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Targeting mechanosensitive transcription factors in atherosclerosis

Niu Niu¹, Suowen Xu^{1,*}, Yanni Xu^{1,4}, Peter J. Little^{2,3}, and Zheng-gen Jin^{1,*}

¹Aab Cardiovascular Research Institute, Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

²School of Pharmacy, The University of Queensland, Woolloongabba, QLD 4102, Australia

³Department of Pharmacy, Xinhua College of Sun Yat-sen University, Guangzhou 510520, China

⁴NHC Key Laboratory of Biotechnology of Antibiotics, National Center for Drug (Microbiology) Screening Laboratory, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), Beijing, China

Abstract

Atherosclerosis is the devastating underlying cause of cardiovascular disease and it preferentially develops at arterial regions exposed to disturbed flow (DF), while much less at regions of unidirectional laminar flow (UF). Recent studies have demonstrated that DF and UF differentially regulate important aspects of endothelial function, such as vascular inflammation, oxidative stress, vascular tone, cell proliferation, senescence, mitochondrial function, and glucose metabolism. DF and UF regulate vascular pathophysiology via differential regulation of mechanosensitive transcription factors (MSTFs) (KLF2, KLF4, NRF2, YAP/TAZ/TEAD, HIF-1 α , NF- κ B, AP-1 and others). Emerging studies show that MSTFs represent promising therapeutic targets for the prevention and treatment of atherosclerosis. Here, we present a comprehensive overview of the role of MSTFs in atherosclerosis and highlight future directions for developing novel therapeutic agents by targeting MSTFs.

Keywords

endothelial cells; shear stress; unidirectional laminar flow; disturbed flow; transcription factors; atherosclerosis

*To whom correspondence should be addressed: Dr. Zheng Gen Jin, Aab Cardiovascular Research Institute (CVRI), Department of Medicine, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box CVRI, Rochester, NY 14642, Phone: 585-276-7691, Fax: 585-276-9829, Zheng-gen_Jin@urmc.rochester.edu, Dr. Suowen Xu, Aab Cardiovascular Research Institute (CVRI), Department of Medicine, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box CVRI, Rochester, NY 14642, Phone: 585-276-7717, Fax: 585-276-9829, Suowen_Xu@urmc.rochester.edu; suowen.xu@gmail.com.

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Atherosclerosis and shear stress

Atherosclerosis (see Glossary) is the major underlying pathology of cardiovascular disease and the leading cause of global morbidity and mortality [1,2]. Atherosclerosis often occurs in medium- and large-sized arteries that consist of endothelial cells, vascular smooth muscle cells (VSMCs), and other vascular cells (Figure 1). The development of atherosclerosis commences with endothelial dysfunction, followed by the retention and modification of low-density lipoproteins (LDL), concomitant with adhesion, rolling, and subsequent transmigration of leukocytes into the sub-endothelial space. Transmigrated monocytes can differentiate into macrophages in the vessel wall and avidly uptake various forms of modified LDL (oxidized LDL in particular), to form “foam cells”, which is considered as the early pathological hallmark of fatty streaks and atherosclerotic plaques [3]. Lineage tracing studies have shown that VSMCs can also form “macrophage-like” cells and foam cells during atherogenesis [4, 5]. The formation and eventual rupture of vulnerable plaques (characterized by plaques with a large necrotic core covered by a thin fibrous cap), eventually leads to arterial thrombosis, vessel occlusion, tissue ischemia and the acute cardiovascular events (such as heart attacks and ischemic strokes) [3].

It has been well established that the distribution of atherosclerotic plaques is not uniform in medium and large-sized arteries, with regions having **disturbed flow** (DF, such as arterial bends and branching points) being more prone to atherosclerosis. The regions with **unidirectional laminar flow** (UF, such as straight parts of aorta) are protected from atherosclerosis [6-14]. The cause of the uneven and focal nature of atherosclerosis lies in the different cellular responses elicited by different pattern of blood flow. Among all the vascular cells in the arterial wall, endothelial cells (the innermost monolayer in the blood vessel) are the major cell type that are constantly exposed to hemodynamic forces from blood flow, including **shear stress**, the frictional dragging force acting on the surface of vascular lumen [12]. Shear stress modulates endothelial structure and function, and hence shear stress is critically involved in cardiovascular development, physiology, and pathology [12].

Shear stress on the vascular endothelium

Endothelial cells are the main cell type that sense (by membrane-localized mechanosensors and mechanosensing complexes) and transduce (by **mechanotransducers**) mechanosignals from shear stress to regulate various signaling pathways and biological functions [12]. In mechanobiology, UF and DF are two important types of shear stress occurring *in vivo*. UF occurs in straight parts of arteries; where blood flow is generally uniform and steady. UF is associated with high laminar shear stress and provides antiinflammatory and anti-atherosclerotic effects by maintaining endothelial cell quiescence, alignment to the direction of flow, and thus preserving endothelial homeostasis [6, 12-14]. DF (also known as turbulent/oscillatory flow) is a complicated flow pattern that has low magnitude of shear stress, and is irregular and non-uniform. Other features of DF include spatiotemporal changes in gradients of shear stress and flow direction [14]. DF normally occurs in regions of arteries that curve sharply or arteries with branching points. DF, in conjunction with other cardiovascular risk factors (such as hyperlipidemia and hyperglycemia), leads to endothelial

dysfunction and focal distribution of atherosclerotic plaques [6, 12-14]. DF-dependent mechanisms promote the initiation of atherosclerosis as well as contribute to plaque rupture. Therefore, DF represents one major biomechanical mechanism for endothelial dysfunction and the progression of coronary artery disease [18, 19].

