Role of autophagy in advanced atherosclerosis (Review)

YU-NING ZHU[1*], WEN-JING FAN[1,2*], CHI ZHANG[1], FANG GUO[1], WEI LI[1], YU-FEI WANG[1], ZHI-SHENG JIANG[1] and SHUN-LIN QU[1]

[1]Institute of Cardiovascular Disease, Key Lab for Arteriosclerology of Hunan Province; [2]Emergency Department, The Second Affiliated Hospital, University of South China, Hengyang, Hunan 421001, P.R. China

Received February 28, 2016; Accepted February 15, 2017

DOI: 10.3892/mmr.2017.6403

Abstract. Atherosclerosis (AS) remains the leading cause for global cardiovascular disease morbidity and mortality, and a major cause of cardiopathy, myocardial infarction and peripheral vascular diseases. Macrophages serve a critical role in atherosclerotic plaque stabilization and rupture, and the selective removal of macrophages may be beneficial in improving plaque stability. Autophagy is a process of self-feeding, during which cytoplasmic proteins or organelles are packaged into vesicles and fused with the lysosome to form an autophagosome. The newly formed autophagosome can degrade internalized proteins, and this process may be used to serve the metabolic and self-renewal requirements of the cell. Autophagy serves an important role in maintaining cell homeostasis and promoting cell survival, and therefore an imbalance in autophagy is closely associated with multiple diseases.

Contents

1. Introduction

Autophagy is a process of cellular repair and survival, during which cytoplasmic components are sequestered into double-membrane vesicles. Previous research has revealed that autophagy is stimulated in advanced atherosclerotic plaques by oxidized lipids, inflammation and metabolic stress (1). Autophagy is beneficial in promoting cellular recovery in adverse environments; it is also valuable in inhibiting apoptosis. In advanced atherosclerosis (AS), macrophage apoptosis, together with defective phagocytic clearance of the apoptotic cells, promotes plaque necrosis, which results in acute atherothrombotic cardiovascular events (2). Basal autophagy is beneficial in early AS, however a detrimental effect is observed in advanced AS plaques (3); autophagy is capable of protecting cells from oxidative stress via the degradation of damaged intracellular material; however, excessively stimulated autophagic activity may result in significant destruction of the cytosol (1). The latter scenario leads to programmed cell death and may cause vascular endothelial cell (VEC) death, which results in plaque destabilization. Endothelial injury or death represents a predominant mechanism of acute clinical events, due to the promotion of lesional thromboses (4). Furthermore, insufficient autophagic activity also reduces free cholesterol and apolipoprotein A-I (5), and this decreases the amount of high-density lipoprotein. Insufficient and excessive autophagy of VECs are therefore both injurious, and the regulation of autophagic homeostasis may be important in the treatment of AS.

2. The molecular mechanism of autophagy

When Ashford and Porter (6) perfused rat livers with glucagon, they discovered a large increase in the number of cellular lysosomes. This phenomenon of self-feeding was referred to as autophagy. This process involves the packaging of cytoplasmic proteins or organelles into vesicles, followed by lysosomal fusion, to form an autophagic lysosome; a cellular event which aids in metabolism and in the renewal of organelles (7). Autophagy is a primary catabolic survival process against various types of stress. During macroautophagy, several substrates, including lipids, pathogens, invading proteins, or even damaged organelles may be sequestered into double-membrane vesicles known as autophagosomes. In order to degrade their cargo, autophagosomes must subsequently fuse with a lysosome, which contains various hydrolases that are capable of degrading the sequestered substrates (8). Autophagy responses are triggered by stress; the elimination of damaged organelles and subsequent release of energy substrates promotes cell survival (9). Under physiological

Correspondence to: Dr Shun-Lin Qu, Institute of Cardiovascular Disease, Key Lab for Arteriosclerology of Hunan Province, University of South China, 28 West Changsheng Road, Hengyang, Hunan 421001, P.R. China
E-mail: qushunlin78@126.com

