Shear Stress in Autophagy and Its Possible Mechanisms in the Process of Atherosclerosis

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Autophagy can eliminate harmful components and maintain cellular homeostasis in response to a series of extracellular insults in eukaryotes. More and more studies show that autophagy plays vital roles in the development of atherosclerosis. Atherosclerosis is a multifactorial disease and shear stress acts as a key role in its process. Understanding the role of shear stress in autophagy may offer insight into atherosclerosis therapies, especially emerging targeted therapy. In this article, we retrospect related studies to summarize the present comprehension of the association between autophagy and atherosclerosis onset and progression.

Keywords: autophagy, shear stress, atherosclerosis, vascular smooth muscle cells, endothelial cells, macrophage

Introduction

ATHEROSCLEROSIS IS A COMPLEX chronic disease of the vasculature initiated by atherosclerotic plaque formation in the arterial walls in response to lipoprotein accumulation that results in a persistent inflammatory response. In a state of chronic inflammation, endothelial cells and vascular smooth muscle cells (VSMCs) are locally activated, supravene adhesion and chemokine molecules, and provoke the enlisting of monocytes, which stick to the endothelium and migrate into the underlying inner membrane. Subsequently, monocyte-derived macrophages accumulate lipids that become trapped in newly formed lipid-laden foam cells. The resulting accumulation of cells, coupled with continuous inflammation, drives chronic recruitment and activation of leukocytes, thereby perpetuating plaque growth and encroachment on the vascular lumen (Lehoux and Jones, 2016).

Atherosclerosis occurs at the bend and branches of arterial, where the flow of blood is disrupted and complex. *In vivo* shear stress is broadly categorized into disturbed or laminar shear stress (LSS). Disturbed shear stress generates abnormal shear stress and turbulent flow, such as oscillating shear stress (OSS) and low SS. Both low SS and OSS promote atherosclerosis effects. Although OSS is characterized by 0 dyne/cm² (Li *et al.*, 2014a), low SS is characterized by <10–12 dyne/cm² that usually takes place in the inner areas of branches and stenosis (Malek *et al.*, 1999). LSS is characterized by 15–30 dyne/cm² at the physiological level, and high shear stress (HSS) is characterized by >30 dyne/cm², which promote protective effects to the endothelium. Shear stress acts on the endothelium and is induced by blood flow, essential for the process of atherosclerosis (Wenning *et al.*, 2014).

Autophagy (literally “self-eating”) is highly conserved in mammals and plays an important role in the intracellular protective process and is induced by various types of stress, such as cellular stress, reactive oxygen species (ROS), starvation, accumulation of protein aggregates, or damaged organelles (Ucar *et al.*, 2012). It can maintain the integrity of cells and cellular dynamic balance through clearance excess and damaged organelles and intracellular pathogens or long-lived proteins (Gibbings *et al.*, 2013; Lu *et al.*, 2015). According to the different transportation mechanisms of cell materials to lysosomes, autophagy is classified as follows: (1) microautophagy, the lysosome membrane-bound proteins are degraded in the lysosome; (2) macroautophagy, in which the source of the endoplasmic reticulum membrane surrounding the material to be degraded is merged with lysosomes to form an autophagosome, which degrades the cellular debris; and (3) autophagy, in which cytoplasmic proteins bind to molecular chaperones and are transferred to lysosomes and then digested by lysosomal enzymes. Macroautophagy is the main type of autophagy (Kovaleva *et al.*, 2012). Autophagy utilizes autophagosome, which is a unique double-membrane vesicle to devour and transfer organelles and cytosolic macromolecules into lysosomes, the contents are degraded by lysosomal acid hydrolases when autophagosomes fuse with lysosomes (Bharath *et al.*, 2014). However, it will cause cell death and self-digestion when excessive autophagy occurs (Jian *et al.*, 2011).

The molecular mechanisms underlying autophagy have been extensively researched from 1950 when the first discovery of chaperone-mediated autophagy was made. Many of autophagy genes (*Atg*) have been identified as essential regulators and/or direct participants in autophagic pathways (Levine and Yuan, 2005). In general, the process of

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TABLE 1. THE MOLECULAR MECHANISMS OF AUTOPHAGY

Target genes	Mechanisms
<i>Atg1-Atg11-Atg17-Atg20-Atg24-Atg29-Atg31/Atg13-8</i> <i>Atg6-Atg14-Vps34-Vps15</i>	This complex, which is mainly regulated by mTOR, is mainly involved in the initial stage of autophagy (Rubinsztein, 2010; Pattingre <i>et al.</i> , 2008). Vps34 can promote the expression of the autophagy-related proteins <i>Atg21</i> and <i>Atg24</i> , as well as binding to the membrane and formation of the former autophagy structure (Yang and Klionsky, 2009; He and Klionsky, 2009).
<i>Atg12-Atg5-Atg16/LC3-II-PE</i>	The complex, which also involves <i>Atg7-Atg10-Atg16-Atg8</i> (LC3)- <i>Atg4</i> , is mainly involved in the elongation phase of the autophagy membrane (Geng and Klionsky, 2008).
Lamp1/Lamp2/P62/SQSTM1	These molecules are involved in the maturation and degradation process of autophagy (Rosenfeldt and Yan, 2009).

Atg, autophagy genes.

autophagy formation can be divided into the induction stage, initial stage, elongation stage, and mature and degradation stage. *Atg* involved in different stages of autophagy and related mechanism are described in Table 1. Apart from autophagy-related genes, signal transduction pathways are also involved in the regulation of autophagy, for example, mTOR and the Beclin-1 complexes (BECN1) (Table 2).

