechanotransduction, which may provide new research opportunities.

lncRNAs are defined as non-protein-coding transcripts longer than 200 nucleotides [89]. At least several thousand lncRNAs likely exist in the mammalian genome. It appears that only one-fifth of transcription across the human genome is involved in protein-coding genes, showing at least four times more lncRNAs than protein-coding RNAs [90]. As epigenetic regulation mechanisms of lncRNAs have gained ample attention in research, new efforts show that shear stress regulates the expression of candidate lncRNAs in ECs, which in turn regulates downstream metalloprotease AMZ2 expression via lncRNA binding to the repressive chromatin mark H3K27me3 [91]. Yao and colleagues found that 68 lncRNAs and 255 mRNAs are up-modulated in the aorta of spontaneously hypertensive rats, whereas 167 lncRNAs and 272 mRNAs are downregulated [92]. Moreover, 15% cyclic strain increases lncRNA XR007793 expression. XR007793 knockdown attenuates VSMC proliferation and migration and inhibits signal transducers and activators of transcription 2 (stat2), LIM domain only 2 (lmo2) and interferon regulatory factor 7 (irf7) [92]. lncRNA n342419, termed MANTIS, is downregulated in patients with idiopathic pulmonary arterial hypertension (IPAH), whereas it is upregulated in the carotid arteries of *Macaca fascicularis* subjected to an atherosclerosis regression diet as well as in ECs isolated from glioblastoma patients [93]. Given the variety of epigenetic mechanisms regulated by lncRNA, it is anticipated that lncRNA regulation of vascular remodeling in response to mechanical stimuli will generate fruitful results.

Mechanoregulation of miRNAs in vascular remodeling

miRNAs are endogenous, non-coding and single-stranded RNAs of 18–22 nucleotides that constitute a novel class of gene regulators [94]. miRNAs bind to the 3′-untranslated regions (3′-UTRs) of their target mRNAs, leading to direct degradation of mRNA or translational repression by a perfect complement in plant cells or imperfect complement in animal cells [94]. The roles of miRNAs in vascular development and diseases have been studied intensively [95,96]. As the changes of cell phenotype, migration, proliferation, hypertrophy and apoptosis, among others, are major events involved in vascular remodeling, very many miRNAs have been shown to regulate these events in the context of mechanoregulation. In the following sections, we review recent findings related to miRNAs in mechanobiology with an emphasis on shear stress effects on ECs and mechanical stretch effects on VSMCs.

Shear stress

Shear stress (SS) exertion on ECs plays significant roles in regulating vascular homeostasis and pathophysiology [6,7]. Laminar SS regulates the expression of miR-126, vascular cell adhesion molecule 1 (VCAM-1), and syndecan-4 (SDC-4) in ECs [97]. miR-126 is increased during long-term exposure to flow and shows a crosstalk between ECs and VSMCs in response to SS, which is mediated

through miR-126 [97,98]. Co-culture of VSMCs with ECs or treatment of VSMCs with conditioned medium from static EC monoculture (EC-CM) increases miR-126 level in VSMCs with concomitant suppression of FOXO3, BCL2 and IRS1 mRNAs and VSMC turnover. These effects are abolished by either inhibition of endothelial miR-126 or the application of laminar SS to ECs. Consistently, depletion of miR-126 in mice inhibits neointimal formation of carotid arteries resulting from cessation of blood flow [98].

In response to laminar SS, miR-23b is induced by the transcription factor Krüppel-like factor 2 (KLF2) [99,100]. Laminar SS also results in the expression of miR-19a, which directly targets cyclin D1, leading to cell cycle arrest at G1/S transition. Thus, miR-19a would be a key regulator of cell cycle progression in response to laminar SS [101]. SS induces expression of miR-30 family members in a KLF2-dependent manner [102]. miR-101 expression is also significantly upregulated in human umbilical vein ECs (HUVECs) exposed to laminar SS at 12 dyn/cm^2. miR-101 targets a mammalian target of rapamycin (mTOR), which in turn causes cell cycle arrest at the G1/S transition and thus suppresses EC proliferation [103]. Co-culturing ECs with VSMCs under static conditions causes initial increases in miR-146a, -708, -451, and -98 in ECs. SS (12 dyn/cm^2) applied to co-cultured ECs for 24 h augments the expression of these four anti-inflammatory miRNAs [104]. These four anti-inflammatory miRNAs are highly expressed in neointimal ECs in injured arteries under physiological flow rather than flow stagnation. Decreased expression of miR-146a can accelerate neointima formation of injured rat carotid artery under physiological flow while overexpression in miR-146a inhibits neointima formation in the rat or mouse [104].

