Editorial

Shear stress regulation of endothelium: A double-edged sword

Liang Bai[1], John Y-J. Shyy[1,2]

[1]Cardiovascular Research Center, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi Province, China;
[2]Department of Medicine/Division of Cardiology, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093, USA

Address for Correspondence:
Prof. John Y-J. Shyy, PhD, Department of Medicine, University of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92093, USA. Email: jshyy@ucsd.edu

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INTRODUCTION

Vascular endothelium, the inner lining of the blood vessel wall, constantly responds to hemodynamic forces from blood flow. Thus, mechanical cues and the subsequent mechanotransduction in vascular endothelial cells (ECs) greatly affect vascular physiology and pathophysiology.[1-3] The molecular mechanisms underlying the flow regulation of endothelial biology have been studied intensively by many labs including our own. In general, the blood flow pattern in the straight part of the arterial tree is less disturbed with high mean shear stress, which induces an array of atheroprotective genes [*e.g.*, AMP-activated protein kinase (AMPK), Krüppel-like factor 4 (KLF4), Krüppel-like factor 2 (KLF2), and Sirtuin 1 (SIRT1)] in ECs.[4-7] Conversely, blood flow in the curvatures and bifurcations of the arterial tree is disturbed with lower shear mean stress. A number of pro-inflammatory genes, such as monocyte chemoattractant protein-1 (MCP-1), sterol regulatory element binding protein 2 (SREBP2), NOD-like receptor family, pyrin domain containing protein 3 (NLRP3) and redox-regulated genes [*e.g.*, NADPH oxidase (NOX)] are activated in ECs in these atheroprone areas, which results in dysfunctional endothelium.[8, 9] In this mini review, we summarize our recent findings in mechanotransduction and the corresponding responses in the endothelium, which result in atheroprotective versus atheroprone phenotype due to the respective atheroprotective or atheroprone flow patterns.

ATHERO-PROTECTIVE FLOW REGULATION OF KLF4-CH25H/LXR AXIS

KLF4 is a key transcription factor regulating the endothelial function promoting vascular homeostasis and conferring an atheroprotective phenotype both *in vitro* and *in vivo*.[10-12] Laminar and/or pulsatile shear stress is potent stimuli to induce KLF4 in ECs. Consequent from KLF4 activation, the expression level of endothelial nitric oxide synthase (eNOS) and eNOS-derived NO bioavailability are elevated.[13] At the upstream, the shear stress induction of KLF4 is mediated through a MEK5/MEF2-dependent signaling pathway.[14,15] Reports from several labs including our own indicate that shear stress regulates gene expression in ECs via epigenetic regulations including DNA methylation, chromatin remodeling, micro RNA, and long non-coding RNA (lncRNA).[16-22] Pulsatile shear stress modifies histone H3K27 acetylation and H3 S10 phosphorylation in cultured ECs.[17,23] Such epigenetic regulation leads to the increased expression of KLF4 and several KLF4 target genes including eNOS. By contrast, the oscillatory shear stress increases DNA methylation of CpG islands at the promoter region of the *KLF4* gene, leading to suppressed expression of KLF4.[18] Consistently, the disturbed flow increases the expression of DNA methyltransferase 3 a (DNMT3A) in swine aortic ECs, which in turn causes hypermethylation in the *KLF4* promoter, thereby decreasing the binding of MEF2 to the promoter, resulting in suppressed KLF4 transcription.[18] Interestingly, DNMT

inhibitors revert the disturbed flow-suppressed KLF4, eNOS, and thrombomodulin, while increasing MCP-1.[18]