*Contributed equally

Key words: autophagy, atherosclerosis, molecular mechanism, vascular, treatment

conditions, autophagy is able to degrade dysfunctional organelles and long-lived proteins; however, autophagic cell death will occur if it is uncontrolled, due to the risk of excessive destruction of organelles and important molecules (10). There is a manner of organelle-selection during autophagy, with selective targeting of lipid droplets, protein aggregates, endoplasmic reticulum, peroxisomes, microorganisms, ribosomes and portions of the nucleus or mitochondria (11). The basic components obtained from cargo degradation are subsequently released into the cytoplasm for recycling. Currently, three primary types of autophagy have been described (12): i) Microautophagy, which involves direct engulfment of cytoplasmic material by lysosomes, via inward invaginations of the lysosomal membrane; ii) macroautophagy, characterized by formation of autophagosomes that fuse with lysosomes; and iii) chaperone-mediated autophagy, which is facilitated by a chaperone complex mediated by lysosomal-associated membrane protein type 2A (LAMP2A), to degrade cytosolic proteins with a specific targeting motif. Microautophagy and macroautophagy may be selective or nonselective, and are observed in yeast and higher eukaryotes; however, chaperone-mediated autophagy is a selective process that has only been described in mammalian cells (13). During chaperone-mediated autophagy, specific protein substrates containing the amino acid sequence KFERQ are recognized by chaperones (14), unfolded and translocated into the lysosome, via LAMP2A. In microautophagy, uptake occurs at the limiting membrane of the lysosome/vacuole, however this process operates by directly sequestering the substrates via invagination of the lysosome/vacuole membrane. Macroautophagy is the most well-studied process out of the 3 types of autophagy (Fig. 1). Multiple autophagy-related genes (ATG) and proteins have been described across the various stages of autophagy (15). Macroautophagy may be dissected into various steps based on the proteins involved: Induction; nucleation of the autophagosome precursor; membrane expansion and maturation of the autophagosome; fusion with the lysosome/vacuole and recycling of the degraded cargo (16). Once autophagy is induced, assembly of the phagophore is initiated by membrane nucleation. In both yeast and mammals, the class III phosphatidylinositol 3-kinases catalyze nucleation of the phagophore by producing phosphatidylinositol 3-phosphate (PtdIns-3-P) and inducing the recruitment of PtdIns-3-P binding proteins. Although membrane nucleation has been established as a key step in the autophagic process, the origin of the membrane that gives rise to the phagophore, and subsequently the autophagosome, remains unclear. Elongation and expansion of the phagophore membrane are key steps in the autophagic process (17). The autophagy related protein Atg12-Atg5-Atg16 and Atg8 conjugation systems, which are inter-related ubiquitin-like conjugation pathways, regulate this stage in both yeast and mammals (18). Previous research discovered that functional cooperation occurs between the Beclin 1-binding proteins Atg14L and Rubicon, and this regulates autophagy at different stages. Atg14L is essential for autophagosome formation, and deficiency of this protein results in defects of autophagic degradation in mouse embryonic stem cells. Furthermore, autophagosome maturation and endocytosis was enhanced and the number of autophagosomes/autolysosomes was increased following knockdown of Rubicon, indicating that Rubicon is negatively involved in the formation of autophagosomes (19).

Autophagy has been described widely in the cardiovascular system; autophagic activity is linked to cardiovascular development, heart and vascular homeostasis and the onset and progression of several cardiovascular diseases. Improved understanding of the molecular mechanism involved may therefore pave the way for novel therapeutic interventions in the treatment of cardiovascular disease.

