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A potential strategy for treating atherosclerosis: improving endothelial function via AMP-activated protein kinase

Feng Gao, Jiemei Chen & Haibo Zhu*

State Key Laboratory for Bioactive Substances and Functions of Natural Medicines, Beijing Key Laboratory of New Drug Mechanisms and Pharmacological Evaluation Study, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

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Endothelial dysfunction is caused by many factors, such as dyslipidemia, endoplasmic reticulum (ER) stress, and inflammation. It has been demonstrated that endothelial dysfunction is the initial process of atherosclerosis. AMP-activated protein kinase (AMPK) is an important metabolic switch that plays a crucial role in lipid metabolism and inflammation. However, recent evidence indicates that AMPK could be a target for atherosclerosis by improving endothelial function. For instance, activation of AMPK inhibits the production of reactive oxygen species induced by mitochondrial dysfunction, ER stress, and NADPH oxidase. Moreover, activation of AMPK inhibits the production of pro-inflammatory factors induced by dyslipidemia and hyperglycemia and restrains production of perivascular adipose tissue-released adipokines. AMPK activation prevents endothelial dysfunction by increasing the bioavailability of nitric oxide. Therefore, we focused on the primary risk factors involved in endothelial dysfunction, and summarize the features of AMPK in the protection of endothelial function, by providing signaling pathways thought to be important in the pathological progress of risk factors.

atherosclerosis, AMP-activated protein kinase, cardiovascular diseases, inflammation, autophagy

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INTRODUCTION

Atherosclerosis is a major cause of cardiovascular disease, which is the leading cause of morbidity and mortality worldwide (Glaudemans et al., 2010). Current reports consider atherosclerosis a disease of metabolic inflammation characterized by the accumulation of oxidized low-density lipoprotein (ox-LDL) and the formation of atherosclerotic plaques in blood vessels (Golia et al., 2014). It has been established that the pathogenesis of atherosclerosis is complex and involves many factors. For instance, endothelial dysfunction increases the release of cytokines, such as monocyte chemoattractant protein-1 (MCP-1) and induces macrophages to adhere to endothelium and infiltrate into the vascular wall, which increases the number of macrophages engulfing ox-LDL, eventually leading to atherosclerotic plaques. Endothelial dysfunction has emerged as a predominant process in the development of atherosclerosis (Tesfamariam and DeFelice, 2007). In addition, endothelial dysfunction-induced chronic inflammation increases endoplasmic reticulum (ER)-stress and production of reactive oxygen species (ROS), which aggravates atherosclerosis. Moreover, disorders of lipid metabolism induce maladaptive inflammation and impair clearance of apoptotic cells, leading to endothelial inflammation (Bories and Leitinger, 2017). Recent advances in the field of atherosclerosis emphasize that improving endothelial function could be a potential target to treat atherosclerosis.

*Corresponding author (email: zhuhaibo@imm.ac.cn)

ENDOTHELIAL DYSFUNCTION IS AN ORIGINATOR OF ATHEROSCLEROSIS

Morphological and functional studies of the early stages of atherosclerosis show that endothelial dysfunction, leukocyte adhesion, and the formation of foam macrophages represent the primary pathogenesis of atherosclerosis. In particular, endothelial dysfunction is believed to be the initial step in atherosclerosis (Sandoo et al., 2015). The endothelium of blood vessels is a monolayer of cells, encased by a basement membrane and a thin protein sheet (Arnaoutova et al., 2009). The role of endothelial cells is to maintain the balance of vascular rhythm and regulate vascular function and proliferation as well as migration of vascular smooth muscle by some bioactive substances, such as nitric oxide (NO), ROS, epoprostenol, and angiotensin II (Maier, 2012). Once endothelial cells are damaged, an imbalance of endothelium-derived substances occurs, which changes the vascular micro-environment and function, resulting in cardiovascular disease (Achari and Jain, 2017). Endothelial dysfunction is characterized by endothelial apoptosis and a reduction of endothelium-dependent vasodilation (Sena et al., 2013). The pathological process of endothelial dysfunction is complex and involves many risk factors, including dyslipidemia, hyperglycemia, ROS production, endothelial inflammation, endothelial nitric oxide synthase (eNOS) dysfunction and ER stress (Porcu et al., 2016). Therefore, improving endothelial function could be a potential strategy for treating atherosclerosis.

