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REVIEW

Shear Stress in Atherosclerotic Plaque Determination

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Atherosclerosis initiates at predictable focal sites near arterial branches and curves, where blood flow is disturbed and shear stress is complex. Endothelial shear stress is the tangential stress derived from the friction of the flowing blood on the endothelial surface of the arterial wall. It is a key factor in modulating endothelial cell gene expression and vascular development and remodeling. Increasing evidences suggest that shear stress patterns have a strong relationship with atherosclerotic features. Moreover, variations in the local artery geometry during atherogenesis further modify flow shear stress characteristics, which contribute to the rupture site at the plaque upstream. In this study, we summarize the mechanistic evidences that associate shear stress patterns with determined atherosclerotic plaque features. An enhanced understanding of the relationship and pathophysiological function of shear stress patterns in atherosclerotic plaque features is essential, which may provide early prediction of clinical risk and guide individualized treatment strategies. In the current review, we analyzed the function of shear stress on the determination of atherosclerotic lesion and provided an update on the mechanotransduction of shear stress, gene expression regulation, and atherosclerotic plaque development and rupture.

Introduction

Atherosclerotic lesions, characterized by the dysfunction of aortic intima and accumulation of lipids and inflammatory cells within the aortic inner walls, are common pathological bases in cardiovascular diseases. These lesions commonly occur at the outer walls of arterial branches and the inner curvatures of tortuous vessels. At these sites, the local flow is disturbed and characterized with low shear stress recirculation, oscillation, or lateral flow (Warboys *et al*., 2011).

In vivo, the endothelial monolayer is directly exposed to blood flow and is a signal transduction interface for blood flow stimuli. Blood flow exerts biomechanical forces on the vasculature and affects vessel physiology and function, especially the intima. Vascular endothelial cell (EC) dysfunction is the initiation step of atherosclerosis development. These hemodynamic forces on ECs include flow-generated endothelial shear stress (ESS) and blood-pressure-derived tensile stress or circumferential stress, with ESS playing the most fundamental role in the regional localization of atherosclerosis.

Flow patterns *in vivo* have been broadly categorized into laminar and disturbed flow. Laminar flow refers to the unidirectional movement of fluid and forms laminar ESS (LSS), contrary to disturbed flow that includes recirculatory (generally found at vascular branch points) and turbulent flows. In the vascular system, the highly variable nature of vessel shapes and surfaces is correlated with the distribution of flow patterns and wall shear stress. Laminar flow changes into turbulent flow and produces abnormal shear stress, including low ESS and oscillating shear stress (OSS). LSS is from a smooth streamlined flow characterized by concentric layers of blood moving in parallel along the course of the arteries. Laminar flow with physiological level of shear stress (15–30 dyne/cm^2 over the cardiac cycle) exhibits protective effects on the endothelium and is atheroprotective. However, nonlaminar flow with low ESS or OSS activates monocytes and platelets as well as promotes EC apoptosis and turnover to create an atheroprone environment (Ravensbergen *et al*., 1998; Woo *et al*., 2011). Low ESS typically occurs at the inner areas of curvatures as well as upstream of stenosis with low time average ($<$10–12 dyne/cm^2 over a cardiac cycle). OSS is characterized by significant changes in both direction (bidirectional) and magnitude between systole and diastole, resulting in a very low time average (usually close to 0 over a cardiac cycle), which occurs primarily downstream of stenoses, at the lateral walls of bifurcations, and in the vicinity of branch points (Chatzizisis *et al*., 2007). High ESS is characterized by a significantly high time average ($>$30 dyne/cm^2 over the cardiac cycle) and occurs at the upstream and most stenotic site of the plaque (Table 1). Numerous studies on the relationship between flow shear stress and atherosclerotic lesions that occur in certain hemodynamic environments have been investigated. The low shear stress theory was first proposed. Then, Matlung *et al.* examined lesion distributions in postmortem

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Table 1. The Different Kinds of Endothelial Shear Stress

Term	*Definition*	*Time average over a cardiac cycle*	*The relationship with atherosclerosis*
Laminar ESS	ESS that is constant with direction and magnitude	$\sim$15 dyne/cm^2	Antiatherosclerosis
Low ESS	ESS that is unidirectional and has low time average	<10–12 dyne/cm^2	Proatherosclerosis
Oscillating ESS	OSS is characterized by significant directional changes and very low time average	$\sim$0 dyne/cm^2	Proatherosclerosis
High ESS	Significantly high time average	>30 dyne/cm^2	Proatherosclerotic plaque rupture

ESS, endothelial shear stress; OSS, oscillating shear stress.

human arteries and proposed the oscillatory shear theory (Matlung *et al*., 2012). To date, the function of low shear stress and OSS as key factors for localizing early atherosclerotic plaques is widely accepted (Peiffer *et al*., 2013). However, an enhanced understanding of the molecular level is imperative to inform future treatment strategies and aiding prevention of atherosclerosis.