UF and DF exert differential effects on the functions of endothelial cells (such as inflammation, oxidative stress, proliferation, cell alignment, mitochondrial function, and glucose metabolism), mainly through **mechanosensitive transcription factors** (MSTFs)-dependent gene expression (Figure 2, Key Figure) [16]. Considering the important role of MSTFs in regulating endothelial cell gene expression, we present an overview of the recent studies examining the role of MSTFs in regulating endothelial function and atherosclerosis. We also highlight possible therapeutic pathways to new cardiovascular drug discovery arising from the targeting MSTFs.

Role of MSTFs in regulating endothelial function and atherosclerosis

As the master regulators of shear stress-induced gene expression in endothelial cells, MSTFs control endothelial function and the development of atherosclerosis. For example, Kruppel like factor 2 (KLF2), Kruppel like factor 2 (KLF4), and nuclear factor (erythroid-derived 2)-like 2 (NRF2) are important MSTFs which are upregulated after exposure to UF [21, 22]. In contrast, transcription factors such as activator protein 1 (AP-1), nuclear factor kappa B (NF- κ B) [23-25], yes-associated protein (YAP)/WW domain-containing transcription regulator 1 (TAZ, also known as WWTR1)/TEA domain transcription factor (TEAD) [26-28], and hypoxia inducible factor 1 alpha (HIF-1 α) [29, 30], are suppressed by UF (Figure 2). However, DF has opposite effects on these MSTFs [6, 12-14, 31]. In the sections below, we review the specific role of these MSTFs in endothelial function and atherosclerosis.

KLF2 and KLF4

Among all the MSTFs, endothelial KLF2 and KLF4 are the best characterized. KLF2 and KLF4 are vascular homeostasis-associated molecular patterns, which regulate the expression of a wide range of antiinflammatory, anti-oxidant, and anti-thrombotic genes in endothelial cells [32]. KLF2 is upregulated by UF, but decreased by DF [33, 34]. Important downstream targets of KLF2 include endothelial nitric oxide synthase (eNOS) and thrombomodulin (THBD) and many others (Figure 2) [10]. Mechanistically, KLF2 increases the expression of target genes via binding to the KLF2 binding sites (consensus binding motif: 5'-C(A/T)CCC-3') in target gene promoters/enhancers [32]. KLF2 also negatively regulates multiple pro-inflammatory, pro-thrombotic, and vasoconstrictive genes, such as vascular cell adhesion molecule (VCAM1), monocyte chemoattractant protein (MCP-1), E-selectin (SELE), endothelin 1 (ET1), and plasminogen activator inhibitor-1 (PAI1) (Figure 2) [10]. KLF2 inhibits the expression of pro-inflammatory genes via recruiting transcriptional co-activator p300 [32] to inhibit the hyperactivation of NF- κ B induced by pro-inflammatory cytokines. KLF4, another important MSTF, also regulates the expression of an appreciable portion of overlapping genes with KLF2 (such as eNOS, THBD, PAI1, and VC AMI) [35].

Further *in vivo* studies have shown that KLF2 and KLF4 protect aortic vessels from atherosclerosis [35, 36]. The protective roles of KLF2 and KLF4 is evidenced by the fact

that KLF2 deficiency and KLF4 deficiency accelerate atherosclerosis in apolipoprotein E deficient (ApoE^{-/-}) mice and LDL receptor deficient (LDLR^{-/-}) mice, two frequently used animal models to assess the effect of genetic manipulations on the development of atherosclerosis [37]. Specifically, KLF2^{+/-}; ApoE^{-/-} mice display an increase of lipid uptake, foam cell formation, and atherosclerotic lesions [36], providing the first direct link between KLF2 and atheroprotection. Similarly, myeloid cell specific knockout of KLF2 also increases the development of atherosclerosis in LDLR^{-/-} mice by increasing the content of neutrophils and macrophages as well as the level of nitroxidative stress (Table 1) [38]. KLF4 has similar atheroprotective functions. Endothelial cell specific deletion of KLF4 increases, while endothelial cell specific KLF4 transgene reduces atherosclerosis in ApoE^{-/-} mice [35], suggesting that activating endothelial KLF2 and KLF4 could confer atheroprotective effects (Table 1).

