Vascular Smooth Muscle Cells Biomechanics

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Cardiovascular diseases are one of the leading causes of global death in developing countries. More than 80% of cardiovascular disease-associated mortality is attributable to atherosclerosis, a chronic inflammatory disease of the vessel wall. During the development of atherosclerosis and other cardiovascular diseases, vascular smooth muscle cells (SMCs) continuously shift from a contractile state towards other phenotypes that differ substantially from differentiated SMCs.

Keywords: smooth muscle cells ; mechanical forces ; cyclic stretch

1. Introduction

During life, cells in the vasculature are continuously exposed to different mechanical forces that regulate their function and homeostasis. These forces include fluid shear stress, cyclic stretch, and hydrostatic pressure [1]. Fluid shear stress is the frictional force from the blood flow, along with the vascular endothelial layer [2][3]. Endothelial cells lining the vessels can sense changes in the blood flow, convert them into biochemical signals, and trigger cellular responses [4]. The importance of mechanical forces on endothelial function has been extensively reviewed [5][6]. Disturbed shear stress influences the site selectivity of atherosclerotic plaque formation and vessel wall remodeling [7]. In healthy vasculature, the endothelium layer prevents the exposure of SMCs located in the vascular media to shear stress. The pulsating nature of the blood flow driven by the heart generates a cyclic stretch, which acts on the medial layers of the vasculature rich in SMCs but can also modulate the endothelial function [8][9]. Early studies in the field have defined that the human aorta experiences approximately a 10% cyclic stretch elongation on its external diameter within each heartbeat under physiological conditions [10][11]. However, during pathological conditions, including atherosclerosis and acute hypertension, blood vessels experience high-magnitude stretches of 20% and above [9][12]. In addition to the shear stress and cyclic stretch, all cellular layers in the vascular wall are subjected to compressive forces in vivo. However, the mechanisms by which compressive forces influence vascular cell phenotypes have not been fully elucidated [13]. Thus, mechanical forces are critical to maintaining a normal and healthy vasculature. However, the loss or excess of these mechanical cues can be detrimental and predisposed to vascular diseases.

2. Vascular Mechanical Microenvironment

2.1 Smooth Muscle Phenotype (SMP)

A significant function of SMCs in the arterial media of large vessels is to synthesize and organize a unique and highly elastic ECM to deal with the mechanical forces imposed by the pulsatile blood flow [14]. The ECM can be defined as the noncellular component that surrounds vascular cells and other organs. It is mainly formed from filamentous and sheet-forming proteins, proteoglycans, and glycosaminoglycans [15]. Recent advances in genetics and proteomics have facilitated our understanding of ECM proteins as potential novel disease biomarkers [16]. SMCs are surrounded by a basement membrane of ECM proteins predominantly formed by laminins, collagens IV and XVIII, perlecan, and other ECM components in healthy vessels. SMCs are also embedded in fibrillar collagen types I, III, and V; fibronectin; and other proteoglycans within the arterial media, known as transitional or interstitial ECM [15]. The changes in ECM degradation and production are recognized as hallmarks of vascular diseases and likely guide the loss of the SMC phenotype and modulation of alternative phenotypes [17]. For example, an altered vascular wall triggers a series of events characterized by the excessive production of poorly organized and highly stiffer collagens (such as collagen I) and other ECM components, such as fibronectin, biglycan, and lumican. These changes in the ECM microenvironment predispose the phenotypic modulation of SMCs [14][17][18]. In vitro work has shown that rat aortic SMCs cultured on collagen I or fibronectin substrates can switch from a contractile to a synthetic phenotype [18][19].