Shear-Stress-Induced Mechanotransduction in ECs

Vascular ECs elicit adaptive changes by sensing mechanical forces and converting them into biochemical signals. This process constitutes a feedback control mechanism to regulate organ development and homeostasis. This mechanosensitive feedback modulates EC functions, such as proliferation, differentiation, migration, and apoptosis (Hahn and Schwartz, 2009). Molecules that were recently identified to be involved in mechanosensitive feedback include ion channels (Barakat, 1999), cell–cell junction (Chiu *et al*., 2004), receptors (Schwartz and DeSimone, 2008), G-proteins (Gudi *et al*., 2003), adhesion molecules and integrins (Jalali *et al*., 2001), the cytoskeleton (Osborn *et al*., 2006), the glycocalyx (Curry and Adamson, 2012), primary cilia (Nauli *et al*., 2013), and caveolae (Yu *et al*., 2006). Two models of mechanotransduction have been proposed to explain sensory mechanisms, which convey integrated information on flow shear stress. The tensegrity theory states that the cellular internal structure is coupled with extracellular matrix via the cytoskeleton. The cytoskeleton generates tension by activating the slide of protein myosin filaments. When ECs are exposed to flow shear stress, tension in the cytoskeleton is redistributed via skeleton rearrangement and cell morphology interchange. Consequently, mechanical signals are randomly transmitted through the entire cell (Stamenović *et al*., 2003). The other mechanoreceptor theory states that mechanoreceptors, such as G protein coupled receptor, integrin, extracellular matrix, force-sensitive ion channels (i.e., Ca^{2+} channel), tyrosine-protein-kinase system, and cell surface proteoglycan, sense and transmit mechanical signals into the cells. Interestingly, recent reports have shown that the nuclei of ECs also act as sensors, which mediate EC polarization against the flow (Tkachenko *et al*., 2013). Studies have demonstrated that mechanosensors might act as a ''molecular switch'' to transfer signals into cells with two patterns. One of these patterns shows that shear stress affects the combination of mechanosensor protein complexes. Consequently, the binding site is exposed, providing a binding site for other signal molecules. However, shear stress affects the movement of mechanosensor proteins on the membrane to transfer shear stress signals (Yamamoto and Ando, 2011). Among these mechanoreceptors, ion channels have been hypothesized to have the key function in mediating endothelial response to shear stress because of their rapid response to flow shear stress. Kefaloyianni showed that low shear stress (5 dyne/cm^2) induced downregulation of the Na^+/Ca^{2+} exchanger gene and upregulation of 18-ion channel subunits, including Ca^{2+}-activated K^+ channels KCa2.2, KCa2.3, CX37, Kv1.5, and HCN2. However, high shear stress (HSS) induced the expression of 30-ion channel subunits, which included KCa2.3, KCa2.2, CX37, Kir2.3, and KCa3.1 (Kefaloyianni and Coetzee, 2011). Hence, the three-way activation of mechanical-sensitive ion channels can be summarized as follows: (1) ion channels are ''pushed'' by shear stress, which is a direct physical approach, and the effect is positively associated with the size of shear stress; (2) ion channels are activated by the changes in the mechanical tension of cytoskeletons; and (3) ion channels are activated because of the change in cell membrane fluidity induced by flow of the shear stress (Butler *et al*., 2001).