AMPK IS A POTENTIAL TARGET OF ATHEROSCLEROSIS

AMP-activated protein kinase (AMPK) is an important metabolic switch that responds to various physiological cues to balance ATP production (Mottillo et al., 2016). AMPK is activated as a heterotrimeric complex, which is composed of an α catalytic subunit containing a Ser/Thr kinase domain, a regulatory β subunit containing a carbohydrate binding domain, and a γ subunit containing cystathionine-β-synthase domains (Hardie et al., 2016). Activation of AMPK through co-activation of peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) transcription modulates mitochondrial biogenesis and then inhibits NADPH oxidase-induced generation of ROS and apoptosis of endothelial cells (Fullerton et al., 2013). In addition, AMPK upregulates the expression of ABCA1 and LXR through sirtuin 1 (SIRT1) to promote cholesterol efflux from macrophage-derived foam cells (Li et al., 2010). AMPK activation upregulates the activity of protein phosphatase 2A, resulting in dephosphorylation of Ser536 within p65; thus, downregulating the expression of lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) to inhibit ox-LDL uptake by macrophages (Chen et al., 2016). Activation of AMPK inhibits CCR2 expression to reduce western diet-induced accumulation of Ly6C hi monocytes in the bone marrow (Wang et al., 2017). However, one study demonstrated that an AMPK alpha1 deficiency attenuates atherosclerosis by inhibiting autophagy-mediated monocyte differentiation and macrophage survival (Wang et al., 2017). Moreover, activation of AMPK/mTOR-induced autophagy induces apoptosis of endothelial cells. Consequently, using AMPK as a target for atherosclerosis treatment remains elusive and requires further study. In this review, we focus on the primary risk factors involved in endothelial dysfunction and summarize the features of AMPK in the protection of endothelial function by providing signaling pathways thought to be important in the pathological progress of risk factors.

AMPK IMPROVES DYSLIPIDEMIA-INDUCED ENDOTHELIAL DYSFUNCTION

Dyslipidemia is a primary factor in endothelial dysfunction and is characterized by elevated LDL cholesterol (LDL-c), triglycerides, or low high-density lipoprotein cholesterol (Haznedaroglu et al., 2017). Some studies have indicated that ox-LDL increases NADPH oxidase activity and the accumulation of intracellular ROS through the LOX-1 receptor to impair endothelial function (Mitra et al., 2011). Dyslipidemia promotes formation of the Nlrp3 inflammasome complex to enhance production of interleukin-1β (IL-1β) and high mobility group box 1, thus impairing endothelial function (Wang et al., 2016). AMPK plays a critical role regulating dyslipidemia and improving endothelial function. For instance, AMPK modulates phosphorylation of acetyl-CoA carboxylase, which is a crucial carboxylase in fatty acid biosynthesis. Activation of AMPK reduces ox-LDL-mediated endothelial injury by negatively regulating the protein kinase C (PKC) signaling pathway that is thought to be important in activation of NADPH oxidase (Bozaquel-Morais et al., 2017). These studies suggest that AMPK improves endothelial function and prevents atherosclerosis by regulating dyslipidemia.

