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REVIEW ARTICLE

Recent advances of mechanosensitive genes in vascular endothelial cells for the formation and treatment of atherosclerosis

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Abstract Atherosclerotic cardiovascular disease and its complications are a high-incidence disease worldwide. Numerous studies have shown that blood flow shear has a huge impact on the function of vascular endothelial cells, and it plays an important role in gene regulation of pro-inflammatory, pro-thrombotic, pro-oxidative stress, and cell permeability. Many important endothelial cell mechanosensitive genes have been discovered, including *KLK10*, *CCN* gene family, *NRP2*, *YAP*, *TAZ*, *HIF-1α*, *NF-κB*, *FOS*, *JUN*, *TFEB*, *KLF2/KLF4*, *NRF2*, and *ID1*. Some of them have been intensively studied, whereas the relevant regulatory mechanism of other genes remains unclear. Focusing on these mechanosensitive genes will provide new strategies for therapeutic intervention in atherosclerotic vascular disease. Thus, this article reviews the mechanosensitive genes affecting vascular endothelial cells, including classical pathways and some newly screened genes, and summarizes the latest research progress on their roles in the pathogenesis of atherosclerosis to reveal effective therapeutic targets of drugs and provide new insights for anti-atherosclerosis.

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Introduction

Atherosclerosis (AS) is a chronic vascular disease formed by fibro-fatty lesions of the arterial wall, which can cause many complications during its development, such as myocardial infarction and stroke.[1–3] One of the early markers of atherosclerosis is endothelial dysfunction, which leads to infiltration of low-density lipoprotein (LDL) into the vascular medium, accompanied by monocyte recruitment and abnormal phagocytosis.[4–6] Macroscopically, vulnerable plaques (characterized by plaques with large necrotic cores covered by thin fibrous caps) form and rupture, thereby leading to arterial thrombosis[7,8] (Fig. 1 and Table 1).

Atherosclerotic plaque forms differently because of the physiological structure of the arteries, which causes the walls of the vessels to receive different fluids. A large number of studies have shown that during the development of AS, changes in blood flow, phenotypic differentiation of smooth muscle cells in the vascular wall, and changes in elastic fiber synthesis affect the biomechanical properties of vascular endothelial cell formation, thereby leading to endothelial cell dysfunction.[9] The formation of atherosclerotic plaques varies depending on the fluid stimulation of the vessel walls. In general, atherosclerotic plaque formation is easier in turbulent flow than in laminar flow (LF).[10] LF can maintain endothelial cell homeostasis and the AS-protective phenotype of endothelial cells, whereas disturbed flow (DF)