Under disturbed flow, the expression of miR-21 is induced in HUVECs [105,106]. Oscillatory SS induces AP-1-dependent miR-21 expression, which directly targets peroxisome proliferator-activated receptor α (PPARα) mRNA, thereby increasing the expression of VCAM1 and C-C motif chemokine 2/monocyte chemoattractant protein 1 (CCL2/MCP1) to promote adhesion of monocytes to ECs. Oscillatory SS induction of miR-21 is a positive feedback loop that increases the pro-inflammatory responses of vascular endothelium [106]. Oscillatory SS also induces miR-663 expression in cultured HUVECs [107]. The disturbed flow also induces the expression of miR-712, which promotes endothelial inflammation and increases endothelial permeability, resulting in a pro-atherogenic phenotype [108].

The expression of miR-126–5p is increased in atheroprone areas in a KLF2-dependent manner. The inhibition of miR-126–5p, but not miR-126–3p, recapitulates the effects of pri-miR-126a knockout, which increases the area of the atherosclerosis lesion, promotes macrophage infiltration, and decreases endothelial repair in mice [109]. On the other hand, miR-10a is drastically decreased in the disturbed flow area under arterial tress, when compared to the laminar flow area. The NF-κB pathway is activated and NF-κB target genes are upregulated when miR-10a is inhibited in ECs *in vitro* [110], showing that the absence of miR-10a in the disturbed flow area causes ECs to be susceptible to inflammation [111]. Interestingly, miR-10a exhibits the lowest expression level among all the examined shear-responsive miRs in ECs under oscillatory SS [112]. In terms of the regulatory mechanism, miR-10a expression is regulated by KLF2 through modulation of RARα–RARE binding, with consequent regulation of GATA6/VCAM-1 in ECs [112]. All these results indicate that miR-10a is downregulated in ECs by disturbed flow via KLF2.

Atheroprone SS induces the expression of miR-92a in concert with oxidized LDL treatment (ox-LDL) in HUVECs [113–116]. As miR-92a targets the 3′-UTR of KLF2 mRNA, atheroprotective laminar flow downregulates miR-92a to induce KLF2 [114]. Interestingly, blockade of miR-92a expression in Ldlr$^{-/-}$ mice restores endothelial function and decreases atherosclerosis [115]. The expression of miR-34 is upregulated by both p53 and oscillatory SS [117]. Blockade of endogenous miR-34a decreases the expression of VCAM-1 and intercellular adhesion molecule-1 (ICAM-1) in ECs. Conversely, miR-34a overexpression increases the level of VCAM-1 and ICAM-1, which promotes monocyte adhesion to ECs [118]. Furthermore, laminar SS increases miR-34a expression levels in human umbilical cord blood-derived endothelial progenitor cells (EPCs). An inverse correlation of miR-34a and Foxj2 expressions is involved in the endothelial differentiation of EPCs [119]. These results indicate that miR-34a is a mechanosensitive miRNA that may have distinct functions in ECs versus EPCs. In addition to the abovementioned miRNAs, there is a panel of SS-sensitive miRNAs that regulate various aspects of endothelial biology.

Cyclic stretch

VSMCs in a vascular medium are loaded with cyclic circumferential strain, i.e., cyclic stretch. Song *et al.* reported that an elevated stretch (16% elongation, 1 Hz) increases miR-21 expression in cultured human aortic smooth muscle cells (HASMCs),