2.2 Different Hardness for Smooth Muscle Phenotype

Changes in the composition of ECM proteins such as elastin and collagen fibers trigger the development of a process known as vascular stiffening. Vascular stiffening increases with age and pathological states such as hypertension and atherosclerosis, as it is often accompanied by fibrosis and increased calcification [20]. To evaluate the effects of stiffness on cellular functions, most in vitro studies culture cells on tunable (soft and hard) gels that can mimic the elasticity of most physiological or pathological conditions within the vessels [21]. To determine the stiffness of solid materials, scientists commonly use the Young's modulus (named after the British scientist Thomas Young). It quantifies the material's resistance to elastic deformation elicited by a given tensile force. The effect of the force is dependent on the area; therefore, equations are defined in terms of stress (most commonly, Pascals; Pa) and divided by strain and the change in length of the materials (unitless) [22]. For example, in vivo stiffness of a healthy porcine aorta has been reported at 8 kPa, while mouse aorta has been at 5 kPa. On the other hand, atherosclerotic changes in the arteries of ApoE-KO mice elicit an increased stiffness of about 28 kPa [23].

An example of how cells can respond to different stiffness conditions was shown by experiments with human SMCs cultured on soft (2 kPa) and stiff (20 kPa) surfaces for 24 h. The expression of SM contractile marker genes such as *MYH11*, *TAGLN*, *CNN1* and *SMTN* was downregulated on stiff substrates. In addition, the genes associated with the proliferation and the synthetic phenotype of SMC were upregulated on stiff substrates compared to soft ones [24]. In contrast, the transcriptome sequencing analysis of mouse SMCs cultured on soft and stiff gels showed the opposite. SMCs cultured on soft substrates (0.17 kPa) increased the expression of a number of genes involved in the synthetic phenotype, such as osteopontin (*OPN*), vimentin, matrix metalloproteinases, and inflammatory cytokines, in comparison to stiff (1.2 kPa) substrates [25]. Interestingly, a more recent study cultured human aortic SMC in soft (1 kPa), medium (40 kPa), and hard (100 kPa) substrates [26]. They observed that SMC cultured on both soft and stiff substrates increased their expression of macrophage *CD68,* galectin 3 *(LGALS3*), and inflammatory interleukin 6 (*IL-6)* and interleukin 1 beta *(IL1β)* markers compared to cells on medium stiffness substrates [26]. Notably, *MYH11* expression, contrary to previous findings, was found upregulated on hard, compared to soft, substrates, thus suggesting that moderate stiffness, a condition closer to the physiological parameters, could be beneficial to SMC function.

Interestingly, the effects on the SMC phenotype elicited by the combination of distinct cues such as different stiffnesses and changes in the ECM proteins associated with stiffening have not been systematically evaluated. Most of the studies have only used gels coated with collagen I or fibronectin to mimic the in vivo microenvironment that SMCs experience in arteries with increased stiffness. For example, a recent study showed that the ECM protein used to coat the gels can differentially affect the SMC phenotype [27]. In this study, the authors observed that rat aortic SMC migration was decreased on stiff gels (103 kPa) coated with collagen I, while it was increased on gels coated with fibronectin [27]. The modulation of the SMC phenotype depends not only on the composition of the ECM but, also, on the physical structure of the matrix presented to the cells. For example, rat aortic SMCs respond with different phenotypes to fibrillar collagen I compared to nonfibrillar collagen I, even though the cell–matrix binding appears to be through the β1 integrin in both cases. It appears that, when collagen fibrils become aligned, the resting tension increases, thus producing a higher Young's modulus. As a result, the cells spread more and proliferate faster on stiffer than on flexible fibrils [28]. Efforts have been made to characterize the stiffness-sensitive transcriptome of human SMCs. Bulk RNA sequencing (RNA-seq) of human SMCs cultured on fibronectin-coated soft physiological (4 kPa) or stiff pathological (25 kPa) substrates was performed [29]. While this study identified 3098 stiffness-sensitive genes, they were focused on long non-coding RNAs (lncRNAs) and provided the first transcriptomic landscape of human SMCs in response to stiffness.

3. Fluid Shear Stress and SMCs

During vascular diseases or surgical interventions such as angioplasty or endarterectomy, vascular endothelium damage can occur, directly exposing SMCs to different patterns and intensities of shear stress [12]. An early work by the Tarbell lab has shown that, even in an intact artery, at least in some conditions, SMCs are continuously exposed to different shear stress magnitudes due to the interstitial flow driven by the transmural pressure gradient [30]. In vitro studies have demonstrated that SMCs directly react to fluid shear stress [31][32]. Therefore, a deeper understanding of the mechanisms by which fluid shear stress modulates the SMC phenotype represents an important scientific question.