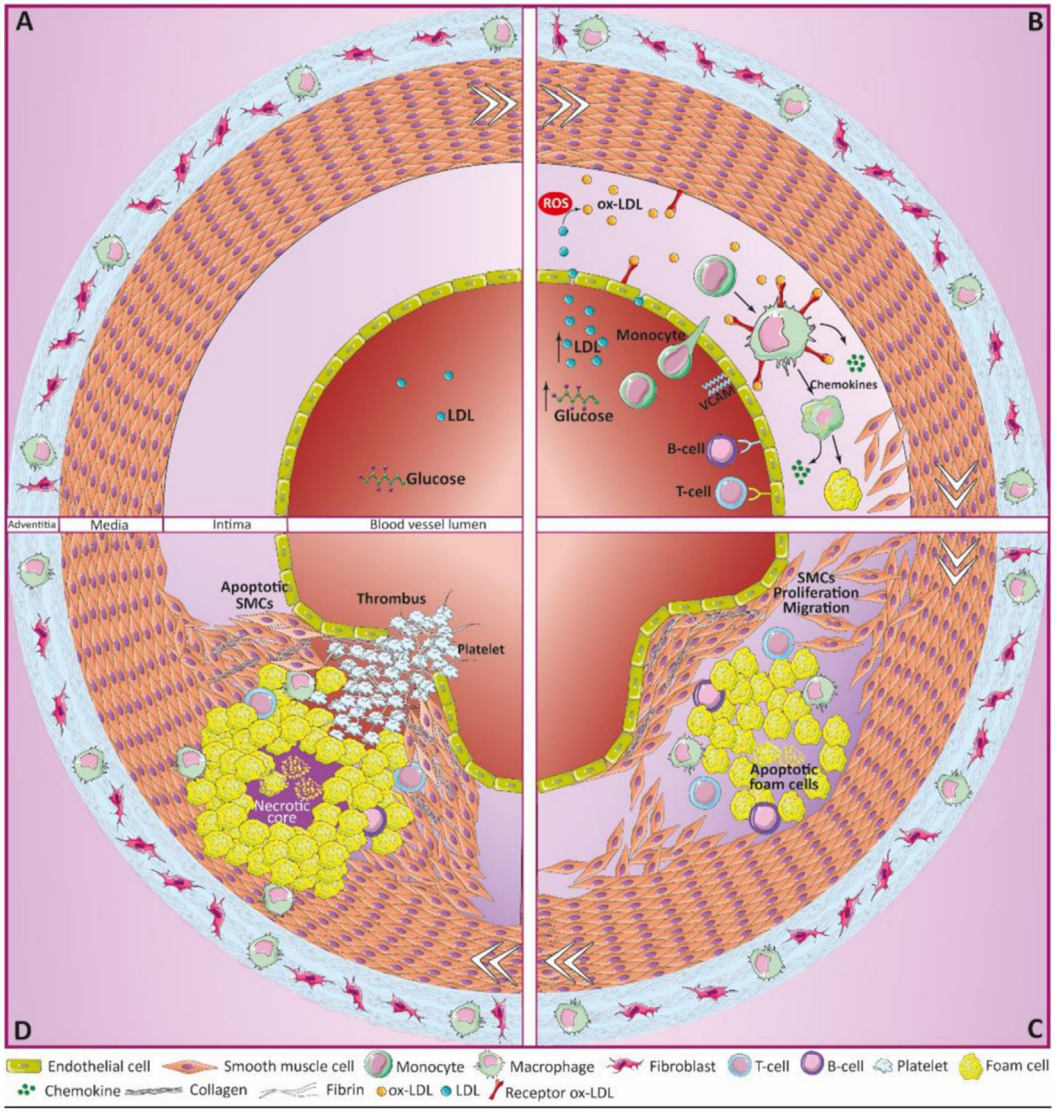

Figure 1 Atherosclerosis progression. **(A)** Different factors contribute to the initiation of atherosclerosis, including hyperglycemia or oxidative stress. **(B)** Phagocytosis of ox-LDL by monocytes/macrophages results from the accumulation and deposition of foam cells and the recruitment of B and T lymphocytes. **(C)** The formation of fatty streaks, along with the proliferation and migration of SMCs to the injured area, creates complex structures, namely, atherosclerotic plaques. Atherosclerotic plaques partially block the lumen of blood vessels, reducing blood flow and oxygen/nutrient supply to surrounding tissues. **(D)** Plaque rupture activates a thrombotic event, completely blocking circulation and potentially leading to stroke or myocardial infarction. Cited from reference[147] with permission from Antioxidants (Basel).

Table 1 Regulation of endothelial mechanosensitive gene activity.

Mechanosensitive gene	Under laminar flow conditions	Under disturbed flow conditions	References
KLK10	Up-regulated; inhibits endothelial inflammation and barrier dysfunction, reduces endothelial and monocyte migration and recruitment	Down-regulated	21—24
NRP2	Activated; participates in endothelial —mesenchymal transition and neovascularization	Suppressed	33—41
YAP/TAZ	Suppressed	Activated; pro-inflammatory	42—51
HIF-1α	Suppressed	Activated; increases vascular permeability; recruits immune cells; promotes endothelial cell proliferation	52—61
NF-κB	Suppressed	Activated; recruits virus cells; pro-inflammatory; induces enhanced transcription of HIF-1α; mediated by TLR-4	62—65
JUN/FOS	Inhibited; protects endothelial cells	Activated; vascular cell function is impaired	66—75
TFEB	Activated; inhibits the activation of *NF-κB*	Inhibited; endothelial cell dysfunction	76—84
KLF2/KLF4	Activated; inhibits the pro-inflammatory transcription factors *NF-κB* and AP-1	Suppressed	85—91
NRF2	Up-regulated; inhibits oxidative stress	Down-regulated; promotes oxidative stress	92—96
ID1	Up-regulated; promotes angiogenesis	Down-regulated; promotes endothelial cell lipid uptake	97—104

enhances endothelial cell pro-inflammatory response and oxidation.[11] Low and disturbed shear stress leads to endothelial damage and chronic inflammation.[12,13] Consequently, increased vascular stiffness resulting from altered smooth muscle phenotypes and reduced elastic fibers are associated with the ability of endothelial cells to recruit monocytes.[14—16] The difference in mechanical force stimulation might be due to the influence of vascular matrix stiffness or different fluid stimulations. Exploring the response of vascular cells to mechanical forces is an essential part of understanding a series of vascular diseases such as AS.

Endothelial cells, as mechanosensory cells, play an important regulatory role in AS development,[1] but the mechanism by which they regulate physiological and pathophysiological responses by decoding the complex mechanical environment remains unclear.[17] This review focuses on some new key genes in the occurrence and development of AS and summarized the latest research in the past two years to provide new targets for AS-related research. Furthermore, this article summarized the known targeted drugs for AS-related genes, hoping to provide new strategies for further AS-targeted treatment.

Endothelial mechanosensitive genes in AS

During the occurrence and development of AS, the mechanical stimulation of intravascular blood flow to the inner wall cells is an important factor affecting AS.[18] Pro-AS shear stress (oscillating or turbulent, non-unidirectional) and anti-AS shear stress (steady or pulsatile, unidirectional LF) stimulate the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are involved in the signal transduction of gene expression in vascular endothelial cells.[19] Non-unidirectional shear stress induces the expression of pro-AS-related genes encoding adhesion molecules and chemokines in a manner that is dependent on superoxide and nitric oxide (NO) production. Under mechanical environmental stimulation, endothelial genes sensitive to mechanical stimulation play an important role in the prevention, development, and treatment of AS.[20] This review of the mechanosensitive transcription factors in AS will provide a comprehensive understanding of the mechanobiological mechanisms that regulate the occurrence and development of AS.

Based on previous studies, in the case of DF, the occurrence and development of AS can be enhanced by stimulating the expression of mechanosensitive genes that promote AS, such as *YAP/TAZ*, *HIF-1α*, *NF-κB*, and *JUN/FOS*. In the case of LF, the occurrence and development of AS can be hindered by activating mechanosensitive genes that protect vascular endothelial cell stability and inhibit the inflammatory response to oxidative stress, such as *KLK10*, *NRP2*, *TFEB*, *KLF2/KLF4*, and *NRF2* (Fig. 2).

KLK10

KLK10 encodes a member of the *KLK* family of 15 secreted serine proteases of the kalstatin-related peptidase,[21] which