AMPK IMPROVES HYPERGLYCEMIA-INDUCED ENDOTHELIAL DYSFUNCTION

Diabetes-induced atherosclerosis often begins with endothelial dysfunction induced by hyperglycemia. Hyperglycemia can cause a series of pathophysiological changes. For example, hyperglycemia inhibits activation of AMPK, resulting in impaired autophagy and ROS production, which impede endothelial function. In addition, hyperglycemia in-

creases the expression of NADPH and PKC, leading to reduction of NO bioavailability (Tsai et al., 2011). Hyperglycemia-induced ROS production and Nlrp3 inflammasome formation exaggerate oxidative stress and vascular inflammation (Kiritoshi et al., 2003). AMPK is an energy sensor that regulates the uptake and catabolism of glucose by critical metabolic regulators, including TBC1D1, PFKFB2, and PFKFB3 (Grahame Hardie, 2014). In addition, pharmacological activation of AMPK prevents endothelial dysfunction in patients with diabetes by improving mitochondrial bioenergetics via the PI3K/Akt/Sirt1 pathway (An et al., 2016). Consistently, AMPK activators enhance the angiogenic function of endothelial progenitor cells in patients with diabetes and improve insulin sensitivity by increasing the expression of heat shock protein beta-1 (Eleftheriadis et al., 2017). AMPK improves palmitate-induced insulin resistance through the insulin receptor substrate-1 (IRS-1)/Akt/eNOS signaling pathway (Liu et al., 2015). Therefore, AMPK may be a promising target for hyperglycemia-induced endothelial dysfunction.

AMPK IMPROVES ROS-INDUCED ENDOTHELIAL DYSFUNCTION

ROS are reactive intermediates of molecular oxygen that play a crucial role in the progression of atherosclerosis. Normal production of ROS transduces intracellular signals and kills bacteria. However, excess production of ROS, such as superoxide (O^{2-}), peroxyl (RO^{2-}), hydroxyl (OH^{-}), and hydrogen peroxide (H_2O_2), leads to oxidative stress, inflammation, and apoptosis (Kim et al., 2016; Nordberg and Arnér, 2001). Notably, activation of AMPK improves endothelial function by reducing ROS production. For example, AMPK prevents lipopolysaccharide-induced apoptosis in human brain microvascular endothelial cells, by reducing NADPH oxidase-induced ROS production (Hu and Liu, 2016). Activation of AMPK enhances FOXO3 binding to the Trx promoter and formation of the transcription activator complex to upregulate Trx expression and reduce ROS production (Li et al., 2009). Moreover, AMPK reduces ROS production to prevent palmitate-induced apoptosis by enhancing the expression of uncoupling protein 2 (Kim et al., 2008). ER stress is a major source of ROS and is widely considered the result of an accumulation of unfolded proteins. Many studies indicate that ER stress generates endothelial dysfunction and apoptosis by inducing the production of ROS and activating TXNIP/NLRP3 inflammasomes (Villacorta et al., 2015). Notably, activation of AMPK reduces ER stress-induced ROS production to protect the endothelium. In addition, activation of AMPK prevents ER stress by inhibiting the expression of inflammatory factors, such as eukaryotic initiation factor 2α, JNK, and IRS-1 (Abais et al., 2015). Activation of AMPK also causes a reduction in ER stress-induced ROS by dynamin-related protein 1 phosphorylation-induced alteration of mitochondria morphology (Sano and Reed, 2013)

Mitochondria are the main source of ATP and generate energy via a multi-step process coupled with oxidative phosphorylation. This process involves production of superoxide anions and leakage of electrons, and mitochondria are considered the main source of ROS (Hu et al., 2017). Of note, AMPK plays a crucial role regulating function and fission of mitochondria. For example, activation of AMPK induces the expression of SIRT3 and phosphorylation of eNOS, which alleviate oxidative stress-induced endothelial dysfunction and apoptosis (Apostolova and Victor, 2015). In addition, activation of AMPK suppresses mitochondrial-induced ROS production by activating manganese superoxide dismutase (Karnewar et al., 2016). One study demonstrated that AMPK increases the expression of mitochondrial fusion proteins 1 and 2 and inner membrane fusion protein optic atrophy type, which enhance proliferation of mitochondria (Wang et al., 2011). Similarly, AMPK regulates mitochondrial fission through dynamin 1-like and phosphorylation of mitochondrial fission factor, which play a crucial role maintaining endothelial homeostasis. Although these studies suggest that activating AMPK reduces ROS production by regulating the mitochondrial electron transport chain, NADPH oxidase, and ER stress, the precise mechanism of how AMPK inhibits ROS production remains elusive.