Previous studies have shown that VSMCs, macrophages, and endothelial cells display characteristics of autophagy when exposed to proatherogenic factors. Moreover, autophagy of vascular cells was found in atherosclerotic lesions, indicating that autophagy is involved in regulating the formation and progression of atherosclerosis. Razani *et al.* revealed that in the atherosclerotic plaques of ApoE^{-/-} mice, the autophagic markers p62 and LC3/*Atg8* are expressed (Maiuri *et al.*, 2013). The increasing evidence about the role of shear stress in autophagy has also been endorsed. Now, we will retrospect the literature concentrating on shear stress in autophagy regulation in the process of atherosclerosis.

Shear Stress and Atherosclerosis Shear Stress in VSMCs

Much evidence shows that during the process of shear stress-induced atherosclerosis, the migration, apoptosis, phenotypic transformation, and proliferation of VSMCs are closely associated with vascular remodeling. However, the predominant mechanism remains to be elucidated.

Accumulated evidence suggests that the increased phosphorylation of protein kinase B (Akt) can cause the proliferation of VSMCs in response to OSS. Furthermore, the increased Akt phosphorylation due to shear stress may be mediated by a phosphatidylinositol 3-kinase (PI3K)-dependent mechanism (Haga *et al.*, 2003; Fitzgerald *et al.*, 2008). A similar study reported that LSS-induced exogenous insulin-like growth factor (IGF)-1 and IGF-1 secretion increased Akt phosphorylation, proliferation of VSMCs thus induced a synthetic phenotype of VSMCs (Wang *et al.*, 2014a), which lead to synthetic phenotype transformation and proliferation. There are studies that show the activation and production of transforming growth factor β 1 (TGF β 1) induced by shear stress, thus inhibiting the growth of VSMCs. In addition, LSS-induced proliferation of VSMCs is also mediated predominantly by TGF β 1. TGF- β exerts its functions by activating downstream signaling pathways, such as PI3K/Akt, and is considered as an important signaling pathway in the regulation of the VSMC phenotype, which is important in vascular biology (Zhu *et al.*, 2015). In the process of VSMC migration and proliferation, platelet-derived growth factor (PDGF) is a potent regulator. *In vivo* enhanced activation of PDGF receptor (PDGFR) in VSMCs by PDGF isoforms secreted by the endothelium is related to the ability that inhibits arterial wall thickening in response to HSS (Palumbo *et al.*, 2002). The binding of PDGF-BB to the PDGFR activates various downstream signaling molecules, including PI3K/Akt (Kang *et al.*, 2016). Furthermore, LSS can enhance the migration and apoptosis of VSMCs, and Rho-GDP dissociation inhibitor alpha (Rho-GDI α) plays a crucial role in this process. LSS induces downregulation of PI3K/Akt pathway and Rho-GDI α then mediates its effect

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TABLE 2. THE MEMBERS AND MECHANISMS OF THE mTOR AND BECN1 COMPLEXES

Target genes	Mechanisms
Beclin-1 (<i>Atg6</i>) complex	The BECN1 complex is composed of Bcl-2, BECN1, UVRAG, and Vps34. (1) Bcl-2, UVRAG, DAPK, and CDK participate in autophagy regulation. (2) Vps34 produced by phosphatidylinositol-3-phosphate (PI3P) promotes the binding of autophagy-related proteins to the membrane to promote autophagy (Backer, 2008).
mTOR complex	(1) The mTOR-mediated signal transduction pathway activates several downstream effectors, such as 4E-BP1 and S6K1 kinase (p70S6 kinase), as well as transcription and translation of genes to control autophagy (Scott <i>et al.</i> , 2004). (2) mTOR kinase directly acts on <i>Atg</i> proteins to regulate the formation of autophagosomes.

BECN1, Beclin-1 complexes; 4E-BP1, 4E binding protein 1.

FIG. 1. Shear stress and atherosclerosis. Shear stress can activate IGF-1, TGF β 1, and PDGF, while increased AKT phosphorylation may be mediated by PI3K. The serine/threonine kinase p70S6k is believed to play a critical role in the regulation of protein synthesis and activation of VSMC differentiation via the PI3K–Akt-dependent activation of the mTOR pathway. PECAM-1 transmits the mechanical signal via VE-cadherin to VEGFR2, which then activates intracellular signaling via PI3K. PI3K further phosphorylates Akt, which leads to eNOS phosphorylation and subsequent NO release. Shear stress-induced phosphorylation of GAB1 and the association with SHP2 are essential for PKA activation, which increases NO production. Shear stress can induce VCAM-1, ICAM-1, E-selectin, MCP-1, IL-8, TNF- α , interferon- γ , VEGF, IL-6, IL-8, and IL-15. KLF-2 can be induced by LSS and inhibit the MAPK pathway via inhibition of JNK and its downstream targets ATF2/c-Jun, and the expression of ICAM-1, VCAM-1, and E-selectin. The suppressive effects of KLF-2 are attributed to inactivation of the proinflammatory AP-1 family of transcription factors by inhibiting phosphorylation and nuclear localization of c-Jun and ATF2, thereby reducing NF- κ B-mediated transcription. LSS can increase MEF2 transcriptional activity via upregulation of the transcriptional activity of ERK5. MEK5 is activated by LSS and catalyzes the phosphorylation of ERK5, which results in activation of MEF2 and subsequent synthesis of KLF-2. ERK5 is a key factor that inhibits endothelial inflammation by ERK5 and TNF. TNF significantly increases NF- κ B activity and VCAM-1 and ICAM-1 expression via Rac-1 activation and ROS generation. LSS increases intracellular antioxidant levels as a result of Nrf2 activation, thereby preventing excess ROS/RNS production. ROS are crucial for NF- κ B signaling downstream of TNF and may also lead to the formation of NACHT, LRR, and PYD domain-containing protein 3 inflammasomes, which activate both IL-1b and IL-18. Nrf2 is activated by HSS. Nrf2 prevents onset of a proinflammatory state via negative regulation of the MAPK pathway. On one hand, Nrf2 suppresses the upstream activators p38 and MAPK kinases 3 and 6, while on the other, it enhances activity of MKP-1, a negative regulator of p38 and JNK, by altering its redox state and promoting the catalytically active, reduced form of MKP-1, which leads to suppressed expression of the adhesion molecule VCAM-1. HSS, high shear stress; LSS, laminar shear stress; ROS, reactive oxygen species; PI3K, phosphatidylinositol 3-kinase; IGF, insulin-like growth factor; TGF β 1, transforming growth factor β 1; PDGF, platelet-derived growth factor; MCP-1, monocyte chemoattractant protein-1; TNF, tumor necrosis factor; IL, interleukin; VEGFR2, vascular endothelial growth factor receptor 2; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intracellular adhesion molecule 1; JNK, Jun NH (2)-terminal kinase; NF- κ B, nuclear factor- κ B; KLF-2, Kruppel-like factor-2; MAPK, mitogen-activated protein kinase; ATF2, activated transcription factor 2; MEF2, myocyte enhancer factor 2; ERK5, extracellular signal-regulated kinase 5; MEK5, methyl ethyl ketone 5; RNS, reactive nitrogen species; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; PECAM-1, platelet endothelial cell adhesion molecule-1; PKA, protein kinase A.