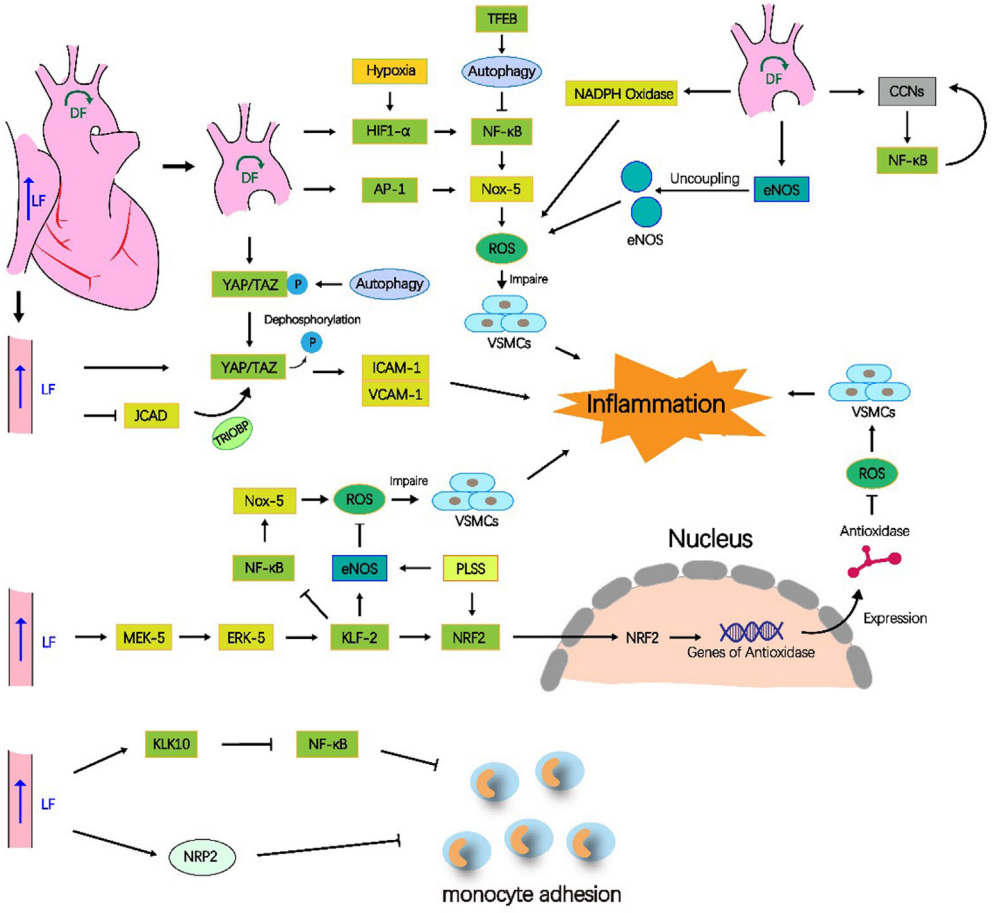

Figure 2 Mechanosensitive gene regulatory networks in atherosclerosis.

can be released into the circulation and is primarily studied as a tumor suppressor.[22] In 2010, researchers found that during AS development, different fluids have different effects on vascular endothelial cells. Such fluids are up-regulated in the LF environment and down-regulated in the DF environment.[23] However, the changes in *KLK10* expression of different fluid environments and the regulatory mechanism among vascular endothelial cells remain unclear. In 2022, the latest research pointed out that the high expression of s-flow *KLK10* can inhibit the expression of *NF-κB*, vascular cell adhesion molecule 1 (VCAM1), and intracellular adhesion molecule 1 (ICAM1), thereby preventing monocyte adhesion.[24] In addition, researchers found that s-flow activation of *KLK10* can protect the permeability barrier of endothelial cells.

Therefore, as a mechanosensitive gene, the activated expression of *KLK10* can inhibit endothelial inflammation and endothelial barrier dysfunction and reduce endothelial cells and monocyte migration and recruitment, without affecting normal cell apoptosis and proliferation. At present, few research results on this gene have been found, and further in-depth research can be expected in the future.

CCN gene family

At present, the role of *CCN* family members (*CCN1*, *CCN2*, and *CCN3*) in the regulation of cholesterol metabolism is not completely understood, but several studies have shown that the CCN family can be used as a new therapeutic option for AS.

CCN1 regulates a variety of cellular processes, including cell adhesion, migration, differentiation, proliferation, survival, apoptosis, and senescence, by binding to and functioning with different integrins.[25,26] Using the $CCN1^{dm/dm}/ApoE^{-/-}$ mice model, some researchers found that *CCN1*/$\alpha 6\beta 1$ was involved in the expression of transcription factors (such as *NF-κB*) *in vitro* and *in vivo*, and *NF-κB* activation further enhanced *CCN1* and integrins.[27] The production of $\alpha 6\beta 1$, through pharmacological inhibition of *CCN1*-$\alpha 6\beta 1$ binding, is a new target for the treatment of AS, such as interference with T1 peptides derived from the $\alpha 6\beta 1$-binding sequence of *CCN1*. Gan et al found that the inhibition of Wnt/β-catenin signaling also reduced *CCN1* expression, which led to endothelial cell inflammation and apoptosis.[28] Moreover, *CCN1* promotes lipid accumulation and foam cell formation by impairing ATP-binding cassette transporters A1 and G1 (ABCA1/G1) mediated cholesterol efflux in macrophages.[29] In endothelial cells, *CCN1* regulates cholesterol metabolism to allow ox-LDL to enter the stroma.[30]

Previous studies have shown that blood flow disturbances up-regulate *CCN1* and *CCN2* expression, and unidirectional LF decreases *CCN1* and *CCN2* expression while increasing *CCN3* expression.[31] The overexpression of *CCN3*

is associated with the control of inflammatory processes and reversal of dyslipidemia during AS, indicating that *CCN3* may be a potential target for AS treatment.[32]

NRP2

Neuropilin 2 (*NRP2*) is a member of the *NRP* gene family, and it is abundantly expressed in neural and vascular tissues.[33] Endothelial-derived *NRP2* can participate in endothelial—mesenchymal transition and angiogenesis by regulating the TGFβ/VEGF/INTEGRIN/SEMA3 signaling pathway.[34,35] Studies have found that the *NRP2* gene can be activated by laminar shear stress (LSS), and the upstream transcription factor GATA2 regulates the expression of *NRP2* during AS development.[36] The loss of *NRP2* increases vascular permeability, thereby aggravating inflammation and lymphedema during inflammatory processes[37] and promoting human endothelial and smooth muscle cell survival and migration in response to VEGF-A and VEGF-C.[38,39] In addition, *NRP2* may interact with monocyte-expressed α5 integrin, mediating monocyte adhesion to the vessel wall.[40] *NRP2* may also positively regulate EndoMT by directly binding to TGFβ1 and complexing with TGFBR1.[41] In addition, NRP2 is highly expressed in the lymphocyte and outer membrane in carotid plaque, promoting lymphangiogenesis and neovascularization.[38]

These results suggest that *NRP2* plays an important pro-atherosclerotic role in AS by regulating apoptosis, indicating that *NRP2* is a potential therapeutic target for the treatment of atherosclerotic diseases.