3. Autophagy in the vascular system

Previous research has indicated that basal autophagy may have a significant impact on the functioning of the vasculature (20). This is induced by oxidative stress, which arises as a result of vascular disease and cell death initiation, particularly during the early stages of AS. Autophagy is a cytoprotective mechanism in the normal vessel wall, and there are several autophagy-signaling pathways involved in the cardiovascular system, which have already been extensively reviewed elsewhere (21), these pathways include the following: i) Mammalian target of rapamycin (mTOR), a highly conserved protein that can integrate several types of extracellular signals, including nutritional signals and growth factors, which are involved in gene transcription, protein translation, ribosome synthesis, and the regulation of cell apoptosis and autophagy. The mTOR protein accordingly serves an important role in cell growth; ii) adenosine monophosphate-activated protein kinase (AMPK) is a conserved heterotrimeric protein kinase, which can stimulate autophagy when adenosine monophosphate (AMP), the cell 'starvation' signal, is increased. AMPK maintains the balance of adenosine triphosphate generation and consumption in eukaryotic cells, via detection of the cellular energy state. Furthermore, AMPK serves a key role in regulating cell growth and proliferation, establishing and stabilizing cell polarity; iii) inositol 1,4,5-trisphosphate and inositol 1,4,5-trisphosphate receptor serve important roles in regulating autophagy; iv) transcription factor tumor promoter p53 has contrasting roles in regulating autophagy, and these are subcellular location-dependent; v) cyclic AMP (cAMP)-dependent protein kinase A (PKA) is composed of two catalytic subunits and two regulatory subunits, binding of cAMP to the regulatory subunit results in a conformational change and subsequent release of the catalytic subunit. Activation of the PKA catalytic subunits impacts on the expression of related genes, and allows detection of the nutritional and growth status of the cell; vi) histone acetyltransferases and histone deacetylases (HDACs) are proteins that regulate chromosome structure and gene expression. HDACs may be targeted by small molecular inhibitors, and this may inhibit pathological cardiac remodeling; vii) glycogen synthase kinase 3β (GSK3β) is a multifunctional serine/threonine kinase, which is commonly found in eukaryotic cells. GSK3β is involved in numerous cellular signaling pathways, the predominant of which involves regulation of glycogen metabolism, cell differentiation, proliferation and gene expression; viii) nicotinamide adenine dinucleotide is a rate-limiting enzyme, which is involved in several physiological activities including cell metabolism, energy synthesis, DNA repair, suppression of apoptosis and stimulation of autophagic flux; ix) microRNAs are non-encoding RNAs that are expressed in eukaryotic cells, and serve a regulatory role in cell proliferation, differentiation and death (21).

Vascular endothelial cells (VECs). VECs are located at the interior surface of the vascular wall, and are an effective permeable barrier between circulating blood and tissues. VECs also participate in the regulation of cellular cholesterol, lipid homeostasis, signal transduction, immunity, inflammation and hemostasis. Dysfunction of the endothelium is a critical inducer for AS and other cardiovascular diseases (20). A recent study demonstrated that oxidized low-density lipoprotein (oxLDL) may induce autophagy in VECs (22), resulting in increased expression of microtubule associated protein 1 light chain 3 (LC3) and B cell lymphoma/leukemia-1 (23). Furthermore, serum starvation induces autophagy, however a reduction in serum VECs induces apoptosis. The role of autophagy in VECs has therefore received significant research attention. A previous study demonstrated that human plasminogen Kringle 5 and endostatin are inhibitors of angiogenesis; these molecules induce apoptosis and inhibit cell proliferation, and can induce apoptosis and promote autophagy in VECs (24).

Smooth muscle cells (SMCs). SMCs demonstrate programmed cell death features in the fibrous cap of advanced AS plaques (25). Features include the formation of myelin figures, the aggregation of ubiquitin inclusion bodies in the cytoplasm and significant vacuolization. Furthermore, myelin figures are formed of phospholipids and plasma membrane fragments arranged in concentric circles, and these reflect autophagic degradation of the membranous cell structure. These autophagic structures are not common in human atherosclerotic plaques; however, they may be observed in cholesterol fed rabbit atherosclerotic plaques (25).

Previous research has speculated that SMCs in the fibrous cap are surrounded by basement membrane, indicating these cells may undergo starvation-induced autophagy (26). However, *in vitro* studies have suggested that there may be alternative pathways of induced autophagy in the atherosclerotic plaque. For example, tumor necrosis factor-α induces the expression of LC3 via the c-jun amino terminal and protein kinase B pathway, and induces expression of Beclin1 via the c-jun amino terminal pathway, thereby leading to SMC death via autophagy (27). Furthermore, mild oxidative stress also may activate autophagy, thus promoting the removal of damaged organelles (28).