AMPK IMPROVES INFLAMMATION-INDUCED ENDOTHELIAL DYSFUNCTION

Endothelial inflammation triggers an increase in cytokines, such as tumor necrosis factor-α (TNF-α) and IL-β, which promote monocyte adherence. In turn, monocyte adherence generates endothelial dysfunction and promotes monocyte infiltration, eventually exacerbating atherosclerosis (Barbour and Turner, 2014). The nuclear factor-κB (NF-κb) transcription family plays a crucial role in the inflammation process and is comprised of five family members, including NF-κB1 (p105), NF-κB2 (p100), RelA (p65), RelB, and c-Rel. NF-κb increases the release of pro-inflammatory factors, including TNF-α and IL-6, to aggravate atherosclerosis (Sprague and Khalil, 2009). Of note, AMPK improves atherosclerosis by inhibiting the release of inflammatory factors. For instance, activation of AMPK inhibits the expression and phosphorylation of NF-κB by regulating protein phosphatase 2A, SIRT1, and PGC-1α (Oeckinghaus and Ghosh, 2009). In addition, AMPK activators, such as AICAR, A769662, and metformin alleviate endothelial inflammation by inhibiting NF-κB expression (Salt and Palmer, 2012). Some studies also indicate that activation of

AMPK reduces kinase-4 phosphorylation to inhibit IL-1β-induced CXCL10 secretion, followed by downregulation of MKK4/JNK and IKK/IκB/NF-Kb signaling. Recent studies show that activation of AMPK directly phosphorylates Ser515 and Ser518 of the Src homology domain within JNK to reduce inflammation (Rutherford et al., 2016).

AMPK IMPROVES ENDOTHELIAL DYSFUNCTION BY STIMULATING PERIVASCULAR ADIPOSE TISSUE

Notably, one study revealed that perivascular adipose tissue (PVAT) is an active endocrine organ. Moreover, PVAT-released adipokines and cytokines directly target endothelial smooth muscle cells to affect atherosclerosis by modulating vasoactivity. In particular, morphological studies of PVAT have reported that two general classes of adipocytes exist in mammals, such as white adipose tissue (WAT) and brown adipose tissue (BAT). It is generally believed that white adipocytes absorb excess energy, which increases the release of MCP-1, leading to an increase in monocyte adhesion and vascular inflammation (Panee, 2012). Brown adipocytes have abundant mitochondria that burn fatty acids to produce heat in response to various stimuli and BAT simultaneously through Nox4-derived hydrogen peroxide to induce activation of cyclic GMP-dependent protein kinase G type-1α, resulting in a reduction in vascular contractility to restore vascular function (Friederich-Persson et al., 2016). Some studies indicate that activation of AMPK increases the expression of BAT markers, such as uncoupling protein 1, PPARA, and PRDM 16. For example, activation of AMPK modulates DNA demethylation of PRDM16 by regulating the concentration of α-ketoglutarate, thus promoting the browning of WAT (Yang et al., 2016). In conclusion, activation of AMPK promotes the browning of WAT, reduces the release of pro-inflammatory factors, and regulates vasoactivity to restore endothelial function.