on migration (Qi *et al.*, 2008). Further investigations revealed that shear stress may be a strong stimulus of mTOR regulation and that only transient activation of mTOR is necessary for initiation of downstream signaling, such as activation of p70S6k, which in turn activates cell growth. The synthesis of protein and activation of VSMC differentiation via the PI3K-Akt-mTOR signal pathway, and the serine/threonine kinase p70S6k is key regulator. In general, the abovementioned revealed that PI3K/Akt/mTOR/p70S6k signaling may be an important mediator of shear stress-induced migration, phenotypic transformation, apoptosis, and proliferation of VSMCs (Rice *et al.*, 2010). This finding is important to elucidate the related mechanism that shear stress regulates VSMC proliferation and should prove helpful to develop new strategies to prevent flow-initiated vascular disease formation (Fig. 1).

Shear Stress in Endothelial Cells

In the development of endothelial dysfunction and atherosclerosis, shear stress is thought to play an important role via the release of inflammatory factors in response to the force acting on the endothelium derived from blood flow (Cheng *et al.*, 2006). Much evidence has shown that low SS and/or OSS contribute to the recruitment of leukocytes in endothelial cells by inducing synthesis of the chemoattractant chemokines monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-8, and E-selectin, and the proinflammatory cytokines interferon- γ , tumor necrosis factor- α (TNF- α), IL-6, and vascular endothelial growth factor (VEGF), and adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule 1 (ICAM-1) (Chiu *et al.*, 2004; Fu *et al.*, 2011). LSS is atheroprotective through induced expression of inflammatory molecules (Fu *et al.*, 2012). LSS causes downregulation of the expression of endothelial cell proinflammatory genes, which is mediated by Jun NH (2)-terminal kinase (JNK) and nuclear factor- κ B (NF- κ B), thus leading to suppression of monocytic cell adhesion. It turned out that LSS inhibits inflammatory and atheroprotective functions in vascular biology (Chiu *et al.*, 2005; Qin *et al.*, 2015). Interestingly, HSS can induce anti-inflammatory response via enhanced expression of cell adhesion molecules, for example, IL-15, IL-8, and IL-6 in endothelial cells and then inhibit the atherogenic processes. These mediators facilitate the aggregation of circulating leukocytes in the endothelial membrane and promote diapedesis into the intima (Andreou *et al.*, 2015). These mechanisms are involved in the process of inflammation and affect the occurrence and development of atherosclerosis. In summary, shear stress can regulate the process of atherosclerosis by mediating the expression of inflammation-related factors, although the underlying mechanisms are not fully understood.

In the initiation and perpetuation of the inflammatory process of atherosclerosis, shear stress is the key regulator. Transcription factors that are closely related to inflammation, including nuclear factor erythroid 2-related factor 2 (Nrf2) and Kruppel-like factor-2 (KLF-2), have been well described in this process. Recent studies found that in human endothelial cells, LSS can increase the expression of CD39, which is atheroprotective. CD39 is regulated by the transcription