Macrophages. Macrophages demonstrate significant phagocytic capabilities, and the cytoplasmic vesicles contained within these cells may therefore result from autophagy, or from phagocytosis of foreign bodies. Autophagic vesicles may be detected using an antibody against a specific marker such as LC3. Cholesterol acyltransferase (ACAT) is a sterol ester enzyme that regulates the free cholesterol in cell membranes to prevent cell death. Phytosterol is a substrate of ACAT, and a recent study revealed that phytosterols may accumulate in macrophages in atherosclerotic plaques, which may induce autophagic death, and necrosis of the plaque (29). High levels of phytosterols were also observed in patients with sitosterol (30), and this may lead to the premature development of a severe coronary AS thrombosis.

There is an increasing body of research (30,31) investigating the biological role of autophagy in the vascular wall (15); it is suspected that autophagic imbalance is associated with many vascular diseases, such as pulmonary hypertension. Furthermore, there is recent evidence that autophagy acts on a series of vascular processes, ranging from angiogenesis to vascular wall calcification. Although the autophagic mechanisms are different in endothelial cells and SMCs, autophagosome formation is related to β-amyloid, which is stimulated by oxidized lipids (32). Notably, the vasoactive substances secreted by endothelial cells have important regulatory effects on autophagy (15).

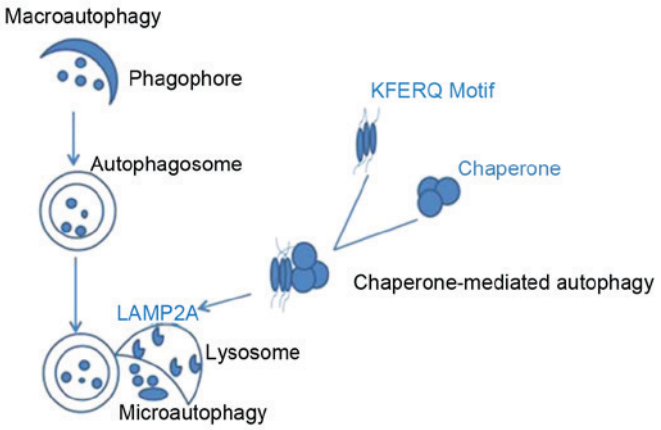

Figure 1. Macroautophagy, microautophagy and chaperone-mediated autophagy. LAMP2A, lysosomal associated membrane protein type 2A.

4. Autophagy in AS

Progressive stages of AS may be characterized by distinctive molecular events. In early AS, cholesterol accumulates to form foam cells, however early foam cell lesions are non-occlusive and this is therefore asymptomatic. In advanced AS, plaque rupture or erosion may cause acute clinical events. However, in early AS with endothelial dysfunction (33), the repair of VEC injuries may prevent the subsequent development of AS. Furthermore, in advanced AS, apoptotic macrophages may be quickly cleaned-up by macrophages, to prevent the progression of inflammation and plaque necrosis, thereby delaying plaque development (29). Autophagy serves an important role at each stage of AS; in early AS, autophagy may reduce lipid accumulation and inhibit the formation of foam cells. As AS progresses, autophagy can remove necrotic cells and delay development of the plaque (34). The specific autophagic mechanisms occurring at each stage are currently unclear, and further research investigating these processes is warranted. In a recent study (35), protein phosphatase magnesium dependent 1D (PPM1D) demonstrated a key role in the regulation of autophagy during the development of AS. The conversion of macrophages into foam cells is a major event in early AS, and PPM1D deficiency inhibits the accumulation of lipid droplets in macrophages, thus preventing the formation of foam cells and delaying the development of atherosclerotic plaques. In early AS stages, cholesterol predominantly accumulates in the cytoplasm as lipid droplets; when macrophage PPM1D is deficient, autophagy is activated and these cytosolic lipid droplets may be fused with autophagosomes and lysosomes, to produce a free cholesterol efflux (35).