AMPK PROTECTS ENDOTHELIUM BY ENHANCING eNOS

eNO plays an important role in signal transduction by activating soluble guanylate cyclase and cyclic guanosine monophosphate. In addition, eNO inhibits expression of xanthine oxidase, NADPH oxidase, and pro-inflammatory factors to resist atherosclerosis. It has been well established that eNOS converts *L*-arginine to *L*-citrulline and NO, which is the main source of eNO (Sukhovershin et al., 2016). Basic and clinical studies have indicated that a reduction of eNO is a hallmark of endothelial dysfunction (Choi et al., 2016). Consecutive studies have demonstrated that targeting eNOS to regulate endothelial function is a potential strategy to treat atherosclerosis. Of note, activation of AMPK phosphorylates ser-1177 within eNOS resulting in increased NO production. Simultaneously, an increase in communication between AMPK and the PI3K-Akt signaling pathway increases eNOS phosphorylation and production of NO (Chen et al., 2016). In addition, AMPK activators, such as AICAR, A769662, and metformin stimulate eNOS activity to regulate NO production. AMPK improves endothelial function by increasing NO concentration.

AMPK PROTECTS THE ENDOTHELIUM BY MODULATING AUTOPHAGY

Autophagy is a lysosomal-dependent self-digestive pathway that maintains basic viability of the cell in response to a nutrient deficiency (Ouyang et al., 2012). It has been established that autophagy eliminates toxic protein and damaged mitochondria to protect endothelial cells (Ding, 2015). Interestingly, AMPK suppresses activation of mTOR by the phosphorylation of TSC2 and Raptor in response to energy stress and then enhances phosphorylation of Ser 317 and Ser 777 within Ulk to heighten autophagy (Kim et al., 2011). AMPK activators enhance autophagy of endothelial cells and increase mitochondrial biogenesis to protect the endothelium. However, in response to hypoxic conditions, CLOCK-induced autophagy damages the endothelium, resulting in apoptosis (Tang et al., 2016). AMPK/mTOR signaling-induced autophagy is a primary factor for cell apoptosis induced by nitrosative stress (Tripathi et al., 2013). These studies suggest that activation of AMPK enhances autophagy to protect damaged endothelium, but the protection of endothelium by enhancing autophagy requires further study and discussion.

AMPK PROTECTS THE ENDOTHELIUM BY REDUCING VASCULAR SMOOTH MUSCLE CELL PROLIFERATION

Shear stress plays a crucial role modulating vascular smooth muscle cell (VSMC) proliferation. Recent studies indicate that shear stress inhibits VSMC proliferation by mediating TGF-β1, diminishing platelet-derived growth factor receptor beta and decreasing matrix metalloproteinase-2. Moreover, more recent research indicates that shear stress enhances AMPK phosphorylation and increases NO production, which prevents pathogenesis in the vascular system (Kim et al., 2017). VSMCs undergo a phenotypic conversion to retain their ability to proliferate and migrate. There is a critical need to elucidate the potential mechanisms of phenotypic conversion of VSMCs. It has been reported that shear stress

modulates phenotypic conversion of VSMCs via AMPK/mTOR/ULK1 signaling pathway-induced autophagy (Sun et al., 2018).

CONCLUSION

Although AMPK has been demonstrated to be a crucial cellular energy sensor and metabolic regulator, recent studies have reported that AMPK protects endothelial cells. AMPK is regarded as an important target for endothelial dysfunction and atherosclerosis. For instance, AMPK inhibits the production of ROS induced by mitochondrial dysfunction, ER stress, and NADPH oxidase. AMPK inhibits the production of pro-inflammatory factors induced by dyslipidemia, hyperglycemia, and PVAT-released adipokines. Moreover, AMPK increases NO bioavailability by stimulating the eNOS/NO-signaling pathway. In addition, AMPK activators, such as adiponectin, resveratrol, and metformin, have great potential for promoting endothelial dysfunction to resist atherosclerosis. However, side effects, such as lactic acidosis, renal toxicity and liver toxicity, restrict the application of AMPK activators in clinical practice. Therefore, a safer and better-tolerated AMPK activator targeting endothelial cells would have substantial value in clinical practice.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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