In recent years, a new signaling network that comprised β1 integrin and caveolin-1 in response to shear stress has been defined. When acute shear stress is applied to cultured ECs, caveolin-1 becomes phosphorylated through an integrin-dependent manner. The phosphorylation of caveolin-1 recruits C-terminal Src-like kinase to the integrin/caveolin-1 complex, which causes additional regulation of Src-family kinase activity and p190RhoGAP tyrosine phosphorylation. Consequently, GTPase RhoA, a key second messenger that allows ECs to adapt to flow, combined with GTP and induced myosin light chain phosphorylation, subsequently causes an exchange of EC phenotype (Yang *et al*., 2011). Moreover, integrin αvβ3 has been proposed to have an important function in the regulation of EC phenotype response to shear stress. Under flow shear stress, αvβ3 combines with adapter molecule Shc and activates the Ras protein through Shc-Grb2-Sos, which increases the downstream signal molecules ERK and JNK (linked to inflammatory gene expression and apoptosis) activity, causing the rearrangement of cell structure and phenotype exchange. JNK2 siRNA significantly inhibits the orientation of ECs along blood flow direction under LSS (Hahn *et al*., 2011).

Shear-Stress-Induced Gene Expression in ECs

Vascular ECs are continuously exposed to blood flow shear stress, which causes the modulation of signaling networks and expression of shear-stress-sensitive genes, resulting in functional regulations. Ohura *et al.* (2003) detected the gene

expression profile of EC under shear stress and indicated that ~3% of gene expression varies; that is, ~600 genes are shear-stress-response genes. Eventually, physiological LSS enhances anti-As gene and anticlotting gene activation to achieve anti-inflammation, anticoagulation, antioxidation, and antiapoptosis responses. However, low ESS and OSS upregulate the expression of proatherosclerosis and prothrombosis genes, which promote inflammation, coagulation, oxidation, apoptosis, and EC proliferation as well as smooth muscle cell (SMC) phenotype transformation from contractile phenotype to synthetic type.

Two key shear-responsive transcription factors, Krüppel-like factor 2 (KLF2) and nuclear factor erythroid 2-related factor 2 (Nrf2), have been identified as being differentially regulated in the endothelium. KLF2 is expressed in ECs in the developing heart, particularly in areas of HSS but not in the downstream regions with disturbed flow. The proximal region of KLF2 promoter, which largely governed its transcription, can be induced by laminar flow and contains a functional consensus-binding site for a family of transcription factors termed myocyte enhancer factor 2 (MEF2) proteins. ERK5 is a kinase that has been well characterized to be activated by laminar flow. Steady laminar flow can increase MEF2 transcriptional activity via upregulating transcriptional activity of ERK5 (Nayak *et al*., 2011). The flow-mediated AMP-activated protein kinase (AMPK) activation is a newly defined KLF2 regulatory pathway in vascular endothelium that acts via ERK5/MEF2 (Young *et al*., 2009). MEK5, a MAP kinase kinase that is constitutively expressed by ECs, is activated by LSS and catalyzes the phosphorylation of ERK5 to result in activation of transcription factors such as MEF2, which in turn leads to synthesis of KLF2 (Clark *et al*., 2011). Further, some reports have indicated that LSS induces histone deacetylase 5 (HDAC5) phosphorylation and nuclear translocation (Lee *et al*., 2012), and then increases KLF2 expression through dissociation of HDAC5 and MEF2 and enhancing MEF2 activity (Wang *et al*., 2010). Atheroprotective flow patterns decrease the level of miR-92a, which in turn increases KLF2 expression to maintain endothelial homeostasis (Wu *et al*., 2011).

KLF2 has been shown to attenuate endothelial activation and leukocyte migration to achieve atheroprotective effects by transcriptional upregulation of endothelial NO synthase (eNOS) thrombomodulin and downregulation of plasminogen activator inhibitor-1 (PAI-1) (Kumar *et al.*, 2013). KLF2 potently inhibits thrombin-mediated induction of multiple cytokines/chemokines (e.g., MCP-1, IL-6, and IL-8) by inhibiting expression of its principal receptor protease-activated receptor 1 (Nayak *et al*., 2011). The induction of KLF2 by LSS leads to the suppression of endothelin-1, a potent vasoconstrictive and mitogenic molecule, thereby precipitating atherosclerosis (Young *et al*., 2009). KLF2 also plays a critical role in vascular homeostasis by inhibiting the expression of proinflammatory genes, such as ICAM-1, VCAM-1, and E-selectin. The suppressive effects of KLF2 were attributed to inactivate proinflammatory AP-1 family transcription factors by inhibiting phosphorylation and nuclear localization of c-Jun and ATF2, thereby reducing NF-κB-mediated transcription (SenBanerjee *et al*., 2004; Fledderus *et al*., 2007). Moreover, KLF2 reduces proinflammatory gene expression by inhibiting the MAPK pathway, such as the inhibition of Jun NH(2)-terminal kinase (JNK) and its downstream targets ATF2/c-Jun (Boon *et al*., 2010). On the other hand, high LSS upregulates the expression of the atheroprotective protein connexin37 (Cx37) in ECs by inducing its transcription factor KLF2 and then increasing intercellular communication (Pfenniger *et al*., 2012).