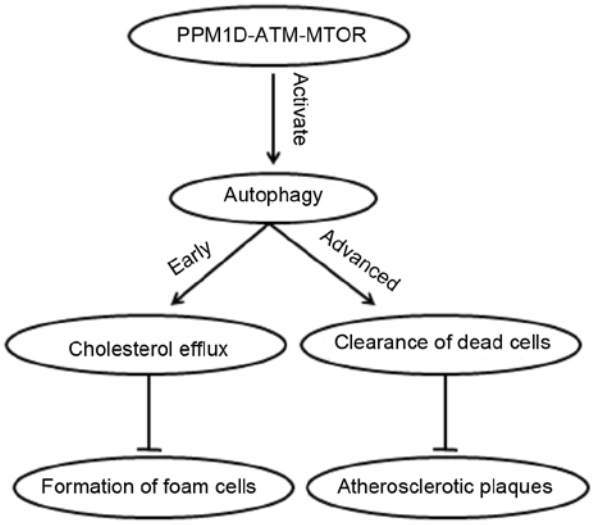

Figure 2. The role of PPM1D in early and advanced AS. PPM1D activates autophagy via the ATM-mTOR pathway, which induces cholesterol efflux and clearance of dead cells in early and late AS, respectively. PPM1D, protein phosphatase magnesium dependent 1D; ATM, ataxia telangiectasia mutated gene; mTOR, mammalian target of rapamycin.

Macrophage apoptosis and defects in the removal of apoptotic cells may promote plaque necrosis in advanced AS, resulting in acute atherothrombotic cardiovascular events (36). Autophagy is activated by the associated stimulation of AS. On the contrary, gathering numbers of apoptotic macrophages inhibits autophagic activity, and this promotes plaque necrosis. Furthermore, macrophage deficiency resulted in an associated reduction in cholesterol efflux, a process that served a significant protective role against the development of AS. Previous research has demonstrated that PPM1D (37), also known as wild-type p53-induced phosphatase (WIP1), functions in a regulatory role in AS development in mice. PPM1D knockdown decreases the lipid accumulation of macrophages and inhibits the formation of foam cells, thus inhibiting the progression of AS plaques. These events are regulated by the ataxia telangiectasia mutated gene (ATM)-mTOR signaling pathway, which controls the efflux of cholesterol, and has a protective effect on early and advanced AS (24) (Fig. 2). A study involving WIP1 deficient mice allowed further insight into the role of this protein in autophagy. In these mice, lipid efflux was increased and autophagic flux was impaired, thereby reducing the conversion of macrophages into foam cells, and preventing the formation of AS. WIP1 regulates autophagy via ATM and mTOR, which indicates that this protein may serve a protective role in advanced AS; inactivation of mTOR may therefore provide a novel therapeutic option in the treatment of AS (22). Other recent studies have investigated the effect of small molecular weight compounds and proteins on AS, including 3BDO, microtubule-associated protein 1 light chain 3 beta (MAP1LC3B) and lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1) (38). 3BDO activation of mTOR serves to protect endothelial cells and to inhibit the development of AS. The mTOR protein is an evolutionarily conserved serine/threonine kinase which has two forms, mTOR1 and mTOR2; mTOR1 predominantly regulates cell growth, cell apoptosis, energy metabolism and autophagy and is rapamycin-sensitive, whereas mTOR2 is associated with cell survival and is not rapamycin-sensitive. Both mTOR forms are able to induce autophagy. The 3BDO-induced inhibition of autophagy is mediated by oxLDL in endothelial cells, and decreased ATG expression and autophagy activity in apoE$^{-/-}$ mice. Conversely, 3BDO barely impacts upon mTOR signaling in macrophages and smooth muscle cells, and therefore 3BDO may selectively protect endothelial cells, promoting atherosclerotic plaque stability via the mTOR pathway (38). MAP1LC3B serves a critical role in limiting the development of AS. Overexpression of MAP1LC3B can stabilize plaque development in AS; the unfolded protein response, which occurs under conditions of stress, activates protective mechanisms by upregulating MAP1LC3B, and this accomplishes a protective effect via the activation of basal autophagy (39). OxLDL contributes to endothelial cell dysfunction, which occurs via the induction of oxidative stress and the subsequent initiation of apoptosis, and this results in AS development and progression (26). Overexpression of the oxLDL receptor 1 (LOX-1) appears to serve a role in attenuating endothelial cell protective autophagy. Previous research has demonstrated that early changes in endothelial cell viability, following incubation with oxLDL, are proceeded by impaired endothelial nitric oxide synthase activity, and subsequent oxidative stress (40). This effect is associated with the overexpression of the LOX-1 receptor, which mediates the attenuation of protective autophagy, and leads to oxLDL-induced bovine aortic endothelial cell death. These studies provided fresh insight into the assessment of the pathophysiological mechanisms underlying EC dysfunction, and allowed the identification of novel therapeutic strategies that target the early stages of AS.