A recent study has shown that deficiency of KLF2 and KLF4 alters the endothelial cell transcriptome [39], and additional shear stress-responsive KLF2- dependent genes have recently been revealed. Fang group reported that KLF2 regulates the expression of a novel flow sensitive gene, phosphatidic acid phosphatase type 2B (PPAP2B), a gene product associated with coronary artery disease and ischemic stroke (CAD/IS). Specifically, PPAP2B is essential for UF-induced endothelial cell alignment and antiinflammatory effects [40]. Moreover, Fang group showed that a genetic variant of PPAP2B, rs17114036, generates a binding site for KLF2 and has CAD/IS-protective effect via regulating endothelial cell responses to hemodynamic changes [41]. In addition, KLF2 is implicated in the regulation of endothelial cell metabolism. A recent study [42] has shown that UF induces KLF2 upregulation, thereby repressing glycolysis and mitochondrial respiration. This study further showed that 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase-3 (PFKFB3), an important gene regulating glycolysis, is a direct target of KLF2, thus extending the functions of KLF2 from maintaining vascular homeostasis to the regulation of endothelial cellular metabolism [42].

Collectively, emerging evidence indicate that endothelial KLF2 and KLF4 represent two major MSTFs that regulate vascular homeostasis and represent promising therapeutic targets to prevent atherosclerosis by pharmacological intervention. Since recent studies have identified that other members of KLF family, such as KLF3 [43] and KLF14 [44] also exhibit similar anti-inflammatory functions as KLF2 and KLF4, it would be interesting to assess whether KLF3 and KLF14 are also mechanosensitive and if so, their potential interaction with KLF2/KLF4 in the regulation of endothelial homeostasis and atherosclerosis.

NRF2

NRF2 is a crucial transcript factor that regulates the expression of a plethora of genes responsible for cytoprotection, phase II detoxification (a process that conjugates drug metabolites or endobiotics via glucuronidation, glutathionylation, and sulphation etc), inflammation, and metabolism in response to oxidative stress. NRF2 functions by binding to a conserved NRF2 binding motif (5'-A/GTGAC^C/GTCAGCA-3') in the target gene promoters [45, 46]. Well-characterized downstream target genes of NRF2 include: heme oxygenase-1

(HO1) and NAD(P)H quinone oxidoreductase 1 (NQO1) [45, 46]. NRF2 expression/activity is controlled transcriptionally, epigenetically, post-translationally, and pharmacologically [45, 46]. NRF2 activation in response to UF is important for endothelial cell adaptation to oxidative and nitrosative stress as well as being anti-inflammatory [10]. In endothelial cells, UF increases eNOS-dependent nitric oxide (NO) production and represses oxidative stress, while DF promotes oxidative stress via NADPH oxidase, xanthine oxidase, eNOS uncoupling, and mitochondrial-derived reactive oxygen species (ROS) [10]. In contrast, NRF2 depletion or knockout reverses UF induced upregulation of cytoprotective enzymes [47].

In addition to the known role of NRF2 in maintaining cellular redox homeostasis via phase 2 detoxification and antioxidant responses, NRF2 has been implicated in metabolism and inflammation related to endothelial cell functions and atherosclerosis. For example, a recent study [48] has revealed that NRF2 binds to the proximal promoter region of IL-6 and IL-1 β genes and suppresses the expression of IL-6 and IL-1 β independent of redox control. In addition, NRF2 activation protects against TNF α -induced activation of VCAM1 gene expression in endothelial cells [49]. NRF2 also regulates the metabolism of carbohydrate, lipid, heme, iron, amino acid, and nucleotides, as well as the generation of NADPH through regulating key enzymes in these metabolic processes, indicating the essential role of NRF2 in dictating metabolic reprogramming under stress conditions [45, 46].

Since both KLF2 [50] and NRF2 [51] function downstream of ERK5 and some NRF2 downstream genes (NQO1 and HO1) are upregulated in KLF2-overexpressed endothelial cells [52], it is plausible that both MSTFs function synergistically to regulate UF-induced endothelial cell gene expression. KLF2 served as a priming factor for UF-induced NRF2 activation since knockdown of KLF2 by small interfering RNA (siRNA) blocks NRF2-dependent antioxidant gene expression in endothelial cells [52, 53]. By analyzing the transcriptomes of endothelial cells exposed to UF, KLF2 overexpression, and NRF2 overexpression, a considerable pool of overlapping genes (76%) were observed between UF treatment and KLF2/NRF2 overexpression in endothelial cells. These results indicate that KLF2 and NRF2 contribute to the vast majority of UF-induced transcriptional networks [52].

YAP/TAZ/TEAD

YAP/TAZ/TEAD in the Hippo pathway emerged as a master regulator of multiple cellular processes, such as cell proliferation, migration, and inflammation [54]. A recent study in YAP/TAZ knockout cells indicates that YAP and TAZ have functionally redundant roles and YAP has an overall stronger influence than TAZ [55]. Connective tissue growth factor (CTGF, also known as CCN2) and cysteine rich angiogenic inducer 61 (Cyr61, also known as CCN1), two well-studied YAP/TAZ target genes, are highly expressed in advanced atherosclerotic plaques [56], indicating the potential pro-atherogenic role of YAP/TAZ activation. In 2016, three independent groups reported that UF suppressed the transactivation of YAP/TAZ/TEAD [26, 27, 57]. Specifically, UF promotes YAP phosphorylation (at serine 127), reduces YAP nuclear localization, and inhibits the expression of YAP-dependent pro-atherogenic genes (CTGF and Cyr61) in cultured human endothelial cells and mouse aortic

endothelium. In contrast, DF promotes the activation of YAP/TAZ in endothelial cells, providing an alternative explanation for the uneven development of atherosclerosis. In addition, DF also promotes YAP activation and endothelial VCAM1-dependent inflammatory responses by increasing YAP phosphorylation (at tyrosine 357) dependent on tyrosine protein kinase activity [28]. In addition to DF pro-inflammatory cytokine TNF α also activates YAP/TAZ/TEAD in endothelial cells [58].