factors KLF-2 and Nrf2 (Fledderus *et al.*, 2008; Kanthi *et al.*, 2015). In endothelial cells, by inducing its transcription factor KLF-2, the expression of the atheroprotective protein connexin 37 is upregulated in response to HSS, which results in increased intercellular communication (Pfenniger *et al.*, 2012). A zinc finger DNA-binding transcription factor KLF-2 plays a critical role for the maintenance of vascular integrity and endothelial homeostasis. LSS was found to induce KLF-2 expression in endothelial cells. Moreover, KLF-2 can inhibit the mitogen-activated protein kinase (MAPK) pathway, as well as JNK and its downstream targets activated transcription factor 2 (ATF2)/c-Jun (Kanthi *et al.*, 2015), which results in inhibition of E-selectin, VCAM-1, and ICAM-1 expression and then reduces proinflammatory response. KLF-2 activates AP-1 family via inhibition of c-Jun and ATF2 phosphorylation and nuclear localization (SenBanerjee *et al.*, 2004). In addition, KLF-2 potentially inhibits the expression of thrombin-mediated induction of IL-8, MCP-1, and IL-6. LSS can induce the KLF-2 promoter, which includes a common binding site for myocyte enhancer factor 2 (MEF2) transcription factors. LSS can upregulate the transcriptional activity of extracellular signal-regulated kinase 5 (ERK5) and then increase the transcriptional activity of MEF2 (Nayak *et al.*, 2011). In endothelial cells, LSS can activate catalyzes the phosphorylation of ERK5 and an MAP kinase kinase methylethylketone 5 to activate transcription factors MEF2 which also lead to KLF-2 synthesis (Clark *et al.*, 2011). MEF2 is a family of transcription factors that comprised four isoforms, that is, MEF2A, -B, -C, and -D. MEF2C acts as an inhibitor that plays a key role in the process of endothelial cell inflammation. The upregulation of proinflammatory molecules and stimulation of leukocyte adhesion to endothelial cells and NF- κ B activation can be induced by the knockdown of MEF2C (Xu *et al.*, 2015). Meanwhile, MEF-2 regulates the p38-dependent pathway and then affects vascular inflammation (Suzuki *et al.*, 2004). ERK5 belongs to the MAPK family and plays a crucial role in cellular differentiation and growth. The ERK5 pathway is equipped with distinctive features: ERK5 plays a key role in cardiovascular development by restraining endothelial inflammation. Increasing evidence shows that in human endothelial cells, ERK5 can inhibit endothelial inflammation act as a key factor contributes to analyze the relationship between TNF inflammatory cascade and ERK5 suggest that Ras-related C3 botulinum toxin substrate 1 (Rac1) and NF- κ B activity induced by TNF, VCAM-1 and ICAM-1 via ROS generation (Wu *et al.*, 2013). In subsequent studies, Dai *et al.* (2007) have shown that HSS can preferentially activate an antioxidant (protective) transcription factor Nrf2. The cytoplasmic Nrf2 separated from suppressor kelch-like ECH-associated protein-1 when exposed to HSS, through the protein kinase C, PI3K-dependent pathway, and MAPK pathways, for example, p38 and JNK extracellular 1/2 (ERK1/2) can lead to Nrf2 nuclear translocation (Dai *et al.*, 2007). In endothelial cells, the MAPK pathway is a negative regulator of Nrf2 and can prevent from exhibiting a proinflammatory state. One side, MAPK kinases 3 and 6 (MKK3/6) were suppressed by Nrf2: on the other side, the MAPK phosphatase-1 (MKP-1), a negative regulator of p38, was enhanced (Zakkar *et al.*, 2009). This will result in the suppression of the VCAM-1 which participates in modulating the inflammatory process in atherosclerosis (Bryan *et al.*, 2014).

In the development of atherosclerosis, inflammation and oxidative stress are the key regulators. In the pathophysiology process of chronic inflammatory disease such as atherosclerosis, ROS are the key products of oxidative stress. The activation of Nrf2 can upregulate the level of intracellular antioxidant in response to LSS in the anti-atherogenic state, therefore inhibiting excessive production of reactive nitrogen species/ROS, necessary for proatherogenic (Takabe *et al.*, 2011). ROS are crucial for NF- κ B signaling downstream of TNF and may also result in the formation of PYD domain-containing protein 3 inflammasomes, NACHT and LRR, which activate both IL-1 and IL-18 (Shi *et al.*, 2015; Blaser *et al.*, 2016). Thus, ROS are the initiating factor of atherosclerosis-related inflammation and the main reason for promoting proinflammatory molecule expression in endothelial cells (Xia *et al.*, 2014).

Nitric oxide (NO) is thought to play regulatory roles at virtually every stage in the development of inflammation. The mechanical stimulation derived from shear stress can be transmit to intracellular signals through vascular endothelial cells (ECs), which eventually results in the production of NO through activation of the endothelial nitric oxide synthase (eNOS) (Li *et al.*, 2016). For example, the phosphorylation of eNOS and activation of PI3K increase NO production in response to LSS (Dixit *et al.*, 2005). When inflammation occurred in endothelial cells, LSS can induce the phosphorylation of platelet endothelial cell adhesion molecule-1 (PECAM-1), which lead to leukocyte transendothelial migration and adhesion (Han *et al.*, 2015). The junctional mechanosensory complex included VEGF receptor 2 (VEGFR2), PECAM-1, and VE-cadherin, regulated in endothelial cell when exposed to LSS (Conway *et al.*, 2013; Li *et al.*, 2016). PECAM-1 can activate intracellular signaling through PI3K by transmitting the mechanical signal from VE-cadherin to VEGFR2 (Tzima *et al.*, 2001). Akt is phosphorylated by PI3K and then results in eNOS phosphorylation and NO release (Kemeny *et al.*, 2013). In endothelial cells, protein tyrosine phosphatase, nonreceptor type 11 (SHP2), and the adaptor protein GRB2-associated binding protein 1 (GAB1) are necessary for the production of eNOS induced by LSS. The increase of NO production is associated with protein kinase A (PKA), which can be activated through the association with SHP2 and phosphorylation of GAB1 induced by shear stress (Dixit *et al.*, 2005). Guzik *et al.* found that NO plays multiple roles in inflammation. Inhibiting the expression of cytokine, chemokine synthesis, and adhesion molecule can reduce the concentrations of NO. The effects of NO are exerted via interactions with cell signaling systems, for example, cyclic adenosine monophosphate (cAMP), G-protein, cyclic guanosine monophosphate, Janus kinase/signal transducers and activators of transcription, and MAPK-dependent signal transduction pathways. Kimbrough *et al.* showed that JNK signaling is essential for the production of NO through cyclic AMP. In summary, shear stress regulates the inflammatory response through a variety of mechanisms and is essential for the development of atherosclerosis (Fig. 1).