The subsequent studies showed that Nrf2, an antioxidant (protective) transcription factor, is preferentially activated by HSS. Exposure to high LSS induces dissociation of cytoplasmic Nrf2 from its suppressor kelch-like ECH-associated protein-1 (Keap1) and Nrf2 nuclear translocation through PI3K-dependent pathway, protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs), such as ERK1/2, JNK, and p38 (Dai *et al*., 2007), leading to increased binding activity through reactive oxygen species (ROS) and the transcriptions of antioxidant-response-element-mediated genes, such as heme-oxygenase 1, NADPH:quinone oxidoreductase-1 (NQO1), and glutathione S-transferase (Hsieh *et al*., 2009). In addition, it has been also shown that KLF2 is able to potentiate the atheroprotective effects of Nrf2 by enhancing its nuclear translocation and activation (Fledderus *et al*., 2008).

LSS maintains the endothelium in an antiatherogenic state via increased intracellular antioxidant levels as a result of Nrf2 activation, thereby preventing excess ROS/RNS production required for proatherogenic gene expression (Takabe *et al*., 2011). Nrf2 prevents ECs at the atheroprotected site from exhibiting a proinflammatory state via negative regulation of the MAPK pathway. On the one hand, Nrf2 suppresses upstream activators of p38, MAPK kinases 3, and 6 (MKK3/6). On the other hand, it enhances activity of MAPK phosphatase-1 (MKP-1), a negative regulator of p38 and JNK, by altering its redox state and promoting the catalytically active, reduced form of MKP-1 (Zakkar *et al*., 2009). This leads to suppressed expression of the adhesion molecule VCAM-1 (Bryan *et al*., 2014) (Fig. 1).

Most recently, epigenetic modifications have been identified to shape the flow-induced EC gene expression. Epigenetics refers to the heritable changes in gene expression and phenotype without alterations in the DNA sequences, which include histone modifications, chromatin remodeling, RNA-based machinery, and DNA methylation modification (cytosine methylation and cytosine hydroxymethylation). OSS (0.5 ± 4 dyne/cm^2) is recently reported to upregulate the expression and nuclear accumulation of types I (HDAC-1/2/3) and II (HDAC-5/7) histone deacetylases through PI3K/Akt signaling pathway in ECs. The highly expressed HDAC-1/2/3 promotes Nrf2 deacetylation, and HDAC-3/5/7 promotes MEF2 deacetylation. Consequently, the expression of antioxidation genes NQO1 and KLF2 is downregulated. Moreover, OSS upregulates the expression of cyclin A and downregulates the expression of $p21^{CIP1}$ to promote EC proliferation through the action of HDAC-1/2/3. However, pulsatile shear stress (12 ± 4 dyne/cm^2) induces nuclear export of HDAC-5/7 (Lee *et al*., 2012).

Mechanosensitive microRNAs (miRs) are also important mediators of endothelial phenotypic adaptations in response to different flow profiles. MiRs are endogenous at ~22 nt RNAs, which are highly conserved, and they negatively regulate the expression of target genes at the posttranscriptional level (Sun *et al*., 2010). In humans, miRs are predicted to regulate the activity of more than 60% of all protein-coding genes and affect many intracellular signal pathways. LSS-induced miRs reduce endothelial inflammation and cell