Recently, the precise role of YAP/TAZ in driving vascular inflammation and atherosclerosis has been addressed *in vivo*. In particular, systemic deficiency of YAP/TAZ (by morpholino oligos) [27], or endothelial cell specific deficiency of YAP (by CRISPR/Cas9 technology) reduces atherosclerosis in ApoE^{-/-} mice [26]. In contrast, overexpression of endothelial YAP, or systemic TAZ, or a constitutively active mutant of YAP/TAZ promotes atherosclerosis in ApoE^{-/-} mice [26, 28]. These series of gain- and loss-of-function studies in mice provide proof-of-concept that YAP/TAZ/TEAD represents a promising therapeutic target to prevent atherosclerosis [26]. Studies in cultured human endothelial cells indicate that activation of YAP/TAZ/TEAD leads to endothelial cell proliferation, cell cycle progression, and inflammation (increased expression of VCAM1 and leukocyte-endothelium interaction) [26-28]. These observations suggest that local and systemic risk factors could drive atherosclerosis by inducing sustained activation of YAP/TAZ/TEAD.

HIF-1 α

Activation of the HIF-1 α pathway drives vascular permeability, leukocyte recruitment, and intraplaque angiogenesis in atherosclerotic plaques from mouse models and human subjects [59, 60]. Specifically, endothelial HIF-1 α aggravates DF-induced atherosclerosis development in ApoE^{-/-} mice after partial carotid ligation, by triggering the miR-19a/CXCL1 (C-X-C motif ligand 1) axis and stimulating leukocyte adhesion to activated endothelial cells [61]. HIF-1 α is activated by DF *in vitro* in both human umbilical vein endothelial cells and human aortic endothelial cells under normoxic conditions [29, 30]. More importantly, HIF-1 α is also activated by DF *in vivo* in areas prone to atherosclerosis of the mouse and porcine aorta (near branches or bends of arteries) [29, 30].

Upon exposure to DF, HIF-1 α expression is increased through a dual mechanism which involves transcriptional activation of NF- κ B as well as HIF-1 α protein stabilization by the deubiquitinating enzyme Cezanne [29]. NADPH oxidase 4 (NOX4)-dependent generation of ROS is also involved in DF-induced HIF-1 α stabilization [30]. Activation of HIF-1 α not only drives the expression of multiple pro-inflammatory and pro-adhesive genes (MCP1, SELE, and VCAM1), represses mitochondria respiration, but also enhances the expression of multiple glycolytic enzymes (such as enolase 2 (ENO2); hexokinase 2 (HK2); PFKFB3, and pyruvate dehydrogenase kinase-1 (PDK-1)) [29, 30]. This evidence suggests that HIF-1 α is critical not only in the initiation of atherosclerosis (by regulating inflammatory responses) [62], but also in advanced stages of atherosclerosis (by regulating intraplaque angiogenesis).

NF- κ B and AP-1

NF- κ B promotes the expression of multiple pro-inflammatory and pro-adhesive genes (VCAM1, MCP1, and SELE) in endothelial cells [24]. Activity of NF- κ B is tightly controlled by its phosphorylation, and subcellular localization. AP-1 is a heterodimer composed of c-Fos, c-Jun, activating transcription factor (ATF) families. Both NF- κ B and AP1 are redox sensitive MSTFs that mediate inflammation of the endothelium [24]. NF- κ B and AP1 are activated by prolonged DF in cultured endothelial cells and endothelium from DF area of mouse aorta [23-25].

Endothelial cell-specific inhibition of NF- κ B reduces and stabilizes atherosclerotic plaques from ApoE^{-/-} mice fed an atherogenic diet or treated with chronic intermittent hypoxia [63, 64]. The mechanism is related to reduced macrophage recruitment via decreased expression of adhesion molecules, and pro-inflammatory cytokines/chemokines [63, 64]. Conversely, UF protects endothelial cells via inhibition of NF- κ B and AP-1 activation induced by TNF α [65]. Overall, these results indicate that NF- κ B and AP-1 as two established pro-inflammatory MSTFs, are differentially regulated by different patterns of blood flow, with important implications in the development of atherosclerosis.

Therapeutic targeting MSTFs in atherosclerosis

As described above, MSTFs regulate the development of atherosclerosis by influencing the expression of genes responsible for endothelial function. In this section, we will overview the effect and mechanism of known MSTFs-targeting pharmacological agents in atherosclerosis (Table 2).