Shear Stress and Macrophages

Macrophages are a significant component of atherosclerosis plaques. Moreover, the activation of adaptive immune

system and macrophages can activate matrix metalloproteinase (MMP) and collagen breakdown, closely related with rupture of vulnerable plaques.

Studies have shown that low SS and OSS are essential to plaque formation. In ApoE^{-/-} mice, low SS can damage vulnerable plaque phenotype, which promotes the formation of thin-cap fibroatheroma, OSS abduction stable lesions (Cheng *et al.*, 2006), and cause stable SMC-rich plaque formation and thin-cap fibroatheroma (Seneviratne *et al.*, 2015). Other factors stimulated by low SS and plaque macrophages include extracellular MMPs in the fibrous thin-cap of vulnerable fibroatheromas (den Dekker *et al.*, 2014). MCP-1 causes the enlargement or rupture of plaque through facilitating macrophage infiltration and exacerbation of inflammation when exposed to low SS and turbulent flow (Aoki *et al.*, 2016). In conclusion, the roles of macrophages in plaque vulnerability and related mechanism need to be further investigated, which contribute to prevent formation of thin cap fibroatheromas.

Autophagy and Atherosclerosis

VSMCs, macrophages, and endothelial cells display characteristics of autophagy when exposed to proatherogenic factors. Moreover, autophagy of vascular cells was found in atherosclerotic lesions, indicating that autophagy participates in the formation of atherosclerosis.

VSMC Autophagy in Atherosclerosis

Atherosclerosis is characterized by the accumulation of oxidized lipoproteins in large arteries. Oxidation of lipoproteins leads to the formation of dozens of new lipids, such as oxysterols, aldehydes, and oxidized fatty acids. These oxidized lipoproteins are thought to promote the progression of atherosclerosis. Oxysterols, such as 7 β -hydroxycholesterol and 7-ketocholesterol (7-KC), are major components of oxidized lipoproteins in human atherosclerotic plaques. Moreover, 7-KC promotes nicotinamide adenine dinucleotide phosphate oxidase 4 (Nox4)-mediated formation of hydrogen peroxidase (H₂O₂), which triggers autophagy through the inhibition of Atg4B activity (He *et al.*, 2013). An analogy report showed that apolipoprotein L6 (ApoL6), the only proapoptotic BH3 member of the B cell lymphoma-2 family, is highly expressed and partially colocalized with activated caspase 3 in regulated autophagy of activated VSMCs in atherosclerotic lesions. Both electron and fluorescence microscopy showed that ApoL6 blocked autophagy flux by decreasing the formation of autophagic vesicles/compartments, possibly through ApoL6-induced time-dependent degradation of BECN1/Atg6, an essential autophagy protein, or ApoL6-induced p62 accumulation in response to BECN1 degradation (Zhaorigetu *et al.*, 2011).

Further studies revealed that capsaicin can activate the transient receptor potential vanilloid subfamily 1 (TRPV1) and play a protective effect in cardiovascular diseases. Meanwhile, autophagy can be induced by TRPV1 and significantly increase cytosolic Ca²⁺ activity (Li *et al.*, 2014b). Reportedly, AMP-activated protein kinase (AMPK) is phosphorylated and activated by Ca²⁺. AMPK can phosphorylate the regulatory-associated protein mTOR and tuberous sclerosis complex 2 (TSC2) and then suppress mTOR activity; phosphorylation of ULK1 (the yeast Atg1

homolog) complex, ULK1 (S555); and phosphorylation of BECN1 to promote phosphoinositide 3-kinase (VPS34) kinase activity, thus positively regulating the process of autophagy. The production of PI3P induced by activation of VPS34 can accelerate the formation of autophagosomes

(Egan *et al.*, 2011). ULK1 can facilitate autophagy through phosphorylates Beclin-1 and strengthen the activity of VPS34 kinase. The phosphorylation of ULK1 (S757) induced by mTOR can activate autophagy, generally because it can restrain the interaction between AMPK and ULK1 and then

FIG. 2. Atherosclerosis and autophagy. 7-KC can increase Nox4-mediated H_2O_2 formation, which triggers autophagy through the inhibition of Atg4B activity. ApoL6 induces degradation of BECN1/Atg6. ApoL6 causes p62 accumulation reciprocal to BECN1 degradation. TRPV1 activation significantly increases cytosolic Ca^{2+} levels. AMPK is phosphorylated and activated by upstream kinases: the tumor suppressor LKB1 and Ca^{2+} . AMPK, which is a positive regulator of autophagy with three layers of regulation, suppresses mTOR activity through phosphorylation of TSC2 and regulatory-associated protein of mTOR; phosphorylation of ULK1 (S555); and promotion of VPS34 kinase activity through phosphorylation of BECN1. Activated VPS34 increases PI3P production. MTOR negatively regulates autophagy through direct phosphorylation of ULK1 (S757) to inactivate ULK1 activity. ULK1 directly phosphorylates BECN1 and enhances VPS34 kinase activity to promote autophagy. mTOR-mediated phosphorylation of ULK1 (S757) also prevents ULK1 interaction with AMPK and thus suppresses the AMPK-induced phosphorylation of ULK1 (S555) to activate ULK1 and autophagy. AMPK regulates autophagy of the ULK1 complex, the most upstream component of the core autophagy machinery that is composed of ULK1, Atg13, FIP200, and Atg101. Moreover, AMPK also regulates the function of the BECN1 complex by directly phosphorylating VPS34 and BECN1, which is essential for autophagosome formation by providing PI3P. RSV increases cAMP levels possibly through activation of ADCY or inhibition of PDE activity, thereby activating the cAMP signaling pathway via PKA and increasing AMPK and SIRT1 activity to ultimately induce autophagy. The activation of JNK may play an important role in autophagic induction by DHEA by phosphorylation of Bcl-2 and dissociation of BECN1 from the Bcl-2/BECN1 complex. ox-LDL blocks autophagic flux resulting in the aggregation of p62/SQSTM1. Then, p62/SQSTM1 regulates gene expression of MMP-9 via NF- κ B-dependent signaling. Moreover, the corresponding downstream effectors of mTOR, S6K1, and 4E-BP1 were also upregulated in ox-LDL-treated macrophages. Telmisartan was found to partially activate PPARc and the induction of autophagy via the PPARc-AMPK-mTOR pathway. Apelin-13 activates the class III PI3K/BECN1-mediated autophagic pathway. Atg, autophagy genes; BECN1, Beclin-1 complexes; cAMP, cyclic adenosine monophosphate; MMP, matrix metalloproteinase; 7-KC, 7-ketocholesterol; TRPV1, transient receptor potential vanilloid subfamily 1; AMPK, AMP-activated protein kinase; TSC2, tuberous sclerosis complex 2; ADCY, adenylate cyclase; ox-LDL, oxidized low-density lipoprotein; 4E-BP1, 4E binding protein 1.