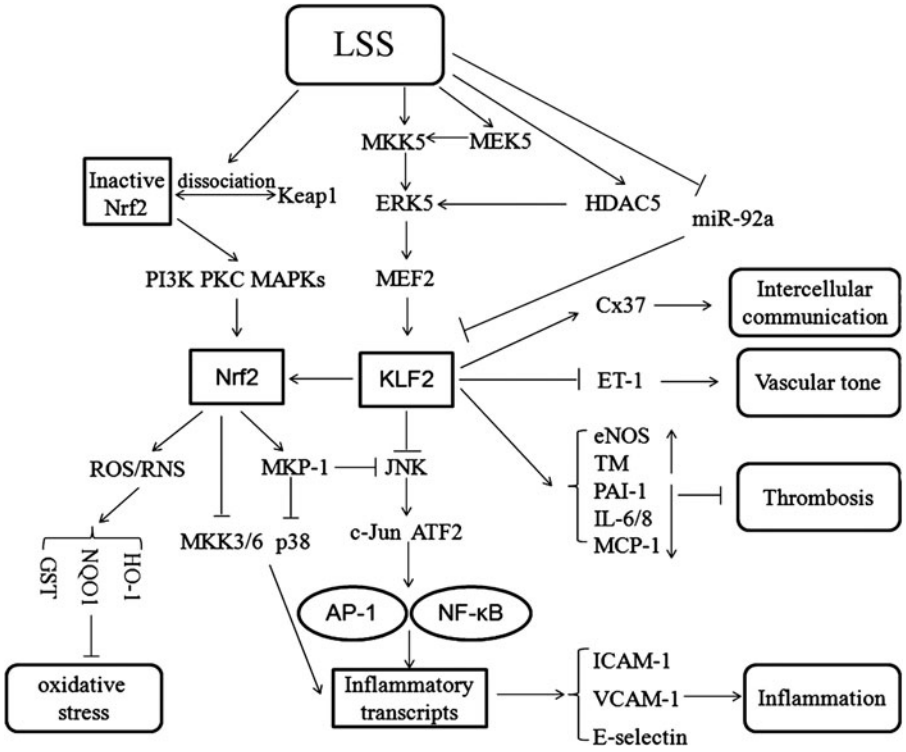

FIG. 1. Laminar shear stress (LSS) induces atheroprotective effects through multiple mechanisms. LSS activates two key shear-responsive transcription factors, Krüppel-like factor 2 (KLF2) and nuclear factor erythroid 2-related factor 2 (Nrf2), through multiple signaling pathways, including phosphatidylinositol-3-kinase (PI3K), protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), histone deacetylase 5 (HDAC5), and microRNA-92a (miR-92a). KLF2 influences the downstream signaling molecules to achieve antithrombotic effects, for example, endothelial nitric oxide synthase (eNOS), thrombomodulin (TM), plasminogen activator inhibitor-1 (PAI-1), IL-6/8, and monocyte chemoattractant protein-1 (MCP-1), as well as connexin37 (Cx37) and endothelin-1 (ET-1), to protect endothelial function. Nrf2 activates the transcriptions of antioxidant response element (ARE)–mediated genes, such as heme-oxygenase 1 (HO-1), NADPH:quinone oxidoreductase-1 (NQO1), and glutathione S-transferase (GST), to resist oxidative stress. These two transcription factors collectively inhibit inflammatory transcripts to resist inflammation through multiple signaling pathways, for example, p38, MAPK kinases 3 and 6 (MKK3/6), nuclear factor-κB (NF-κB), and activator protein 1 (AP-1).

cycle progression, providing potent atheroprotective effect. Members of the miR-23-27-24 cluster are increased by LSS, and miR-23b specifically blocks cell cycle progression, whereas miR-27b reduced EC repulsive signals (Boon *et al.*, 2012). LSS increases miR19a expression, which inhibits cyclinD1 expression and arrests the cell cycle at the G1/S phase (Qin *et al.*, 2010). Recently, a positive feedback loop has been demonstrated, in which PI3K mediates in the shear stress regulation of miR-19a expression and MAPK mediates in miR-23b, 27b. In turn, these miRs can modulate the shear-induced PI3K and MAPK activation and EC proliferation (He *et al.*, 2012). Conversely, oscillatory flow elicits the opposite networks. Studies have shown that miR-663 upregulated by OSS plays a key role in monocyte adhesion and inflammatory responses of ECs by mediating the expression of inflammatory genes (Ni *et al.*, 2011). Moreover, OSS induces the expression of miR-21 to inhibit the translation of peroxisome-proliferator-activated receptor-α and upregulate AP-1 activation and then promoting the expression of VCAM-1 and MCP-1, the adhesion of monocytes to ECs (Zhou *et al.*, 2011). MiR-21 expression decreases the expression of the proapoptotic gene PTEN and plays a role in EC apoptosis through enhancement of the PI3K/Akt/eNOS signaling pathway (Weber *et al.*, 2010). Recently, miR-92a has been identified as an athero miR, whose expression is preferentially upregulated by the atherogenic low ESS. Further, specific knockout of miR-92a expression can reduce endothelial inflammation and alter the development of atherosclerosis, decreasing plaque size and promoting a more stable lesion phenotype (Loyer *et al.*, 2014). MiR-712 was also identified as a mechanosensitive miR, which was upregulated by disturbed flow in ECs and contributed to decrease the tissue inhibition of metalloproteinase 3 (TIMP3) expression and stimulate proatherogenic responses, endothelial inflammation, and permeability (Son *et al.*, 2013). Shear-stress-sensitive miRs and their mechanism will be further clarified, which will contribute to the in-depth exploration of how EC phenotype adaptation responses to different shear stress patterns (Fig. 2).