Activators of KLF2 and KLF4

KLF2 and KLF4 elicit potent anti-inflammatory effects in both endothelial cells and macrophages [32]. Therefore, pharmacological activators of KLF2 and KLF4 are promising agents to treat endothelial dysfunction and atherosclerosis. Many pharmaceutical agents have been identified/repurposed as activators of KLF2 and/or KLF4, such as statins, resveratrol, suberanilohydroxamic acid (SAHA), tannic acid (TA), and many others.

HMG-CoA reductase inhibitors (statins) are well characterized activators of KLF2 and KLF4 both *in vitro* and *in vivo*. Statins activate KLF2 via the mevalonate- [66] and MEF2 (myocyte enhancer factor-2)-dependent pathways [67] since exogenous addition of mevalonate or mutation of the MEF2 binding site in the KLF2 promoter reverses the effects of statin-mediated KLF2 upregulation.

Resveratrol is a natural and pharmacological activator of both KLF2 and KLF4 in endothelial cells. Resveratrol activates the KLF2 via SIRT1 (Sirtuin 1)/MEK5 (MAP2K5 mitogen-activated protein kinase kinase 5)/MEF2 [68] and AMPK α (AMP-activated protein kinase alpha)/ERK5/MEF2 pathways [69]. Resveratrol also activates KLF4 via the MEK5/MEF2-dependent signaling pathway in endothelial cells [22]. SAHA (also known as vorinostat), a pan-inhibitor of histone deacetylases, has been identified as a pharmacological activator of KLF2 by luciferase reporter assay-based high throughput drug screening [70]. SAHA exhibits potent anti-inflammatory effects and reduces monocyte adhesion to activated

endothelial cells. The protective effects against inflammation of the endothelium are reversed in KLF2 deficiency or depletion, suggesting that SAHA attenuates inflammation in a KLF2-dependent manner. Furthermore, luciferase assays indicate that SAHA activates KLF2 via the MEF2-dependent pathway [70].

In addition, TA has recently been identified as a new pharmacological activator of KLF2 [71]. TA activates KLF2 via the ERK5/MEF2 pathway, thereby reducing monocyte adhesion to activated endothelium in a KLF2-dependent manner [71]. Most importantly, both SAHA [70] and TA [71, 72] have protective effects against atherosclerosis in ApoE^{-/-} mice fed a high fat diet.

A recent study from the García-Cardena laboratory [73] reported another KLF2 reporter system using green fluorescence protein (GFP). This screening system is also compatible with high throughput drug screening by assessing GFP fluorescence using a microplate reader. This reporter system has been used successfully to validate current known KLF2 activators (simvastatin) or treatments (UF or infection with constitutively active mutant of MEK5) [73]. The use of KLF2-luciferase reporter assays and GFP-KLF2 reporter assays, is potentially useful for identifying new KLF2 activators. It remains to be determined whether or not compounds identified by these methods also activate KLF4. In addition, the potential side effects of systemic KLF2 and KLF4 activators needs to be carefully assessed.

NRF2 activators

Despite the controversial role of NRF2 in atherosclerosis in pre-clinical animal models of atherosclerosis, pharmacological activators of NRF2 have shown overall atheroprotective effects [74]. One such NRF2 activator is sulforaphane, which is a bioactive natural product from broccoli. An elegant study [75] from Evans' laboratory has shown that NRF2 is activated in endothelial cells at sites of great curvature (atheroprotective sites) of C57BL/6J mouse aorta by *en face* immunostaining. Pharmacological activation of NRF2 by sulforaphane reduces endothelial cell activation at atheroprone sites (inner curvature) by suppressing p38/VCAM1 signaling pathway in a KLF2-dependent manner [75]. Additionally, statins also activate the NRF2 pathway, and NRF2-dependent expression of various cytoprotective genes, such as HO-1. Simultaneous treatment with UF and atorvastatin has synergistic effects in protecting against H₂O₂-induced oxidant injury in endothelial cells [76].

YAP/TAZ/TEAD inhibitors

Verteporfin, also known as Visudyne, is a benzoporphyrin derivative photosensitizer widely used in photodynamic therapy in patients with macular degeneration. Verteporfin is the first identified pharmacological inhibitor of YAP by disrupting the YAP/TEAD interaction [77]. Intra-arterial administration of verteporfin combined with photoactivation reduces atherosclerosis and macrophage apoptosis in ApoE^{-/-} mice [78]. In addition, many existing FDA-approved drugs, such as statins, have anti-atherosclerotic functions partially by inhibiting YAP/TAZ transactivation [26, 27]. These lines of evidence indicate the therapeutic potential of the use of YAP/TAZ/TEAD inhibitors to treat atherosclerosis.