whereas a moderate stretch (10% elongation, 1 Hz) decreases expression. Because miR-21 is involved in HASMC proliferation, the complex of miR-21 and programmed cell death protein 4 (PDCD4) regulates stretch-induced apoptosis [120]. Cyclic stretch also modulates the VSMC phenotype through several other miRNAs. In VSMCs of the portal vein, the stretch-induced mRNA expression of contractile markers is reduced in the absence of miR-143/145 [121]. In stretched portal veins and in pressurized carotid arteries, the expression of miR-144/451 is downregulated, which is inversely correlated with the expression and phosphorylation of AMPK [122]. In human aortic VSMCs cultured on collagen I, 16% stretch suppresses miR-145 expression in connection with reduced expression of contractile markers of VSMCs. miR-145 overexpression can partially recover the expression of these markers in the stretched cells. Furthermore, the stretch-activated extracellular signal-regulated kinase 1/2 (ERK1/2) and upregulated angiotensin-converting enzyme (ACE) account for the inhibition of miR-145 expression [123]. VSMCs exposed to physiological waveforms differentiate further compared with those under static or sinusoidal cyclic strain. Increased expression of miR-143, -145, and VSMC markers desmin, calponin and SM-22 are found in these more differentiated cells. Uniform dynamic stretch not only increases the expression level of miR-143 and -145, but also increases that of miR-221 [124].

Once a vein graft is transplanted into the arterial system, such as a saphenous vein in a coronary artery bypass graft, the transplanted vessels are exposed to the arterial mechanical environment. Arterialized cyclic stretch will affect VSMC functions in the grafted veins, including excessive proliferation and migration, which causes neointima formation and ultimately leads to vein graft failure. Huang *et al.* reported a novel mechanism by which miR-33 mediates mechanical stretch-induced venous VSMC proliferation and neointimal hyperplasia. Thus, miR-33 targeting might be a novel therapeutic strategy to prevent vein graft failure and neointimal hyperplasia [125]. Embedded in the intronic sequences of genes encoding sterol regulatory element-binding proteins (SREBPs), miR-33 has been shown to modulate the proliferation of several cell types *in vivo* and *in vitro* [126–128]. Huang *et al.* first reported that in a graft vein rat model, neointimal hyperplasia and cell proliferation is significantly increased (Fig. 3a). Furthermore, miR-33 expression is decreased one, two and four weeks post-grafting [125]. In contrast, the expression of bone morphogenetic protein 3 (BMP3), a putative target of miR-33, and the phosphorylation of smad2 and smad5, which are potential downstream targets of BMP3, are all increased in the grafted veins. While miR-33 mimics attenuate, miR-33 inhibitors accelerate VSMC proliferation. Moreover, recombinant BMP3 increases VSMC proliferation and phospho-smad2 and -smad5 levels. By contrast, BMP3 siRNAs have the opposite effect [125]. To explore the mechanism on a cellular molecular level, venous VSMCs were exposed to mimic arterial cyclic stretch by a cell stretch loading system *in vitro*. The arterial stretch shows an increase in proliferation and repression of miR-33 expression. Additionally, BMP3 expression and smad2 and smad5 phosphorylation are enhanced (Fig. 3b). Perivascular multi-point injection of agomiR-33 in the graft vein rat model *in vivo* not only attenuates BMP3 expression as well as smad2 and smad5 phosphorylation, but also clearly accelerates neointimal formation and cell proliferation in the grafted veins (Fig. 3c). These effects of agomiR-33 on grafted veins can be reversed by local injection of BMP3 lentivirus [125].

Although the work by Huang *et al.* demonstrates that miR-33 affects vein graft-induced neointimal hyperplasia, a number of important questions remain unanswered, including the molecular mechanism and mechanosensors that control miR-33 expression in response to arterial stretch of VSMCs and vascular injury. Whether miR-33 is involved in human vein graft adaptation would be another interesting research topic. Interestingly, the human genome encodes two miR-33 isoforms, namely, miR-33a and miR-33b, which are, respectively, co-expressed with SREBP2 and SREBP1 [129]. Thus, additional studies using human samples or animal models such as non-human primates that express miR-33a and miR-33b will be important to translate these findings to the intimal hyperplasia observed in human vein grafts [126]. The mechanism of miRNAs in mechanotransduction has not been fully elucidated. Specifically, miRNA can regulate multiple target genes in the cell signaling network, greatly influencing biological pathways, cell functions and the dynamic balance of the vessel wall. miRNA as a biomarker or therapeutic target will be superior to the existing biomarkers or treatment drugs for cardiovascular disease [130–133].