YAP and *TAZ*

As early as 2011, studies have shown that the proteins encoded by *YAP* and *TAZ* genes are sensors and mediators of mechanical signals dictated by the cellular microenvironment.[42—44] In 2016, studies confirmed that the transcriptional activity of *YAP*/*TAZ* was inhibited under the long-term mechanical activation of LF. Conversely, upon turbulent stimulation, the phosphorylation level of *YAP*/*TAZ* was reduced, which resulted in enhanced transcriptional activity.[45,46] *YAP*/*TAZ* activity has been shown to be associated with genes that promote AS. For example, enhanced *YAP*/*TAZ* activity can increase the expression level of *CCN1*,[47,48] promote some important pro-inflammatory factors such as ICAM-1 and VCAM-1, recruit immune cells, and mediate the inflammatory response of macrophages.[49]

On the contrary, knockout of *YAP* and *TAZ* will reduce the probability and degree of AS development in mice, whereas the overexpression of *YAP*/*TAZ* will lead to aggravation of the occurrence and development of AS in animal models.[50] As a classical mechanosensitive signaling pathway, Hippo-*YAP*/*TAZ* is regulated by many upstream and downstream signals. Among them, current research shows that the novel coronary artery disease risk gene (JCAD) can inhibit the activation of the *YAP*/*TAZ* pathway and the expression of downstream pro-AS genes (including *CCN1*).[51] Particularly, JCAD regulates *YAP*/*TAZ* activation by interacting with the actin-binding protein TRIOBP to stabilize stress fiber formation, and endothelial JCAD expression is increased in mouse and human atherosclerotic plaques. The abovementioned data from genomic proteomics and animal model studies show that targeting the expression of *YAP*/*TAZ* and the regulation of upstream and downstream gene molecules and transcription products is a potential treatment plan for AS.

HIF-1α

HIF-1α can restore oxygen homeostasis by inducing glycolysis, erythropoiesis, and angiogenesis under hypoxia, and the loss of *HIF-1α* reduces hypoxia-induced vascular endothelial growth factor (VEGF) expression.[52,53] In addition, the high expression level of *HIF-1α* leads to the activation of multiple inflammatory genes, including *NF-κB* signaling.[54] With the development of AS plaques, the lesion area (under the fibrous cap) is in a hypoxic environment; *HIF-1α* is further activated; the inflammatory response intensifies and leads to vascular proliferation, and the rate of plaque rupture is accelerated.[55] *HIF-1α* activation affects the expression of factors such as *OCT4*, whose transcriptional activation is dependent on *HIF-1α* and *KLF4* in vascular smooth muscle cells (VSMCs). Studies have demonstrated that the conditional knockout of *OCT4* in VSMCs in $ApoE^{-/-}$ mice results in increased lesion size and changes in lesion composition, which is consistent with reduced plaque stability (it is characterized by thinning of the fibrous cap, an increase in the necrotic core area, and an increase in intraplaque hemorrhage). Therefore, *HIF-1α* regulates the effect of *OCT4* on AS.

HIF-1α is up-regulated in low-shear-stress regions of porcine and murine arteries, and *HIF-1α* promotes vascular endothelial cell proliferation and inflammatory activation under low-shear stimulation by inducing glycolytic enzymes.[57] A mouse partial carotid artery ligation model demonstrated that turbulent flow up-regulates the expression of *HIF-1α*, glycolytic enzymes, and inflammatory genes and enhances endothelial cell proliferation.[58] In $ApoE^{-/-}$ mice, the endothelial cell-specific gene deletion of *HIF-1α* inhibits inflammation and endothelial cell proliferation in partially ligated arteries, indicating that *HIF-1α* promotes inflammation and vascular dysfunction in regions of low shear stress.[59] Studies have shown that SIRT6 overexpression promotes the invasion, migration, and proliferation of human umbilical vein endothelial cells (HUVECs) under normoxic and hypoxic conditions by transcriptionally activating *HIF-1α* and preventing deubiquitination and degradation of K37 and K532.[60] These findings indicate that the *HIF-1α* gene plays a key role not only in the initiation of AS (by regulating the inflammatory response) but also in the advanced stages of AS (by regulating intraplaque angiogenesis).[61]

NF-κB

NF-κB is a key regulatory gene for immunity, stress response, apoptosis, and differentiation.[62] During *NF-κB* activation, multiple stimuli combine to mediate various transcriptional programs.[55] The activation of *NF-κB* can promote the expression of multiple pro-inflammatory and pro-adhesion genes (VCAM1, MCP1, and SELE) in endothelial

cells.[63] The activity of *NF-κB* is regulated by Toll-like receptors (TLRs), tumor necrosis factor receptor, and interleukin-1 (IL-1R), and it plays an important role in the immune response.[64] *NF-κB* signal transduction is activated in response to mechanical activation in turbulent regions, and in *ApoE*$^{-/-}$ mice or *Ldlr*$^{-/-}$ mice models, N*F-κB* activation and *NF-κB*-induced gene expression were shown to be up-regulated after lipopolysaccharide treatment or high-fat feeding. The expression of *NF-κB* is activated in endothelial cells under a high-cholesterol environment, and the endothelial cell-specific inhibition of *NF-κB* can reduce the expression of pro-inflammatory and pro-adhesion genes and monocyte chemotactic protein, thereby reducing the recruitment of macrophages and stabilizing AS plaques of *ApoE*$^{-/-}$ mice.[65] This phenomenon may help to locate AS lesions in the future using the location of high steady-state expression levels of *NF-κB*/*IκB* components.