lead to the phosphorylation of ULK1 (S555) inhibited through AMPK. The important upstream ingredients of the autophagy ULK1 complex consist of ULK1, Atg101, FIP200, and Atg13 and can be mediated by AMPK. Moreover, the autophagosome formation is dependent on the AMPK, due to direct phosphorylation of VPS34 and BECN1, and then regulates the activity of BECN1 complex (Kim *et al.*, 2013). Taken together, when cellular energy is depleted, AMPK can regulate mTOR, ULK1, and VPS34-BECN1 complex and then guarantee the activation of autophagy (Ding, 2015). In VSMCs, TRPV1 induced by capsaicin can activate autophagy and then decrease the accumulation of lipid through decreased uptake of lipid and promote cholesterol efflux. Importantly, emerging evidence has demonstrated that autophagy deficiency in VSMCs contributes to atherosclerosis onset and progression, thereby shedding new light on potential therapeutic targets for treatment of vascular disorders. Further understanding of how autophagy regulates the function of lesion cells will undoubtedly be helpful to determine whether the modulation of this mechanism would be a viable option for atherosclerosis therapy (Fig. 2).

Endothelial Cell Autophagy in Atherosclerosis

Autophagy plays a vital role in the maintenance of endothelial cell populations in response to a series of extracellular insults. Nevertheless, it remains uncertain whether the mechanisms underlying autophagy activation have any influence on the participation of endothelial cells in atherosclerotic plaque formation. In the process of autophagy, various downstream signaling pathways were triggered by the insults. Recent literatures show that resveratrol (RsV) can increase cAMP levels by activation of adenylate cyclase or inhibition of phosphodiesterase (PDE) activity, thus activating cAMP signaling via sirtuin (SIRT1) and activity PKA and increasing AMPK, and then ultimately inducing autophagy and attenuating endothelial inflammation (Chen *et al.*, 2013). Additionally, in endothelial cells a similar study reported that delphinidin-3-glucoside stimulating autophagy by activate the AMPK/SIRT1 signaling pathways, and then weaken the injury induced by oxidized low-density lipoprotein (ox-LDL) (Jin *et al.*, 2014). A further study revealed dehydroepiandrosterone (DHEA) with the effects of antiatherogenic (i.e., decreased autophagic flux and inhibition of autophagosome/lysosome fusion) in endothelial cells exposed to linoleic acid (LA). Moreover, DHEA can stimulate autophagy and restrain endothelial senescence induced by LA. The activation of JNK exerts a critical role in DHEA-induced autophagy, possibly although Bcl-2 phosphorylation and BECN1 separate from the BECN1/Bcl-2 complex (Jin *et al.*, 2014).

In addition, mTOR, a key regulator of autophagy, thus modulating the balance of mTOR signaling could be of great significance in the development of novel therapies against atherosclerosis. Cat L is a cysteine protease that was recently implicated in the instability of advanced atherosclerotic plaques. ox-LDL was found to upregulate Cat L protein levels and activation of endothelial cells in a concentration-dependent manner, as well as stimulate autophagy and apoptosis of endothelial cells, and increase the permeability of endothelial cell monolayers (Wei *et al.*, 2013). Also, ox-LDL inhibited mTOR and increased the protein levels of Atg13 and promoted its dephosphorylation, thereby inducing au-

tophagy of endothelial cells (Peng *et al.*, 2014). The activation of endothelial cells by ox-LDL subsequently increases endothelial permeability in the early stages of atherosclerosis. Recent work also indicated that phosphatidylcholine-specific phospholipase C negatively regulated autophagy of endothelial autophagy independent of mTOR in endothelial dysfunction and several inflammation processes (Wang *et al.*, 2013). This new knowledge gained from the above-cited studies confirms that autophagy plays important roles in endothelial cells and should advance current understandings of mechanisms of atherosclerosis and provide new targets for treatment of cardiovascular diseases (Fig. 2).

Macrophage Autophagy and Atherosclerosis

Evidence showing that atherosclerosis progression is accompanied with increasingly defective macrophage autophagy provides further enthusiasm regarding the possibility that enhancement of macrophage autophagy may be an effective way to diminish or even reverse atherosclerosis (Leng *et al.*, 2016). The following mentioned are mainly focus on the autophagy modulate cellular lipoprotein metabolism in macrophages.