Recently, we showed that pulsatile shear stress increases the expression of cholesterol-25-hydroxylase (Ch25h) and liver X receptor (LXR) via KLF4 in ECs *in vitro* and *in vivo*.[17] At the transcriptional level, KLF4 transactivates Ch25h and LXR via directly binding to the respective promoters (Figure 1). At the epigenetic level, the pulsatile shear stress decreased DNA methylation, but enhanced the modifications of active histone marks, namely, H3K4me3 and H3K27ac in the upstream region of *Ch25h*, which facilitated Ch25h induction. *In vivo*, the increased expression of Ch25h in the mouse thoracic aorta (atheroprotective flow area) was in line with the enriched H3K4me3 and H3K27ac but had attenuated DNA methylation.[17] The pulsatile flow-induced KLF4-Ch25h/LXR axis contributed to an atheroprotective phenotype of endothelium, which was supported by increased atherosclerosis in the thoracic aorta of the Ch25h$^{-/-}$/ApoE$^{-/-}$ double knockout mice fed a western diet.[17]

ATHEROPRONE FLOW REGULATION OF SREBP2

Disturbed flow pattern at arterial branches and curvatures causes inflammatory and oxidative responses in endothelium. These atherogenic events include the induction of interleukin 1β (IL-1β), NOX, MCP-1, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) etc., production of reactive oxygen species, endothelial dysfunction, and monocyte recruitment.[1,3] Our recent study shows that the disturbed flow *in vivo* and oscillatory shear stress *in vitro* activate SREBP2, which in turn induces the NLRP3 inflammasome in ECs.[9] Functioning as a transcription factor, SREBP2