FOS and *JUN*

The common heterodimeric protein transcription factor AP-1, which is composed of c-*FOS* and c-*JUN* with different proteins of the JDP and ATP families, is involved in the regulation of gene expression in response to various external signals (*e.g.*, cytokines, growth factors, environmental stress, and bacterial and viral infections). It is also closely related to the regulation of cellular processes such as cell differentiation, cell proliferation, and apoptosis.[66,67] Previous evidence suggests that AP-1 couples with *NF-κB* to form a complex during the activation of inflammatory genes. The AP-1/NF-*κB* signaling pathway is an important initiating factor for activating the inflammatory pathway mediated by TNF-α.[68] Recent studies have shown that ERK/p38 activation is an important feedforward signal of the AP-1/NF-*κB* pathway.[69] In animal models of cardiovascular diseases, the primary accumulation of ox-LDL activates the ERK/p38 signaling pathway and induces its phosphorylation, thereby stimulating the AP-1/NF-*κB* pathway. *NF-κB* inhibits this activation, but it does not affect ERK/P38 activation, whereas the AP-1/NF-κB pathway is affected because phosphorylation is inhibited. Further studies showed that the down-regulation of ox-LDL resulted in a decrease in AP-1 activity and phosphorylation of c-*JUN* N-terminal kinase 1/2 (JNK 1/2), a process that prevented the activation of inflammatory pathways.[70] In another study, investigators used troxagliptin (SRY-472 trelagliptin succinic acid) to inhibit vascular inflammatory factors, including MCP-1, chemokine 1, and interleukin (IL-6). Similarly, the data suggest that the level of activation of AP-1/NF-*κB* is down-regulated with the increase of trolagliptin concentrations, thereby regulating inflammatory processes and monocyte adhesion.[71] During the migration of VSMCs, NOX family proteins are important factors in regulating oxidative stress damage, and recent studies have demonstrated that the AP-1 signaling pathway can induce the overexpression of NOX family proteins, thereby promoting the generation of free radicals in VSMCs and the development of vascular inflammation.[72,73] A recent study showed that VSMCs proliferation, migration, and AS plaque development are accelerated with AP-1 up-regulation. The use of upstream inhibitors results in the loss of AP-1 and delays the development of AS.[74] Combined with evidence from multiple studies, AP-1 has been identified as an important hub for regulating vascular inflammation.[75]

TFEB

TFEB is a main regulatory gene regulating lysosomal fusion ability. Under negative nutrient conditions, changes in cellular conditions induce the translocation of TFEB proteins from the cytoplasm to the nucleus. The overexpression of *TFEB* cascades downstream signals that regulate lysosomal initiation, extracellular secretion, and autophagy.[76–79] In addition, *TFEB* and *TFE3* play direct regulatory roles in innate and adaptive immune systems.[80]

In vitro, *TFEB* knockdown resulted in the dysregulation of multiple inflammatory factors, such as major histocompatibility complex-2 and interleukin-1 (IL-1).[78] In vascular inflammation model mice, the exogenous overexpression of *TFEB* can reduce the level of aortic vascular inflammation and the expression of endothelial cell chemokines in mice. This study also demonstrated that *TFEB* can inhibit the *NF-κB* signaling pathway by inhibiting the activity of kappa B kinase. *TFEB* knockout increased the number of mice dying from inflammation.[81] Studies in endothelial cells have found a similar phenomenon that the *NF-κB* pathway is inhibited by *TFEB*, thereby inhibiting the occurrence of vascular inflammation and increasing vascular endothelial dysfunction.[82] Furthermore, recent evidence suggests that *TFEB* is involved in the regulation of lipid homeostasis in cardiovascular models. Its expression level is positively correlated with lipid transporters and lipid mediators, and *TFEB* knockout leads to abnormal intravascular lipid levels.[83] A specific piece of evidence suggests that the up-regulation of LF levels increases *TFEB* protein abundance during LF prevention of AS development and prevents the development of endothelial dysfunction and endothelial inflammation to a certain extent. The up-regulation of *TFEB* reduces the degree of oxidative stress in vascular endothelial cells and the recruitment of leukocytes to endothelial cells, thereby preventing the development of AS.[84] In addition, the up-regulation of *TFEB* has been used as a clinical strategy to treat cardiovascular diseases.

KLF2/*KLF4*

KLF2 and *KLF4* are vascular homeostasis-related genes involved in regulating the expression of various anti-inflammatory, antioxidant, and antithrombotic genes in endothelial cells.[85] *KLF2* also regulates various proinflammatory, prothrombotic, and vasoconstrictor factors, such as VCAM1, MCP-1, E-selectin (SELE), and endothelin 1 (ET1). *KLF2* inhibits the activation of *NF-κB* and the expression of pro-inflammatory genes by recruiting transcriptional activators.[86] Based on previous reports, *KLF2* is critical for shear stress-induced cell alignment, fiber assembly with shear, and JNK and its downstream target *ATF2*/c-*Jun*.[87] *KLF2* also represses inflammatory genes by inhibiting the pro-inflammatory transcription factors *NF-κB* and AP-1. The mechanism of LF-mediated AS protection is the mitogen extracellular signal-regulated kinase 5 (MEK5)-ERK5-*KLF2* pathway.[88] In addition, studies have shown that

KLF2 improves the nuclear localization of *NRF2*. *NRF2* potently induces anti-inflammatory/antioxidant enzymes.[89] Furthermore, *in vivo* studies have shown that *KLF2* and *KLF4* coregulated several genes to protect the aorta from AS.[49] The development of AS was accelerated in $ApoE^{-/-}$ mice and $Ldlr^{-/-}$ mice after *KLF2* and *KLF4* gene knockout, indicating that the activation of *KLF2* and *KLF4* in endothelial cells may induce AS protection.[90] Moreover, SSRE and *KLF* elements are key fluid sensors required for transcriptionally permissive, hypomethylated eNOS promoters in endothelial cells under chronic shear stress *in vivo*. The expression of eNOS is regulated by a blood flow-dependent epigenetic mechanism, which provides a new mechanism for the regulation of eNOS genes in AS.[91] *KLF2* and *KLF4* of endothelial cells, which are mechanosensitive genes that primarily regulate vascular homeostasis, are potential therapeutic targets for the prevention of AS through pharmacological intervention.