Studies investigating the effects of shear stress have mainly been performed on cultured monolayers of ECs seeded on flat and stiff substrates [5]. The same principles and devices have been applied to studying the effects of shear stress on the SMC phenotype. The most common method is parallel plate flow chambers where the cells are subjected to a constant fluid shear, typically from a warm cell medium moved by a peristaltic pump at a certain speed and pattern. The Ibidi Pump System can mimic various in vivo shear stress conditions, such as the laminar flow typical of atherosclerosis-

protected vessels or the oscillatory flow typical of atherosclerosis-prone areas. Parallel plates are made of plastic or glass and coated with various proteins, such as collagens I and IV, and fibronectin. Further studies are required to determine the effect of soft stiffness substrates and different protein substrates on the SMC phenotype in the presence of shear stress.

Early studies have used DNA microarrays to determine the global expression profile of human aortic SMCs under fluid shear stress [33]. Cells cultured on glass slides coated with fibronectin were exposed to laminar shear stress (12 dynes/cm 2) for 24 h and compared to the cells under static conditions. Among the top regulated were the genes involved in the cell cycle and death, cell adhesion, and ECM. In the same study, they confirmed by BrdU labeling that laminar shear stress promotes human SMC proliferation compared to static controls [33]. Unfortunately, no information about the expression of SM contractile marker genes was found or stated in this screen. Multiple other studies, however, have shown that the exposure of rat aortic SMCs to laminar shear stress (8 or 14 dynes/cm 2) for extended periods of time (15–24 h) reduced the expression of some classical SM markers when compared to static controls [32][33][34][35]. In one of these, the exposure of rat cerebral artery SMCs to a laminar flow (15 dynes/cm 2) for 6, 12, and 24 h resulted in the time-dependent downregulation of Acta2 and Tagln , while matrix metalloproteinase 2 (Mmp-2) and tumor necrosis factor-alpha (Tnf- α) were upregulated. Phenotypic switching in this study was also accompanied by the enhanced proliferation and migration of SMCs after shear stress [35]. Thus, this and other studies suggest that laminar shear stress induces the dedifferentiation of SMCs compared to cells under static conditions.

Overall, the physiological relevance of the in vitro responses of SMCs to laminar shear stress is unclear. The patterns of shear stress at sites of endothelial cell injury in vivo do not necessarily mirror the continuous laminar shear stress addressed by several studies. Disturbed or turbulent shear stress patterns have been shown to induce atherosclerotic plaque formation in vivo and activate inflammatory signaling on endothelial cells in vitro [36]. However, the in vitro effects of disturbed or turbulent shear stress on the SMC phenotype have not been well-characterized. Pioneer studies have shown that bovine aortic SMC increased their DNA synthesis and proliferation capacity when exposed to oscillatory shear stress (14 dynes/cm 2) for 3 or 5 days compared to the static controls [37], but the degree to which this was accompanied by changes in the SMC phenotypic markers was not analyzed. A more systematic characterization of the phenotype and function of SMCs exposed to a greater range of shear stress forces and patterns on relevant substrates are further required.