Therapies based on ncRNAs represent one of new frontier in human disease treatment. There are still many unknown areas in the research of ncRNAs under mechanotransduction. Multiple transcriptional factors/co-activators/co-suppressors involved in the regulation of miRNA expression under mechanical regulation need to be elucidated. The unknown area is not only a challenge but also an opportunity, and scientists and

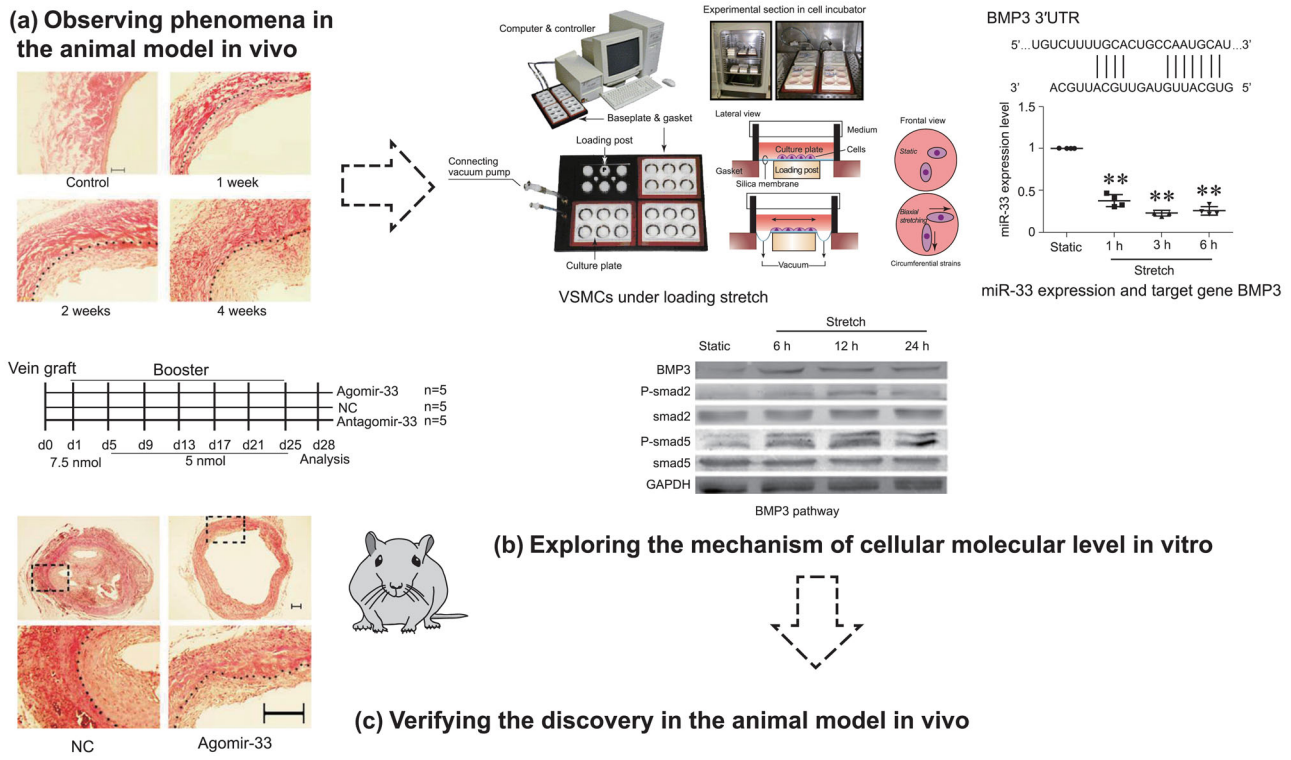


Figure 3. Schematic outlining the mechanobiological study of the roles of miRNAs and their target gene for exploring biomarkers. (a) Pathological outcomes in an animal model *in vivo*. Neointimal hyperplasia and cell proliferation are increased significantly, and miR-33 expression is decreased in rat vein grafts one, two and four weeks post-surgery. (b) Exploration of the biomechanical mechanism at the cellular and molecular levels *in vitro*. Use of a cyclic strain loading model of venous VSMCs and computation prediction of the miRNA target gene. The arterial stretch increases venous VSMC proliferation, represses miR-33 expression, and enhances target gene, BMP3, expression and phosphorylation of its downstream molecules smad2 and smad5, which are involved in VSMC proliferation. (c) Verifying the discovery in the animal model *in vivo*. The perivascular multi-point injection in the graft vein rat model demonstrates that agomiR-33 not only attenuates BMP3 expression and smad2 and smad5 phosphorylation, but also attenuates neointimal formation and cell proliferation in grafted veins.

clinicians need to work together to overcome the difficulties.