HIF-1 α inhibitors

HIF-1 α is mainly induced by hypoxic stress which is very common during the development of atherosclerosis. The activation of HIF-1 α promotes the development and enhances the vulnerability of atherosclerotic plaques [59, 60]. Therefore, down-regulation of HIF-1 α expression and signaling holds promise for treating atherosclerosis. For example, evodiamine, a bioactive anti-inflammatory alkaloid isolated from the medicinal herb *Evodia rutaecarpa*, can repress hypoxia-induced cyclooxygenase 2 (COX-2) expression by inhibiting HIF-1 α [79]. Digoxin, a drug traditionally used in treating cardiac arrhythmia and heart failure, also inhibits HIF-1 α activation. Specifically, digoxin treatment decreases HIF-1 α protein expression in endothelial cells, and consequently digoxin inhibits endothelial cell growth and survival both *in vitro* and *in vivo* [80]. Importantly, digoxin has a dose-dependent inhibitory effect on atherosclerotic lesion formation and plasma lipid levels [81]. 2-Methoxyestradiol (2ME2) is an orally bioavailable small molecule inhibitor of HIF-1 α with anti-tumor and anti-angiogenic activities. 2ME2 inhibits HIF-1 α by depolymerizing microtubules and blocking the nuclear accumulation and transcriptional activity of HIF-1 α [82]. In ApoE^{-/-} mice, 2ME2 (66.6 μ g/day) reduces atherosclerotic lesions [83], suggesting 2ME2 could be a potential anti-atherosclerotic drug.

NF- κ B inhibitors

The NF- κ B signaling pathway plays a crucial role in driving endothelial inflammation and dysfunction by orchestrating the expression of inflammatory cytokines/chemokines involved in atherosclerosis. In this scenario, endothelium-restricted inhibition of NF- κ B, reduces atherosclerotic plaque formation in ApoE^{-/-} mice [63, 64], indicating that blocking NF- κ B-dependent pro-inflammatory gene expression in the arterial wall could retard the progression of the atherosclerotic process. In this regard, many NF- κ B inhibitors, such as BMS-06 and vinpocetine, have been shown to reduce atherosclerosis in mice [84].

Concluding remarks and future perspectives

Studies in the past several decades have elucidated multiple mechanisms of atherosclerosis, such as endothelial dysfunction, oxidative stress, chronic inflammation, lipid deposition, and epigenetic dysregulation [2, 3, 15]. Despite significant advances in the understanding of pathogenesis of atherosclerosis and emerging pharmacotherapies, atherosclerosis and its clinical sequelae remains the major cause of morbidity and mortality worldwide. A deeper understanding of molecular and cellular mechanisms of atherogenesis and its progression will lead to the identification of novel therapeutic targets for atherosclerosis and accelerate the discovery process of target-based new cardiovascular drugs.

MSTFs are important master regulator of endothelial gene expression. Researches in the past decade have identified multiple MSTFs that are involved in regulating endothelial function and atherosclerosis. In this review, we mainly focused on the role of main MSTFs in regulating endothelial function, atherosclerosis and targeted pharmacotherapies. In addition to the MSTFs described above, there are several other MSTFs in endothelial cells, for example, early growth response-1 gene (Egr-1) [87], Hand2 [88], and Snail (which mediates endothelial mesenchymal transition (EndoMT)) [89]. In-depth elucidation of the regulation

and roles of MSTFs in atherosclerosis is critical for designing effective therapeutic strategies against atherosclerosis.

However, there are several key questions (see Outstanding Questions) and challenges in translating findings from mechanobiological studies into new MSTF-targeted therapeutics. For example, the potential interaction network of multiple MSTFs regulates the expression of important genes in oxidative stress, inflammation, and metabolism in endothelial cells. Important interactions, such as interaction of KLF2 with NRF2 [51, 52, 90], NF- κ B with HIF-1 α [29, 30] have been described recently. Firstly, KLF2 potently inhibits the expression and activity of HIF-1 α , while HIF-1 α is also involved in DF-induced KLF2 downregulation [62]. Secondly, redox-independent regulation of inflammatory and metabolic gene expression by NRF2 [45, 91, 92] further highlights the potential interaction between KLF2 and NRF2. Thirdly, glucose metabolism also regulates inflammation in endothelial cells evidenced by the fact that PFKFB3-mediated increased glycolysis promotes endothelial inflammation [93]. Therefore, the interplay of oxidative stress, inflammation, and glucose metabolism (glycolysis) in endothelial cells is complex and may represent a new direction to study the effect of hemodynamic forces on endothelial function [29, 30, 51, 52, 90, 93] (Figure 3).

In light of the important role of MSTFs in regulating endothelial function and atherosclerosis, the identification and elucidation of new endothelial MSTFs seem to be a pressing issue. Fortunately, recent biotechnological advances, such as genome-wide **transcriptional profiling** [94] could possibly assist us in identifying new MSTFs and accelerate MSTFs-based drug discovery (Figure 4). The increasing ease of accessibility to transcriptional profiling datasets in public repositories (such as NCBI Gene Expression Omnibus and Arrayexpress) will also assist in defining MSTF-regulated gene signatures. Further validation of the role of new MSTFs in atherosclerosis using genetically engineered mice will be needed for potential development of MSTFs targeting therapeutics (Figure 4). Last but not least, the pharmacokinetic/pharmacodynamic profile and potential side effects of MSTFs-targeted therapeutics is another important consideration in translating preclinical studies into effective therapeutics of atherosclerosis.