Shear-Stress-Induced Phenotypic Adaptations in ECs

ECs are exposed to different shear stresses and cell phenotypic adaptations as response to shear stress patterns (Uzarski *et al.*, 2013). Phenotypic adaptations include short-term responses and long-term effects. Short-term responses include cytoskeleton reorientation and intracellular protein relocalization, and stimulation of enzyme activation (Rouleau

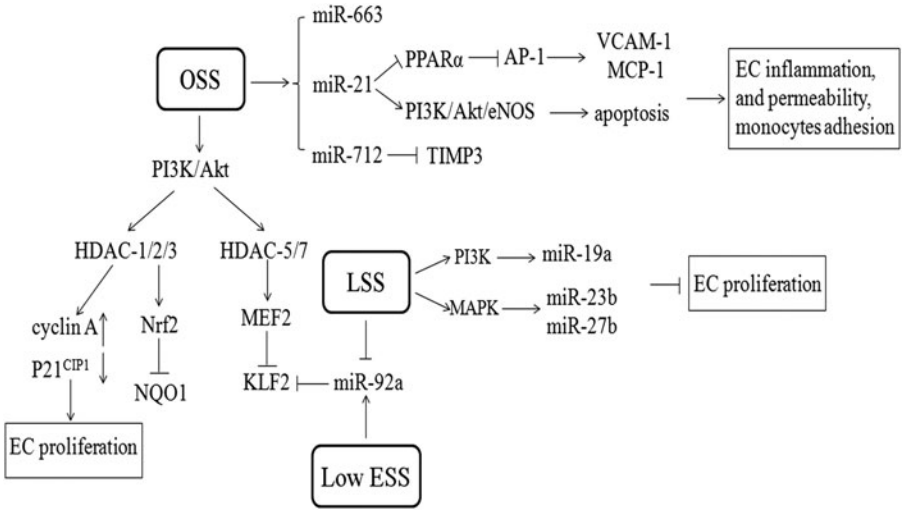

FIG. 2. Epigenetic modifications of shear stress in endothelial cells (ECs). Oscillating shear stress (OSS) upregulates the expression and nuclear accumulation of types I (HDAC-1/2/3) and II (HDAC-5/7) histone deacetylases through PI3K/Akt signaling pathway. Then, they, respectively, promote Nrf2 and MEF2 deacetylation to downregulate the expression of NQO1 and KLF2. OSS promotes EC proliferation through regulating the expression of cyclin A and $p21^{CIP1}$ after enhancing HDAC-1/2/3. MiRs (e.g., miR-663, 21, and 712) induced by OSS increase EC inflammation, permeability, and monocyte adhesion through multiple signaling pathways, such as AP-1 and PI3K/Akt. However, LSS promotes miR-19a, 23b, and 27b through PI3K and MAPK to inhibit EC proliferation. LSS upregulates KLF2 expression through downregulating miR-92a, which can be promoted by low endothelial shear stress (ESS).

et al., 2010). Long-term response involves protein synthesis and secretion, structural reorganization, proliferation, viability, and vascular phenotype. Endothelial phenotypes in atheroprone regions are subtly different from those located at atheroresistant sites. ECs have polygonal morphology under static condition. When exposed to physical LSS, observed at the straight part of the aorta, these factors exhibit an atheroresistant phenotype, such as antiproliferative (Malek *et al*., 1999), anti-inflammatory (Yamawaki *et al*., 2003), and antithrombotic phenotypes (Malek *et al*., 1994). This atheroresistant phenotype features elongated and oriented long axis consistent with the direction of shear flow by thickening the stress fibers, decreasing peak cell height, increasing cell mechanical stiffness, downregulating inflammatory cell adhesion molecules and cytokines, and conferring an atheroprotective, anticoagulant, and anti-inflammatory phenotype. However, when exposed to disturbed shear stress such as OSS observed at branch points and other regions of complex geometry, ECs exhibit a unique atheroprone phenotype. This atheroprone phenotype features circular and increased expression of atherogenic transcription factors, such as NF-κB, and a prooxidant and proinflammatory state (Dardik *et al*., 2005).