The main source of foam cells is macrophages, and it is necessary for the cholesterol efflux in the process of atherosclerosis (Shao *et al.*, 2016). It has already been shown that macrophage autophagy exerts a protective role against the progression of atherosclerosis through cholesterol efflux. Current findings indicate that autophagy can degrade intracellular lipid droplets and contribute to cellular lipid metabolism, the process known as lipophagy (Sergin and Razani, 2014). Autophagy in the form of lipophagy contributes to macrophage cholesterol homeostasis through cooperation with lysosomes by presenting free cholesterol for apolipoprotein A-I-mediated cholesterol efflux (Leng *et al.*, 2016). The marked increase in p62/sequestosome 1 (SQSTM1) levels in plaque macrophages suggests a vast disruption to autophagy-mediated degradation. The disruption of autophagy in cultured macrophages either chemically (chloroquine) or genetically (*ATG5* deficiency) abrogates cholesterol efflux to apolipoprotein A-I (Sergin and Razani, 2014). However, the mechanisms underlying macrophage autophagy regulation of lipoprotein metabolism remain unclear. Throughout the studies in the past shows that ox-LDL induction autophagy of macrophages and subsequent attenuation of lipid accumulation. Nevertheless, autophagic flux was blocked by abundance of ox-LDL, led to the accumulation of p62/SQSTM1 that is related to NF- κ B, and then participated in the expression of MMP-9, which plays a crucial role in atherosclerotic plaque (Zhou *et al.*, 2016). Meanwhile, simvastatin can weaken the accumulation of lipid through strengthening macrophage autophagy induced by ox-LDL (Huang *et al.*, 2015).

Apart from the aforementioned mechanisms, other signaling pathways are involved in the cholesterol flux process. Macrophage autophagy plays a crucial role in plaque stabilization, owing to reduced inflammation in the middle and late phase of atherosclerosis (Maiuri *et al.*, 2013; Zhai *et al.*, 2014; Yuan *et al.*, 2016). In the present study, macrophage autophagy selectively restrains the signaling pathway of PI3K/Akt/mTOR and then a marked effect on atherosclerotic plaque inflammation (Zhai *et al.*, 2014). An important negative regulator of ataxia telangiectasia mutated (ATM)-

signaling WPP domain interacting protein 1 (WIP1), also critical for the accumulation of fat and the process of atherosclerosis. It can suppress macrophage conversion into foam cells when knocked down and may rely on selective activation of autophagy by mTOR in the regulation of cholesterol efflux in an ATM-dependent manner (Le Guezennec *et al.*, 2012). Moreover, the corresponding downstream effector transcription initiation factor 4E binding protein 1 (4E-BP1) and ribosomal protein S6 kinase beta-1 (S6K1) of mTOR were also upregulated in ox-LDL-treated macrophages. Besides, PPAR γ can be activated by telmisartan to a certain extent (Benson *et al.*, 2004; Schupp *et al.*, 2004) and the PPAR γ -AMPK-mTOR pathway was in-

involved in the process of inhibiting the accumulation of lipid (Matsumura *et al.*, 2011) and inducing autophagy by macrophages (Li *et al.*, 2015a). mTOR can enhance lipid-laden macrophage foam cell formation by inhibiting the ULK1-mediated autophagic pathway, thereby facilitating the pathological process of atherosclerosis. This finding suggests that the mTOR pathway is activated during macrophage foam cell formation, indicating a potential pro-atherosclerotic role of mTOR (Wang *et al.*, 2014b). In addition, cholesterol efflux can be induced by adipokine apelin-13 resulting in the activation of the classIIIPI3K/BECN1-regulated autophagy pathway in macrophages and then exerts antiatherosclerotic effects (Yao *et al.*, 2015).

FIG. 3. Shear stress and autophagy. OSS mediates LC3-II to LC3-I ratios to promote autophagosome biogenesis and p62/SQSTM1 accumulation. Shear-induced autophagy is mediated by BMPRs and BMPR-specific Smad1/5 in a pathway that is independent of BMPs. LSS-induced autophagy is regulated by p38 MAPK. The BMPR/Smad1/5/p38 MAPK cascade modulates expression of LC3B-II proteins. LSS increases phosphorylation of AMPK. Activated AMPK may inhibit mTOR. In addition, AMPK can be activated by LSS via a pathway that is independent of PECAM-1 and distinct from that resulting in AKT activation. Meanwhile, AKT-mediated phosphorylation of BECN1 enhances its interactions with 14-3-3 and vimentin intermediate filament proteins. Akt suppression of autophagy can also be mediated by mTOR activation, which inhibits the autophagy-initiating ULK1 kinase complex. LOX-1 plays an important role in LSS-induced autophagy via ROS production. ROS regulates autophagy via various signaling pathways, including ROS-FOXO3-LC3/BNIP3-autophagy, ROS-NRF2-P62-autophagy, ROS-HIF1-BNIP3/NIX-autophagy, ROS-TIGAR-autophagy, as well as regulation mainly by inhibition of Atg4 activity. NO impairs autophagy by inhibiting the activity of the S-nitrosylation substrates JNK1 and IKK- β . Inhibition of JNK1 by NO reduces Bcl-2 phosphorylation and increases Bcl-2/BECN1 interactions, thereby disrupting Vps34/BECN1 complex formation. Additionally, NO inhibits IKK- β and reduces AMPK phosphorylation, leading to mTORC1 activation. OSS, oscillating shear stress; BMPR, bone morphogenetic protein receptor; IKK- β , inhibits of nuclear factor kappa-B kinase subunit beta.