NRF2

NRF2 is an emerging cellular antioxidant-regulated gene. *NRF2* controls the basal and inducible expression of antioxidant response element-dependent gene arrays to modulate the physiological and pathological outcomes induced by peroxidation, and it is a key regulator of cellular defense mechanisms against foreign organisms and oxidative stress.[92] Previous studies revealed that *NRF2* plays a role in regulating antioxidant levels and anti-AS by stabilizing shear stress.[93] Pulsed laminar shear stress (PLSS) induced the expression level of adhesion molecules, and chemokines are enhanced in *NRF2*-siRNA-treated HUVECs and isolated arterial endothelial cells from *NRF2* knockout mice.[94] Thus, PLSS leads to an increase in intracellular antioxidant levels through *NRF2* activation, thereby maintaining the state of endothelial cells during AS development and preventing excessive ROS/RNS production required for pro-AS gene expression.[94] LF activates the expression of *NRF2*, which is important for endothelial cells to adapt to oxidative and nitrosative stress. In vascular endothelial cells, increased blood flow shear stress induces eNOS-dependent NO production and inhibits oxidative stress. By contrast, perturbation promotes oxidative stress through the uncoupling of NADPH oxidase, xanthine oxidase, and eNOS, and *NRF2* knockdown reverses the laminar-induced up-regulation of cytoprotective enzymes.[95]

The inhibition of *KLF2* blocks the expression of *NRF2*-dependent antioxidant genes in endothelial cells; therefore, *KLF2* can serve as a promoter of *NRF2* activation. Moreover, *KLF2* and *NRF2* synergistically regulate LF-induced endothelial gene expression. *KLF2* and *NRF2* are involved in the vast majority of LF-induced transcriptional networks.[96] Therefore, understanding the regulation of *NRF2* gene activity and downstream pathways has great implications for human health.

ID1

ID1 encodes a DNA-binding inhibitor, also known as an inhibitor of differentiation (Id), a member of the helix-loop-helix transcription factor family. Given the lack of a DNA-binding basic domain, it can inhibit transcription. The factor binds to DNA and inhibits the activation of basic helix-loop-helix transcription factors.[97] *ID1*, a member of the *ID* family, can participate in angiogenesis by accelerating the cell cycle and inducing cell proliferation and migration. HUVECs overexpressing *ID1* could promote capillary formation through cytoskeletal reorganization and cell contraction.[98] *ID1* may affect the stability of atherosclerotic plaques through inflammation and angiogenesis. Hypoxia, VEGF, TNF-α, NAD(P)H oxidase, and other plaque-forming factors can up-regulate the expression of *ID1*.[99] Studies showed that ID1 overexpression increased the proportion of EPCs in the S/G(2)M phase and significantly increased the expression level of cyclin D1, whereas knocking down *ID1* inhibited the expression level of cyclin D1 and blocked the cellular process of EPCs in the G(1) phase. In addition, *ID1* up-regulates the expression level of integration site family member 2 (Wnt2) of wingless murine mammary tumor virus and promotes β-catenin accumulation and nuclear translocation, thereby promoting cell cycle progression in EPCs.[100] Zhang et al found that low oscillatory shear stress (OSS) promotes lipid uptake by down-regulating the expression of *ID1* by using the $ApoE^{-/-}$ mice ligation model *in vivo* and applying OSS *in vitro*. *ID1* overexpression and knockdown experiments showed that *ID1* can bind to sterol regulatory element-binding protein 1 (SREBP1) to regulate LDLR expression and affect lipid uptake.[101] When exposed to ox-LDL, the expression and distribution of *ID1* protein are regulated by shear stress and lipoproteins, and high shear stress promotes *ID1* expression and angiogenesis.[102,103] Recently, Valanti et al proposed that the use of a recombinant HDL form containing human apolipoprotein E (rHDL-*ApoE*3) could activate the overexpression of *ID1* and subsequent activation of MEK1/2 and PI3K and its downstream targets ERK1/2, AKT, and p38 MAPK to improve vascular permeability in the body.[104] These findings indicate that the *ID1* gene is an important hub for the development of AS.

Activators and inhibitors of mechanosensitive genes

YAP/TAZ inhibitors

SNO-GNAI2 promotes AS by binding to CXCR5 to activate *YAP*-dependent endothelial cell inflammation.[105] TRAF6, a downstream effector of interleukin 1β (IL-1β), triggers *YAP* ubiquitination at K252, blocks the interaction between *YAP* and angiomotin, and enhances *YAP* nuclear translocation. Recombinant IL-1 receptor antagonists reduce the formation of atherosclerotic lesions.[106] Yuan et al found that SIRT1 expression was enhanced in atherosclerotic vessels in mice receiving rapamycin-induced autophagy, whereas *YAP* was inhibited, and LF-induced endothelial autophagy and SIRT1 expression contributed to the inhibition of the Hippo/*YAP* signaling pathway and inhibited the formation of atherosclerotic plaques.[107] Sal-b is an effective water-soluble substance extracted from the roots and rhizomes of *Salvia miltiorrhiza*. It has the chemical structure of phenolic acid; the molecular formula is C36H30O16, and the molecular weight is 748. The anti-AS action pathway is

related to the regulation of the *YAP*/*TAZ*/JNK signaling pathway.[108] Naringin reversed ox-LDL-triggered HUVEC apoptosis, EndoMT, and inflammation by inhibiting the YAP pathway. Therefore, Naringin may have therapeutic effects on endothelial injury-related diseases.[109] Methotrexate (MTX) exerts AS protection through the amp-dependent kinase (AMPK)-*YAP*/*TAZ* pathway.[110] Coenzyme Q10 (CoQ10) improves mitochondrial function, inhibits ROS production, and attenuates AS by activating the AMPK-*YAP*-OPA1 pathway.[111] This evidence indicates that the use of *YAP*/*TAZ*/TEAD inhibitors to treat AS has potential therapeutic by inhibiting the transactivating portion of *YAP*/*TAZ* with anti-AS function.

HIF-1α inhibitors

Benzopyranoyl 1,2,3-triazole serves as a HIF-1 inhibitor by increasing *HIF-1α* hydroxylation, subsequent ubiquitination, and proteasomal degradation. It is synergistic with the epidermal growth factor receptor inhibitor gefitinib.[112] BIX01294 is a dizapazolamide derivative initially identified as a neutral histone-lysine n-methyltransferase 2 (EHMT2)/G9a inhibitor in a small-molecule chemical library screen. BIX01294 increases the hydroxylation of HIF-1α by increasing the expression of PHD2 and pVHL.[113] In addition, the natural drug cadolactone, which is isolated from the latex and fruit of plantain, inhibits HIF-1 transcriptional activity.[114] Glycerin (a mixture of glycerin I, II, and III) is a group of phytoalexins that exert HIF-1 inhibitory effects in two ways. First, the translation of *HIF-1α* was blocked by inhibiting the PI3K/AKT/mTOR pathway under hypoxic conditions. Second, they interfere with the binding activity of HSP90, thereby reducing the stability of *HIF-1α*.[115] MPTOG157 developed an indole-3-ethylsulfamoylbenzeneacrylamide compound based on the core structures of HDAC inhibitors, namely, PXD101 and LBH589. MPTOG157 has a strong inhibitory effect on HDAC1, 2, 3, and 6, resulting in the hyperacetylation of HSP90 and HIF-1α, the degradation of HSP90, and a strong anti-inflammatory effect.[116] Pyraclostrobin analogs inhibit hypoxia-induced *HIF-1α* transcriptional activation, and they promote ubiquitin-dependent proteasomal degradation of *HIF-1α* by increasing intracellular oxygen tension under hypoxic conditions.[117]