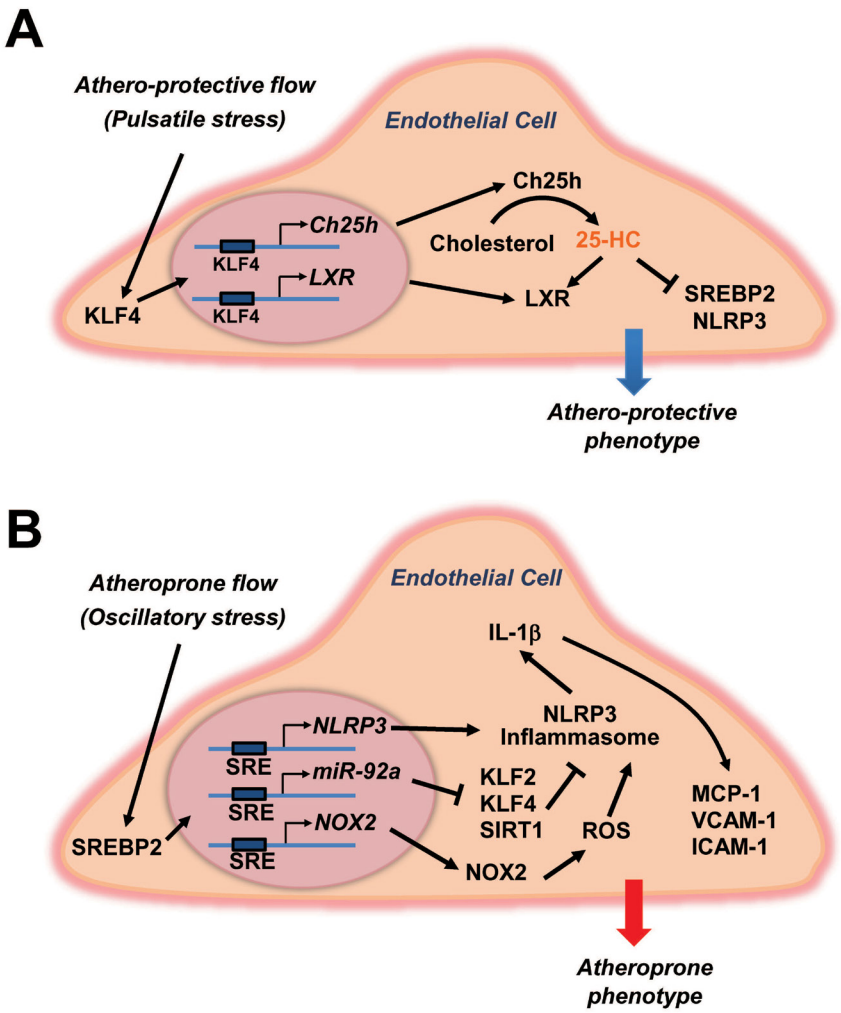

Figure 1: Shear stress regulation of endothelium. (A) Non-disturbed pulsatile flow induces KLF4-Ch25h/LXR axis. In endothelial cells, such shear stress induces KLF4, which in turn transactivates Ch25h and LXR, thus inhibiting SREBP2 and NLRP3 inflammasome and contributing to an athero-protective phenotype. (B) Disturbed and oscillatory flow induces SREBP2. Shear stress of this type activates SREBP2. SREBP2 then transactivates NLRP3, miR-92a, and NOX2. NLRP3 inflammasome activation causes IL-1ß production, while miR-92a targets KLF2, KLF4, and SIRT1. Oxidative stress is increased because of NOX2 induction. Together, these detrimental pathways lead to a dysfunctional endothelium, which presents an atheroprone phenotype.

plays a key role in regulating cholesterol homeostasis.[24,25] In addition to its canonical role in cholesterol biosynthesis, SREBP2 activates NLRP3 inflammasome in various cell types.[9,26] As NLRP3 inflammasome activation leads to IL-1β production,[27] our results demonstrate that a disturbed flow-induced SREBP2 is sufficient and necessary for the increased innate immune responses in endothelium.

Micro RNA-33 (miR-33) is an intronic miR co-expressed with SREBP2. miR-33 targets mRNAs encoding ATP-binding cassette transporter A1 and G1 (ABCA1 and ABCG1) that are essential for reverse cholesterol transport (RCT).[28,29] Intriguingly, LXR transactivates ABCA1 and ABCG1 to facilitate RCT. In hepatocytes and macrophages, cholesterol homeostasis is maintained through an intricate balance between the LXR-mediated RCT via ABCA1/ABCG1 and the SREBP2-mediated cholesterol biosynthesis. Our own research demonstrates that disturbed flow induces miR-33 in concert with the increased expression of SREBP2 in ECs.[9] The impaired LXR-ABCA1/ABCG1 axis in ECs has been suggested to be pro-inflammatory in ECs.[17] SREBP2 activation not only increases the level of miR-33, but also transactivates miR-92a.[30] It is well documented that miR-92a targets KLF2, KLF4, and SIRT1.[30, 31] These three molecules are crucial for endothelial homeostasis, in part via the induction of eNOS and the downregulation of IL-1β.[4,13]

Thus, the disturbed flow-activated SREBP2 may impair the EC function through several mechanisms: (1) SREBP2 activation of NLRP3 inflammasome, resulting in increased production of IL-1β; (2) miR-33 targeting ABCA1 and ABCG1 with consequent suppression of RCT; (3) miR-92a targeting KLF2, KLF4, and SIRT1, which leads to dysfunctional endothelium (Figure 1). In sum, these pathophysiological events contribute to an atheroprone phenotype of ECs. This thesis involving SREBP2 being a master regulator under a disturbed flow is strongly supported by the increased atherosclerosis seen in the thoracic aorta of an $ApoE^{-/-}$ mouse line, in which the activated form of SREBP2 is overexpressed in the endothelium.[9]

CONCLUSIONS

In summary, several recent studies synergistically reveal that shear stress is a double-edged sword, depending upon the endothelial location in the arterial tree. Pulsatile, unidirectional flow activates the KLF4-Ch25h/LXR homeostatic axis in ECs, thereby playing an atheroprotective role (Figure 1). By contrast, the disturbed flow activates SREBP2 with consequent induction of NLRP3 inflammasome, miR-33, and miR-92a in ECs. Disturbed flow being atheroprone, it increases innate immune response and decreases EC function (Figure 1). With respect to translational implication of these results, statins, the cholesterol lowering drug, activate the KLF4-Ch25h/LXR axis in endothelium[17] and the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) uses anti-IL-1β to reduce the risk of cardiovascular events.[32] The molecular basis underlying the common beneficial effect of cardiovascular medicine and shear stress may provide new therapeutic strategies for prevention and treatment of atherosclerosis.

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Conflict of Interests

The authors declare no conflict of interests.

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