Therefore, activation of the macrophagic autophagy possibly provides the potential therapeutic strategy for atherosclerosis plaque stability (Fig. 2).

Shear Stress in Autophagy

Recent studies revealed that the complex network of externality factors regulated autophagy. The roles of shear stress in autophagy are essential for the process of atherosclerosis. Studies show (Komatsu *et al.*, 2007) that disturbed flow-mediated OSS regulated autophagy through the increase of autophagosome induced by LC3-I conversion to LC3-II ratios and the impaired autophagic flux induced by the accumulation of p62/SQSTM1. In the aortic arch, impaired autophagic flux is derived from the accumulated p62/SQSTM1 in circumstance of OSS (Komatsu *et al.*, 2007). Therefore, in the disturbed flow regions, the impaired autophagic flux is in favor of the initiation of endothelial dysfunction (Li *et al.*, 2015). However, the involvement of shear stress in autophagy remains to be elucidated.

A number of signal pathways involved in the process of atherosclerosis, such as shear stress regulation of autophagy, should be further investigated. It has been shown that bone morphogenetic protein receptors (BMPRs) induce the occurrence of autophagy (Chang *et al.*, 2008). Shear-induced autophagy is mediated by BMPRs and BMPR-specific Smad1/5 in a pathway that is independent of BMPRs. LSS-induced autophagy is regulated by p38 MAPK (Lien *et al.*, 2013). A recent study demonstrated that during the process of autophagy, the BMPR/Smad1/5/p38 MAPK signal pathways upregulated microtubule-associated protein 1 light chain 2 (LC3II) protein expression and downregulated p62/SQSTM1 expression (Eisenberg-Lerner *et al.*, 2009). Young *et al.* showed that AMPK pathways are essential for the process of LSS-induced autophagy in endothelial cells because LSS increased phosphorylation of AMPK and inhibits a key positive regulator of autophagy mTOR-dependent signaling pathways (Meley *et al.*, 2006; Kim *et al.*, 2011; Mao and Klionsky, 2011). In addition, AMPK can be activated by LSS via a pathway that is independent of PECAM-1 and distinct from that resulting in AKT activation (Dixit *et al.*, 2008). At the same time, the phosphorylation of BECN1 induced by AKT promoted the combined vimentin intermediate filament proteins with 14-3-3. Furthermore, when knocked down vimentin can restrain the transformation of Akt-driven and the increase of autophagy. Therefore, Akt-induced phosphorylation of BECN1 is critical for inhibition of autophagy through autophagy inhibitory BECN1/14-3-3/vimentin intermediate filament complex. The activation of mTOR, which autophagy-initiating ULK1 kinase complex also be able to regulated the Akt suppression of autophagy (Wang *et al.*, 2012).

Furthermore, there are studies which show that oxidative stress can be regulated by shear stress, for example, the production of ROS and NO. In endothelial cells, current evidence shows that LSS is a major determinant factor for LOX-1. Murase *et al.* indicated that LOX-1 is essential for the autophagy induced by LSS and LOX-1 is essential for autophagy through ROS (Murase *et al.*, 1998; Ding *et al.*, 2015). Li *et al.* revealed that ROS–NRF2–P62–autophagy, ROS–TIGAR–autophagy, ROS–HIF1–BNIP3/NIX–autophagy, ROS–FOXO3–LC3/BNIP3–autophagy signal pathways are essential for the autophagy induced by the abundance of

ROS (Li *et al.*, 2015c). Besides, TSC1, TSC2, and Rheb consist of the TSC signaling node which a Rheb GTPase-activating protein localized in peroxisome functions inhibit mTORC1 and then induce autophagy in response to ROS production (Zhang *et al.*, 2013).

Further study revealed that oxidative stress maintained the NO dynamic balance, and shear stress increased the production of ROS in endothelial cells (Bharath *et al.*, 2014). In recent years, found that autophagy related mediator such as NO, a potent cellular messenger, restrains the formation of autophagosome through various pathways. On the one hand, NO reduces phosphorylation of AMPK, leading to mTORC1 activation by TSC2 though IKK- β and inhibiting the activity of the S-nitrosylation substrates JNK1 and then ultimately impairs autophagy. On the other hand, NO promotes the interaction BECN1/Bcl-2 and reduces the phosphorylation of Bcl-2 though inhibition of JNK1, and then damaging the formation of Vps34/BECN1 complex, eventually affect autophagy (Shibamoto *et al.*, 1990). From above mentioned concluded that autophagy also plays an important roles in balance of oxidant and antioxidant ex-cepts for maintaining NO (Fig. 3) (Bharath *et al.*, 2014).

Issue and Perspectives

Flow shear stress anomalies are important factors that promote atherosclerosis, but underlying mechanisms have not yet been fully elucidated. Shear stress reportedly regulates the autophagy-related genes, for example, LC3, P62/SQSTM1, and then affects the development of atherosclerosis. However, lots of problems remain further illuminated. Hence, future investigations focus on the accurate intracellular signaling mechanisms, such as the contribution of defect autophagy to vascular disability. Markedly, the role of shear stress in autophagy has been promulgated by many studies in recent years and needs to be further studied; it will offer us a thorough comprehension of related roles of pathophysiology similarly with innovate strategies in atherosclerosis therapeutics. In specific phases of atherosclerosis, the cotargeting of the autophagic pathway may prove to be beneficial data, despite current studies not providing an obvious consequence for autophagy in clinical application. Thus, further exploration of the molecular mechanism of shear force regulation of vascular cell autophagy will provide new therapeutic targets and strategies for further understanding the mechanism of atherosclerosis.

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