NF-κB/*NRF2* activators and inhibitors

Azithromycin is a macrolide antibiotic that inhibits bacterial protein synthesis and quorum sensing and reduces biofilm formation. The initial stimulatory effects of azithromycin on immune cells and epithelial cells include interaction with phospholipids and Erk1/2, followed by the regulation of transcription factors AP-1 and *NF-κB*, inflammatory cytokines, and mucin release. The delayed inhibition of cellular function and high lysosomal accumulation is accompanied by the disruption of protein and intracellular lipid trafficking, and the regulation of surface receptor expression, macrophage phenotype, and autophagy.[118] BMS-06 and vinpocetine could block *NF-κB*-dependent pro-inflammatory gene expression in the arterial wall and delay the progression of AS.[119] IFNγ is a potent inducer of *NF-κB* and AP-1, which are transcription factors that control many pro-inflammatory signaling cascades associated with the vascular injury response.[120]

Anti-inflammatory effects of flavonoids *in vitro* or cellular models include the inhibition of the synthesis and activity of various pro-inflammatory mediators, such as eicosanoids, cytokines, adhesion molecules, and C-reactive protein. Flavonoids also inhibit upstream regulators of inflammation, such as the transcription factors *NF-κB*, AP-1, and *NRF2*.[121] Luteolin isolates and luteolin-enriched plant extracts exhibited anti-inflammatory activity. The mechanism of action of luteolin is different, in which Src is located in the nuclear factor *NF-κB* pathway, and MAPK is located in the AP-1 pathway. *In vitro*, *in vivo*, and clinical studies have shown that the main pharmacological mechanism of luteolin is its anti-inflammatory activity, which is derived from the regulation of *NF-κB*.[122]

KLF2/*KLF4* activators

KLF2 and *KLF4* have potent anti-inflammatory effects in endothelial cells and macrophages. Therefore, pharmacological activators such as *KLF2* and *KLF4* are promising drugs for the treatment of endothelial dysfunction and AS. Many drugs have been identified or repurposed as activators of *KLF2* and/or *KLF4*, including statins such as HMG-CoA reductase inhibitors, resveratrol, histone deacetylase inhibitors (SAHA), and tannic acid (TA)[123] In addition, *KLF2* is activated by a recently identified transcriptional activator of eNOS and endothelial cell inflammatory inhibitor ERK5. Recently, TA has been identified as a novel pharmacological activator of *KLF2*, activating *KLF2* through the ERK5/MEF2 pathway. It was protective against AS in *ApoE*$^{-/-}$ high-fat diet mice.[124] A novel long non-coding RNA, AF131217.1, was found to be up-regulated after LSS treatment in HUVECs. AF131217.1 inhibited fluid-mediated monocyte adhesion by down-regulating the expression of VCAM-1 and ICAM-1 and the expression of *KLF2* and eNOS in response to fluid stimulation. The knockdown of AF131217.1 promoted the expression of ICAM-1 and VCAM-1 and TNF-α-induced changes in monocyte adhesion and *KLF2* and eNOS expression. Mechanistic studies showed that AF131217.1, as a competitive endogenous RNA of miR-128−3p, was involved in regulating the activity of its target gene *KLF4*.[125] Fluid shear stress reduces the expression of CSE in human and mouse endothelial cells, and it is negatively correlated with *KLF 2*. CSE (cystathionine γ lyase) was identified as a direct target of *KLF2*-regulated microRNA, miR-27b, and the high expression of CSE in endothelial cells, and it was negatively correlated with KLF2 and miR-27b levels. Therefore, the regulation of CSE expression by shear stress/DF is dependent on *KLF2* and miR-27b.[126,127]

Summary and future perspectives

AS is a high-incidence chronic disease worldwide. Despite several basic and clinical research results, drugs such as statins and monoclonal antibodies have been developed to fight the disease.[128] However, with the rapid development and wide application of RNA sequencing, proteomics, lipidomics, and metabolomics, we can easily obtain information on related regulatory genes and can rapidly screen new

regulatory genes.[129] Many new approaches have also been developed in AS therapy, such as gene editing, induced pluripotential stem cell methodology, or nano-drugs.[130–134] Therefore, we verified the role of endothelial dysfunction and biomechanical stimulation in AS to control the occurrence and development of AS and summarized the newly discovered regulatory genes and new mechanisms of classical pathways to facilitate the development of effective targeted drugs in the future.

Changes in blood flow can directly affect the function of vascular endothelial cells, and endothelial cell phenotype changes and endothelial cell dysfunction caused by abnormal fluid stimulation are considered important driving factors of AS.[135] Mechanosensitive factors transmit shear stress to the nucleus, thereby affecting its cellular behavior. Several studies in the past few decades have outlined the biological mechanisms by which many mechanosensitive genes regulate the phenotype, function, and behavior of endothelial cells in response to hemodynamic change, but the role of some of these signaling pathways remains unclear. In addition, new mechanosensitive genes are constantly being discovered, and therapies targeting these new mechanosensitive genes are constantly being updated. Therefore, identifying and elucidating more genes sensitive to endothelial mechanics are necessary. Although Niu et al[55] have also proposed inhibitors or activators of certain endothelial mechanosensitive genes, in the past two years, with the deepening of related research, more targeted inhibitors or activators of corresponding genes have been identified (Fig. 3). Moreover, our mechanistic study should not be limited to the scope of proteins and coding RNAs, some non-coding and micro-RNAs also play important roles in AS.[136] For example, for some mechanosensitive transcription factors, targeted drug delivery regimens using small interfering RNA (siRNA) and micro-RNA have been developed as potential treatments for AS.[137,138] At present, the development of some targeted drugs is hindered by the time and dosage of administration. Based on the patient age, disease course, and other types of complications, individualized and precise evaluation is required, which is also an important aspect of