These phenotypic adaptations might be heterogeneously caused by mechanical signals, which are associated with differences in focal adhesion arrangement and differential involvement of signal kinases. Recent studies have shown that a junctional mechanosensitive complex consisting of vascular endothelial cadherin (as an adaptor), vascular endothelial growth factor receptor 2 (activates phosphatidylinositol-3-OH kinase), platelet EC adhesion molecule (PECAM; directly transmits mechanical force), and other elements has an important function in flow-dependent phenotypic adaptations of ECs. Among those molecules, the complex PECAM has more attention. The complex PECAM induces PI3K activation and integrin transformation from a low-binding activity to a high-binding activity. Activated integrin results in the activation of Cdc42 and small GTP enzymes, such as Rac and Rho, mediating adaptive rearrangement of vascular ECs (Tzima *et al*., 2005).

Shear Stress Versus Atherosclerotic Plaque Vulnerability

The high mortality of cerebrovascular events associated with atherosclerosis can be ascribed to the rupture of vulnerable plaques in the carotid arteries. The morphology of vulnerable plaques consists of three characteristic compositions: a large lipid core, a thin fibrous cap, and an accumulation of inflammatory leukocytes, with reduced collagen content and SMCs, increased activity of matrix metalloproteinase (MMP) and cathepsin, and a large number of angiogenesis (Krams *et al*., 2006; Fenning and Wilensky, 2014). Studies have illustrated that shear stress not only raises the expression of adhesion molecules and chemokines of ECs to promote inflammatory cell aggregation (Yuan *et al*., 2013), but also increases the expression and activation of MMP (Gambillara *et al*., 2005), elastolytic enzymes (Chatzizisis *et al*., 2011), and cathepsin (Yamamoto and Ando, 2011), which degrade the extracellular matrix and contribute to unstable plaque rupture. Cheng *et al.* (2006) demonstrated that patterns of fluid shear stress determine atherosclerotic lesion size and vulnerability in an abnormal shear stress mouse model (Koskinas *et al*., 2009). Low shear stress not only promotes continued local lipid accumulation, inflammation, oxidative stress, matrix breakdown, and eventually further plaque progression and excessive expansive remodeling, but also plays a crucial role in vascular remodeling and transition of early, stable plaques to high-risk atherosclerotic lesions,

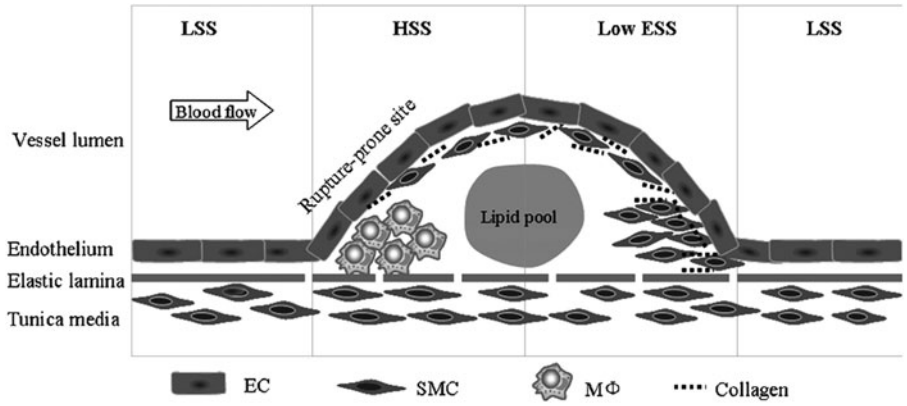

FIG. 3. Shear stress around the rupture site of atherosclerotic plaque. It forms different patterns of shear stress, such as laminar shear stress (LSS), high shear stress (HSS), and low shear stress (low ESS), around atherosclerotic plaque. The upstream region (particularly in the shoulder of plaque) is prone to rupture with higher number of macrophages, lower smooth muscle cells (SMCs), and collagens where it is HSS area. The downstream region with low ESS is not easy to rupture with higher number of SMCs and collagens and lower macrophages.