SUMMARY AND PERSPECTIVES

Vascular remodeling is a common pathophysiological process in cardiovascular diseases and mechanostimuli, including SS and cyclic strain, which are critically important factors regulating vascular physiology and pathology. Vascular cells, mainly ECs and VSMCs, can sense the various forms of mechanical signals, transform them into intracellular biochemical signals i.e., mechanotransduction, and then initiate cascades of cellular responses that ultimately regulate vascular functions (Fig. 4). Vascular mechanobiology elucidates the molecular and cellular basis of responses in ECs and VSMCs under mechanical conditions. With the advancement of high-throughput technologies, gene-editing methods and computational biology, there are new and exciting research opportunities in the area of mechanobiology for researchers from different disciplines. An improved understanding of mechanobiology in vascular remodeling may facilitate the development of novel therapeutic approaches targeting vascular impairments. The potential targets include, but are not limited to, mechanosensors, key proteins, miRNAs and lncRNAs in ECs and VSMCs. Thus, identification of these key molecules would be a priority in subsequent investigations for clinical translation.

The core concept of the 'stress-growth' theory is the interplay and synergism between the mechanical micro-environment and chemical micro-environment within cells. The results from mechanobiology studies, including mechanical, biochemical, cellular and molecular mechanisms, will provide valuable information revealing the major mechanical and chemical factors in the organism. The mechanobiological study should include the following: experimental approaches using animal models (or clinical data) *in vivo*; mechanistic studies at the cellular and molecular level *in vitro;* validation using gene manipulation approaches in disease models or model animals

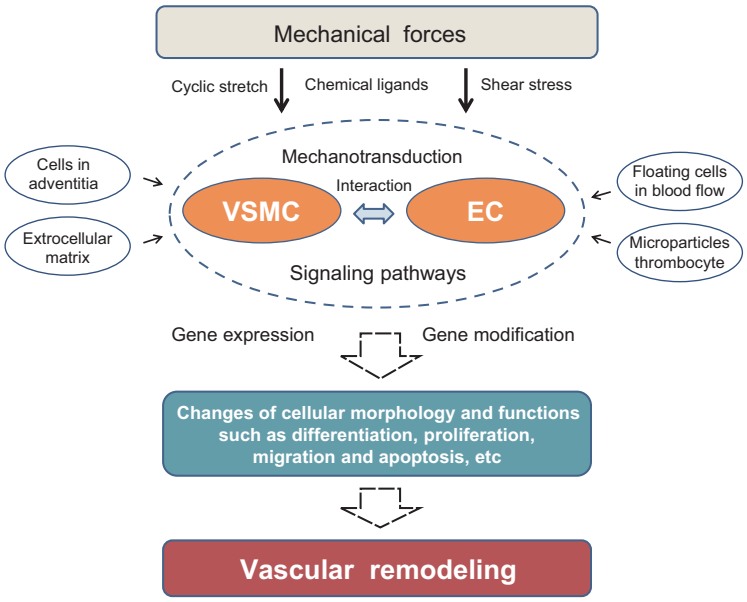


Figure 4. Schematic outlining mechanobiology in vascular remodeling. Mechanical forces, including SS and cyclic strain, are critically important factors regulating vascular remodeling. Vascular cells, mainly ECs and VSMCs, can sense the various forms of mechanical signals, transform them into intracellular biochemical signals, i.e., mechanotransduction, and then initiate cascades of cellular responses that ultimately regulate vascular functions. The interaction between ECs and VSMCs is also involved in vascular remodeling, in addition to chemical factors, microparticles, thrombocytes and the floating cells in the blood flow, as well as extracellular matrix and cells in the adventitia.

in vivo; further revalidation and implication using clinical samples; and gradual achievement of clinical transformation. This frontier field of research will play a unique role in elucidating key scientific issues in human physiology and disease, and produce important theoretical and clinical findings.

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