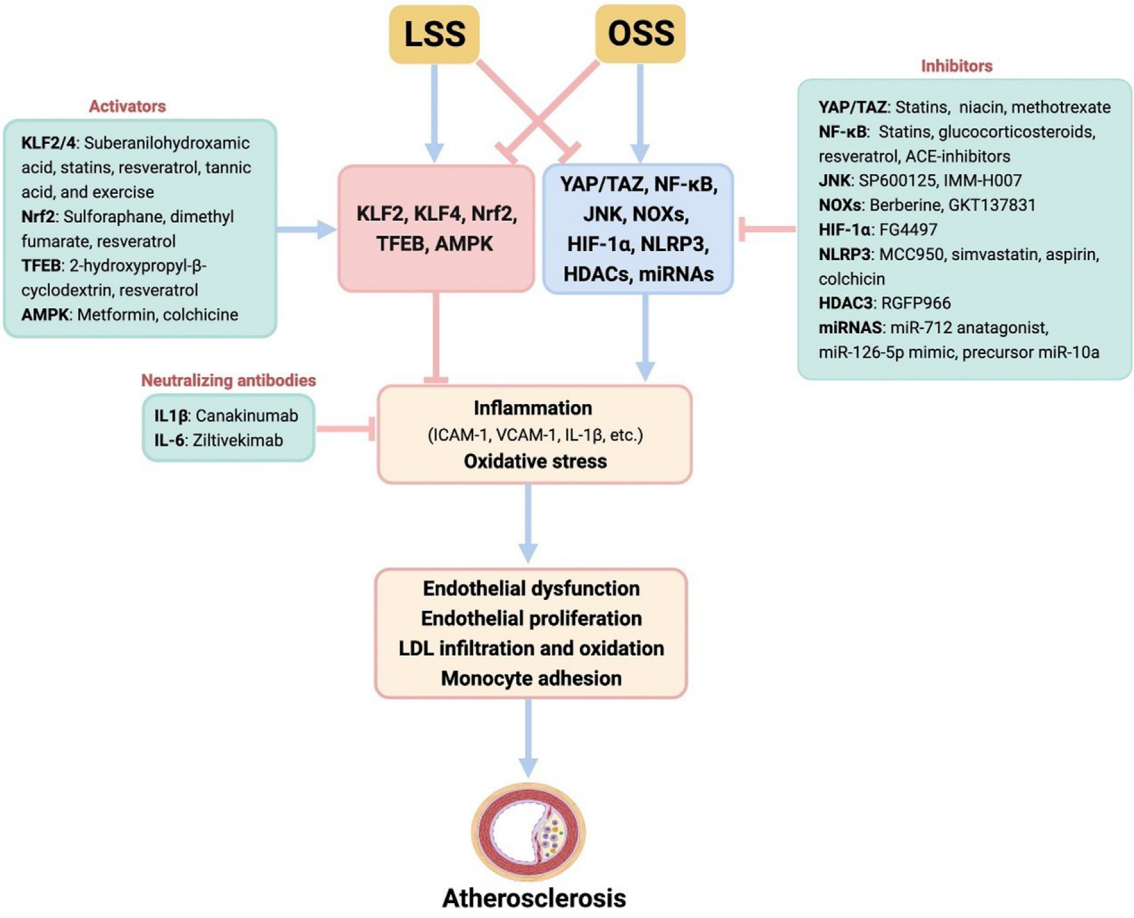

Figure 3 Role of shear stress signaling in the regulation of endothelial cell inflammation and oxidative stress. Inflammation and oxidative stress are the most important pathophysiological processes in the development of atherosclerosis. LSS increased, whereas OSS suppressed the expression of many anti-inflammatory and antioxidant transcription factors, such as *KLF2/4*, *NRF2*, and *TFEB*. Activating some of these genes has been shown to have clear beneficial effects on atherosclerosis. By contrast, OSS induced the expression of proinflammatory and prooxidative stress genes, such as *YAP/TAZ*, *HIF-1α*, and *NLRP3*. Antagonizing these deleterious signals may also alleviate atherosclerosis by improving endothelial function, reducing LDL infiltration and oxidation, and inhibiting endothelial–monocyte attachment. Cited from reference[148]. Copyright 2022 Elsevier B.V., with permission from Pharmacol Ther.

future research.[139] In addition, the research results based on database analysis can be used as targets for future research and treatment. For example, plasma lipoprotein(a) (Lp(a)) levels are primarily related to LPA single-nucleotide polymorphisms and related apolipoprotein subtypes.[140—142] The increased mRNA expression level of the LPA gene is a mechanism of cardiovascular diseases, and it is considered that the targeted reduction of Lp(a) level is an important direction for the treatment of AS.[143] However, the regulatory mechanism of the LPA gene related to hemodynamics deserves further study.[144] Apart from directly acting on endothelial cells, the stimulation of blood flow on the migration of monocytes and smooth muscle cells must be further studied, including whether the mechanical responses of different cells affect one another, the order and extent of the effects, and the intermediate transmission medium.[145,146]

Further research on AS can be conducted in accordance with the following three aspects: (i) further clarify the regulatory mechanism of force regulators in atherosclerotic cardiovascular diseases, (ii) screen new mechano-regulated genes through new technologies and new analytical methods, and (iii) use known high-throughput drug screening of mechanosensitive genes to identify new drugs. The multidisciplinary research of cell physiology, biomedical engineering, pharmacology, and functional genomics must be promoted to find early and precision treatment for AS in the future.

Author contributions

G.W. and G.Z. designed the study. S.L. and Z.X. drafted the manuscript. Y.W., L.C., X.W., Y.Z., D.L., and G.Z. participated in data collection and manuscript revision and provided input to the discussion. G.W. provided supervision, funding supporting, writing the review, and editing. X.W. and Y.Z. made critical revisions to the manuscript, and G.W. approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflict of interests.

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