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Glossary

Atherosclerosis:

Atherosclerosis is a common lipid-driven disease in which lipids are retained and accumulated in the vessel wall. As a result, the vessel lumen become narrowed and hardened. At initial stage, atherosclerosis is asymptotic. When atherosclerosis progresses to more advanced stage, it causes severe symptoms, such as: heart attacks, stroke, and so on.

Many risk factors (such as hyperlipidemia, hyperglycemia, physical inactivity, smoking, aging, and obesity) can lead to this disease.

Disturbed flow (DF):

DF (also known as turbulent/oscillatory flow) is a complicated flow pattern that is irregular and non-uniform, including spatiotemporal changes in gradients of shear stress and flow direction. DF typically includes periods of reciprocating flow reversal that generates oscillatory wall shear stress. DF is associated with endothelial dysfunction (such as vascular inflammation), and focal distribution of atherosclerotic plaques.

Unidirectional laminar flow (UF):

UF is also called as steady laminar flow. It is a well-ordered normal flow pattern in the blood vessel. UF is associated with high shear stress and is antiinflammatory as well as atheroprotective.

Mechanotransducer:

In biomedical engineering, mechanotransducer indicates the specific molecules through which endothelial cells and other mechanotransducing cells sense and respond to mechanical cues (such as shear stress or cyclic strain) by converting them to biochemical/electrochemical signals that can elicit specific cellular responses.

Shear stress:

Shear stress is the mechanical force generated by flowing blood fluid. The magnitude of shear stress is defined as force/wall area (e.g., dyn/cm²) and the direction of shear stress is parallel to the direction of blood flow.

Mechanosensitive transcription factors (MSTFs):

Specialized DNA binding proteins that respond to biomechanical treatments and control the rate of transcription of genetic information from DNA to mRNA. MSTFs regulate downstream gene expression by binding to specific sequences of DNA.

Transcriptional profiling:

Transcriptional profiling, also known as expression profiling, refers to high-throughput sequencing techniques that are used to detect the expression amount of many transcripts (such as genes) in cells or tissues at the same time. The most common methods of transcriptional profiling include DNA microarray and RNA-sequencing.

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Outstanding questions

- How to identify novel mechanosensitive transcription factors (MSTFs)?
- How do MSTFs crosstalk with each other to regulate vascular pathophysiology?
- How are MSTFs regulated epigenetically (by DNA methylation, histone modification, and non-coding RNAs)?
- How to identify novel atheroprotective drugs by targeting MSTFs?

Highlights

- Unidirectional laminar flow and disturbed flow differentially modulate several mechanosensitive transcription factors (MSTFs) in endothelial cells.
- MSTFs regulate multiple aspects of endothelial function and vascular homeostasis, such as inflammation, proliferation, thrombosis, oxidative stress, and endothelial cell metabolism.
- MSTFs crosstalk with each other and functionally regulates endothelial gene expression and function.
- Targeting MSTFs could be a promising therapeutic strategy for atherosclerosis.

Figure 1. Vascular components of the artery

Blood vessels are usually composed of three layers: the tunica intima, tunica media, and tunica adventitia. The intima consists of an endothelial cells monolayer lining the vessel lumen. Often, the internal elastic lamina separates the tunica intima from the tunica media. The tunica media is mainly composed of vascular smooth muscle cells. The external elastic lamina separates the tunica media from the tunica adventitia. The tunica adventitia is primarily composed of connective tissues made up of fibroblasts, macrophages, and associated collagen-rich matrix proteins.

Figure 2, Key figure. Role of MSTFs in regulating endothelial phenotype

A. Image showing the focal distribution of lesions in a mouse model of atherosclerosis. The lesions preferentially develop at regions with disturbed flow (DF), such as branches and bends of the blood vessel (indicated by white arrow), but much less in regions of unidirectional laminar flow (UF), such as the straight part of the blood vessel (unpublished data). Note: color saturation of the image is enhanced for presentation purposes.

B. Major MSTFs that are regulated by UF and DF in ECs. The downregulation is shown by blue arrows and upregulation is shown by red arrows.

C. The genes that are regulated by each of the MSTFs in DF and UF treated endothelial cells, are shown.

D. Flow-dependent endothelial gene expression regulates EC phenotype. DF treated endothelial cells show a pro-inflammatory, pro-oxidant, pro-proliferative response, and enhanced endothelial to mesenchymal transition (EndoMT) phenotype. By doing so, DF causes endothelial cell activation and ensuing dysfunction. In contrast, UF treated ECs show anti-inflammatory, anti-oxidant, and anti-proliferative phenotype along with reduced EndoMT and glycolysis, leading to endothelial cell quiescence and vascular homeostasis.