resulting in an acute coronary syndrome (Cheng *et al*., 2006). Low shear stress induced higher expression of proatherogenic vascular endothelial growth factor, MMP, interleukin 6, C-reactive protein, and interferon-γ than OSS. Hence, atherosclerotic lesions at low shear stress region showed a vulnerable plaque phenotype (with fewer SMCs, less collagen, and more lipids), while atherosclerotic lesions at OSS regions induced the growth of more stabilized plaques (with a thick fibrous cap containing similar relative amounts of macrophages but fewer lipids) (Phinikaridou *et al*., 2013; Shami *et al*., 2013). Additionally, Chatzizisis *et al.* (2011) showed that low shear stress regions contribute to the breakdown of balance between interstitial collagenases and elastases, such as MMP-2/9/12, cathepsin (CAT) K, and CAT S, with their endogenous inhibitors, resulting in endothelial discontinuity, intense infiltration of activated inflammatory cells, severe internal elastic lamina fragmentation, reduced collagen content, and vulnerable plaque with a thin fibrocap. Recently, Olivon indentified that shear stress is a key biomechanical regulator of arginase and inhibition of vascular arginase decreases the size of atherosclerotic lesions induced by low ESS (Olivon *et al*., 2013). Notably, the decrease in total vascular ROS, the amount of apoptosis rate, macrophages, and lipid and collagen contents in abnormal shear stress regions may be more pronounced.

Shear Stress Versus Rupture Site of Atherosclerotic Plaque

Once the advanced plaques protrude into the lumen, the local hemodynamic conditions and shear stress exposed to plaque become dramatically altered, which contributes to the plaque composition and the location of plaque rupture. Thrysøe *et al.* (2010) showed that peak is asymmetrically distributed longitudinally, with 50% occurring proximal to the maximal stenosis and 25% at the point of maximal stenosis with a longitudinal two-dimensional computational model combined with MRI data. Then, these differences in shear stress load, along with the atherosclerotic plaque, affect cellular composition and plaque vulnerability through a complex network of hemodynamic and biochemical feedback mechanisms (Dirksen *et al*., 1998). Moreover, the shoulder of the upstream plaque experiences the highest local pressure and a steeply increasing wall shear stress, where a significantly increased expression of proteolytic enzymes and the proapoptotic protein Bax is observed. Computational results showed that the shear stress over the proximal end of stenosis, where plaque rupture mostly occurred, is elevated, whereas shear stress at the downstream of the stenosis is decreased. Compared with the downstream (distal) atherosclerotic plaque, the upstream plaque components show vulnerable plaque features with higher number of macrophages, higher MMP-9 activity, lower SMC, and increased occurrence of neovascularization and hemorrhage (Segers *et al*., 2007; Fagerberg *et al*., 2010; Koskinas *et al*., 2013). Consequently, plaque rupture is observed to occur most frequently at the upstream part of a lumen-intruding plaque. Interestingly, HSS (>70 dyne/cm^2), which is generally considered to prevent atherogenic processes via inducing anti-inflammatory and antithrombotic actions, not only generates platelet-derived microparticles to enhance expression of cell adhesion molecules, for example, IL-8, IL-1β, and IL-6, in ECs (Nomura *et al*., 2001), but also destabilizes the plaque by cap weakening leading to ulceration through antiproliferative effect (Groen *et al*., 2008) (Fig. 3). In a review, a number of biological pathways were proposed, which could explain the important role of HSS in destabilization of the vulnerable plaque (Slager *et al*., 2005).

Summary

Mechanisms that link local ESS and atherosclerotic lesion development and progression remain complicated and difficult to dissect. Further studies are required to obtain more details about these ESS-mediated processes, which may deepen our understanding of the pathobiology of coronary artery disease. The application of such information can help to develop innovative methods to assess the clinical risk and novel therapeutic target to counter disease progression and improve clinical outcomes. In addition, drugs that mimic the effect of atheroprone flow to stabilize plaques and reduce clinical events may be an alternative treatment strategy.

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