4. Smooth Muscle Cell Mechanotransduction

The cellular process of converting mechanical cues into biochemical signals is known as cellular mechanotransduction. This aspect has been reviewed extensively in other vascular cells [38]. However, the precise mechanisms of cellular mechanotransduction on SMCs upon stretching are still not completely clear. In general terms, external mechanical forces can be transmitted to a cell in different ways, primarily by activating the integrin signaling pathway but also by G protein-coupled receptors (GPCRs), by nonselective cation channels, or by the coordinated and synergistic interactions of some or all of them [39]. The cytoplasmatic domain of integrins is functionally linked to various intracellular proteins such as talin, focal adhesion kinase (FAK), zyxin, paxillin, and vinculin. These proteins are organized as a focal adhesion complex to regulate the biochemical cascades initiated by mechanical forces [38]. The exposure of SMCs to physiological stretching (10%) for more than 6 h has shown increased levels of both the αv and β3 integrin subunits [40]. Experiments on rat SMCs have shown that stretching can induce cell adhesion kinase β, a highly related protein to FAK. Interestingly, this response seems to be partly mediated by the sodium and calcium ion channels [41]. At higher magnitudes of stretching (13% for 1 h), focal adhesion proteins such as zyxin are activated and translocated to the nucleus [42]. Other evidence suggests that integrins activate cellular responses upon stretching in coordination with the growth factor receptors [43]. The exposure of physiological cyclic (10%, 24 h) stretching on rat SMCs can also inhibit the Notch 3 receptor expression [44]. GPCRs have also been proposed to function as mechanoreceptors in SMCs. In particular, the angiotensin II type I receptor (AT 1R) can be activated by excessive mechanical stretching (20%) and induce ERK signaling, which leads to increased migration and protein synthesis [45][46]. Different studies have shown that stretching can activate nonselective cation channels in SMCs. The stretch-induced increase of cytosolic calcium concentration in SMCs results from the release of intracellular calcium stores [47]. Cyclic stretching significantly decreases the transient receptor potential cation channel C4 (TRPC4) protein expression in SMCs [48]. Further studies are required to determine how these or potentially new mechanotransducers are regulated under normal and pathological stretching conditions on SMCs.

Different studies have investigated the potential intracellular pathways induced by mechanical stretching on SMCs. For example, transforming growth factor-b (TGF-β) signaling has been implicated. The TGF-β1 levels were increased in the supernatant of rat SMCs exposed to 10% stretching for 24 h compared to the static controls [49]. Together with high TGF-β1 levels, the protein levels of ACTA2, CNN1, and TAGLN were also increased upon physiological cyclic stretching [49]. This study also showed that TGF-β1 could activate Smad2/5, leading to an increase of SIRT6 (a member of the sirtuin

family). High levels of SIRT6 in the nuclei then mediate the upregulation of SM markers and, thus, a more contractile phenotype after stretching. Another member of the sirtuin family, SIRT1, was also shown to be upregulated during the physiological levels of stretching (10% for 24 h). SIRT1 promotes both the activation of forkhead transcription factor 3a (Foxo3a) and inhibition of Foxo4, resulting in a more contractile phenotype on stretched rat SMCs than the static controls [50]. The role of mechanical force-induced epigenetic modifications in vascular gene expression has been extensively studied in endothelial cells [51] and less in SMCs [52]. In rat smooth muscle cells, physiological stretching for 48 h (10%, 1 Hz) significantly regulated the expression of histone deacetylases, particularly HDAC3, 4, and 7, compared to static cultured cells.

These changes in the expression of stretched cells accompanied a reduced migration compared to the static controls [53]. The other mechanisms by which shear stress and stretch induce the expression of epigenetic factors to modulate SMC functions have been recently reviewed [52].

Effectors of the Hippo pathway, YES-associated protein (YAP), and the transcriptional coactivator with a PDZ-binding motif (TAZ) are also involved in the stretch-induced phenotypic modulation of SMCs. YAP/TAZ activation after 24 h of cyclic stretching (13%) was linked to an increase in proliferation and proinflammatory gene expression (TNF-α, IL-6, IL-8, and IL-1B) in human umbilical artery SMCs compared to the static controls [54]. Human aortic SMCs subjected to stretching (16%) for 12 h increased their expression of angiotensin-converting enzyme (ACE), which, in turn, activated extracellular signal-regulated kinase-1 (ERK1). Phosphorylated ERK1 then blocked miR-145 and reduced the levels of the contractile marker genes inducing a phenotypic switch. In rat SMCs, the release of proinflammatory cytokine IL-6 was increased in cells subjected to 15% of cyclic stretching (from 3 to 24 h) compared to the static controls [55]. The authors described that this effect is mediated by a mechanism involving the Ras/Rac/p38 and NFKB signaling pathways. Early works by others have also described the stimulation of RhoA by mechanical stress, but the mechanism is unknown [56][57].

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