Figure 3. Interaction network of MSTFs

The interaction of multiple MSTFs represent a complex interaction network that regulates the expression of genes involved in oxidative stress, inflammation, and metabolic programming (such as glycolysis) in ECs. Three biological processes-oxidative stress, inflammation and metabolism cross talk with each other. The KLF2/NRF2 crosstalk, and NF- κ B/HIF-1 α crosstalk are two examples. Glycolysis promotes endothelial inflammation, while KLF2 suppresses vascular inflammation, glycolysis, and HIF-1 α expression/activity. It is plausible that KLF2 may negatively regulate DF induced upregulation of HIF-1 α . On the other hand, HIF-1 α could also possibly be involved in DF induced KLF2 downregulation. Additionally, redox-independent regulation of inflammatory and metabolic gene expression by NRF2 further pinpoints the potential interaction network between KLF2 and NRF2. The elucidation of new MSTFs (transcription factor X) and their cross regulation with other MSTFs are critical for further understanding flow-induced transcriptional program and functional consequences.

Figure 4. Discovery pipeline of novel MSTFs and targeted therapeutics

Transcriptional profiling (such as RNA-sequencing or microarray) is commonly used to identify novel MSTFs. *In vivo*, intimal RNA samples from regions of DF and regions of unidirectional UF are collected for RNA-sequencing or microarray. *In vitro*, human ECs are exposed to UF, or DF before RNA is collected for transcriptional profiling. New MSTFs or gene regulation pathways can be identified by downstream bioinformatics analysis of differentially expressed genes. The newly identified MSTFs need to be validated both in cultured cells and in pre-clinical animal models. After validation, drug-screening system can be established to identify novel/repurposed pharmacological modulators of MSTFs, which can be further tested pre-clinically and clinically.

Abbreviations: HAEC, human aortic endothelial cells; HCAECs, human coronary artery endothelial cells; HUVECs, human umbilical vein endothelial cells; IPA, ingenuity® pathway analysis; KEGG, Kyoto encyclopedia of genes and genomes

Table 1Role of MSTFs in atherosclerosis *in vivo*

Animal model	Effect on atherosclerosis	Observed atherosclerotic characteristics	References
KLF2 ^{+/-} ; ApoE ^{-/-} mice	↑	↑ Foam cell formation	[36]
KLF2 ^{Mye} ; LDLR ^{-/-} mice	↑	↑ Lesional neutrophils and macrophages	[38]
KLF4 ^{EC} ; ApoE ^{-/-}	↑	↑ Endothelial inflammation and thrombosis	[35]
KLF4 ^{Tg(EC)} ; ApoE ^{-/-}	↓	↓ Endothelial inflammation and thrombosis	[35]
NRF2 ^{-/-} ; ApoE ^{-/-}	↓	↓ Foam cell formation	[95, 96]
NRF2 ^{-/-} ; LDLR ^{-/-} ApoB 100/100	Mixed	↓ Atherosclerotic lesion size ↑ Atherosclerotic plaque vulnerability	[97]
NF-kB ^{EC} ; ApoE ^{-/-}	↓	↓ Adhesion molecules and inflammation	[63]
I-κBmt ^{OE(EC)} ; ApoE ^{-/-}	↓	↓ Chronic intermittent hypoxia induced atherosclerosis; NF-kB activity	[64]
HIF-1α ^{EC} ; ApoE ^{-/-}	↓	↓ Monocyte adhesion	[61]
HIF-1α ^{Mye} ; ApoE ^{-/-}	↓	↓ Inflammatory gene expression	[98]
YAP/TAZ ^{KD} ; ApoE ^{-/-}	↓	↓ Promotes EC proliferation, inflammation	[27]
YAP ^{OE(EO)} ; ApoE ^{-/-} ; YAP ^{Tg(EC)} ; ApoE ^{-/-} ; YAP/TAZ ^{CA} ; ApoE ^{-/-}	↑	↑ Monocyte adhesion, macrophage accumulation	[26]
YAP ^{OE(EC)} ; ApoE ^{-/-}	↑	↑ VCAM1 dependent monocyte adhesion	[28]

Abbreviations:

KD, knockdown; OE (EC), endothelial cell-specific overexpression; Tg (EC), endothelial cell specific transgene; Mye, myeloid cell-specific knockout; EC, endothelial cell-specific knockout

Table 2

Examples of atheroprotective drugs that target MSTFs

Name of drug	Effect on atherosclerosis	Targeted MSTFs	References
Statins	↓	(+) KLF2; (+) KLF4; (+) NRF2; (–) NF- κ B; (–) AP1; (–) YAP/TEAD; (–) HIF-1 α	[26, 27, 66, 67, 99-102]
Resveratrol	↓	(+) KLF2; (+) KLF4; (+) NRF2; (–) NF- κ B	[22, 68, 69, 103, 104]
Tannic acid	↓	(+) KLF2	[71, 72]
SAHA	↓	(+) KLF2	[70]
Sulforaphane	↓	(+) NRF2; (–) NF- κ B	[75, 105]
Verteporfin	↓	(–) YAP/TEAD	[77, 78]
Evodiamine	↓	(–) HIF-1 α	[79, 106]
Digoxin	↓	(–) HIF-1 α	[80, 81]
2-Methoxyestradiol	↓	(–) HIF-1 α	[82, 83]

(+) activate; (–) inhibit.