5. Conclusions

Recently, Han *et al* reported that curcumin and resveratrol induced autophagy to protect VECs (41). Autophagy is an evolutionarily conserved mechanism that serves a critical role in the regulation of lipid metabolism in normal cells. Therefore, autophagy may provide new therapeutic targets for lipid metabolism disorders, including AS. Previous research has demonstrated that PPM1D may inhibit the formation of foam cells by the ATM/mTOR signaling pathway, via the regulation of autophagy-dependent cholesterol efflux (42). Autophagy-mediated inhibition of foam cell formation is induced by oxLDL, this results in stimulation of transient receptor potential vanilloid type 1 (TRPV1) by capsaicin, and the AMPK signaling pathway is activated by oxLDL to repair autophagic injury, with ultimate inhibition of the formation of foam cells. Consequently, the role of autophagy and TRPV1 in the formation of vascular smooth muscle cell foam cells, may provide a novel therapeutic target for the treatment of AS (43). Therefore, current research indicates that atherosclerotic development is associated with the dysfunction of autophagy, an event which results in vascular oxidative stress, inflammation and plaque necrosis; this knowledge may provide a novel mechanism through which the progress of AS may be inhibited (44). This disease may be treatable via an autophagic signal, and a few drugs that can regulate autophagy have been identified. Autophagy is not only protective, but it may also have an impaired impact on the cell; however, there is limited

clinical data to demonstrate its curative role, and currently the treatment of cardiovascular disease generally involves the downregulation of autophagy (45).

In conclusion, macrophage apoptosis combined with defective phagocytosis to clear the apoptotic cells promotes plaque necrosis, thus leading to acute atherothrombotic cardiovascular events in advanced AS. Inhibition of autophagy increases apoptosis in macrophages, and treatment of macrophages with AS stimulators of apoptosis resulted in the induction of autophagy (46). Defective macrophage autophagy results in oxidative stress, plaque necrosis, macrophage apoptosis and defective phagocytic clearance in AS (47). Previous research discovered that the inhibition of autophagy, *in vitro* or *in vivo*, resulted in increased apoptosis and reduced recognition of the apoptotic cells by phagocytes (12). However, although autophagy is beneficial in AS, excessive autophagy may promote plaque rupture and the precipitation of acute clinical events, suggesting that both insufficient and excessive autophagy of VECs is detrimental, and the regulation of autophagic homeostasis is critical in the treatment of AS. Currently these autophagic functions have not been investigated in the context of vascular inflammation, and as these studies progress more can be identified about whether autophagy is a suitable target for therapeutic intervention in AS.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant no. 81670424), the National Natural Science Foundation of Hunan Province (grant no. 2015JJ2118), the Key Scientific Research Fund of Hunan Provincial Education Department (grant no. 15A166), the Scientific Research Fund of the National Security Agency (grant no. hunan-0013-2016AQ), the Scientific Research Fund of Hunan Provincial Health and Family Planning Commission (grant no. B2016087), the Ph.D. Programs Foundation of Ministry of Education of China (grant nos. 20114324120004 and 20124324110003), the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Hunan Province (grant no. 2008-244), the Construct Program of the Key Discipline in Human Province (grant no. 2011-76) and the Postgraduate Innovation Project of Scientific Research of University of South China (grant no. 2015XCX38).

References

1. Liao X, Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS, Robbins J, Martinez J and Tabas I: Macrophage autophagy plays a protective role in advanced atherosclerosis. Cell Metab 15: 545-553, 2012.
2. Dong Z, Wang L, Xu J, Li Y, Zhang Y, Zhang S and Miao J: Promotion of autophagy and inhibition of apoptosis by low concentrations of cadmium in vascular endothelial cells. Toxicol In Vitro 23: 105-110, 2009.
3. Liu H, Cao Y, Tong T, Shi J, Zhang Y, Yang Y and Liu C: Autophagy in atherosclerosis: A phenomenon found in human carotid atherosclerotic plaques. Chin Med J (Engl) 128: 69-74, 2015.
4. Wang L, Li H, Zhang J, Lu W, Zhao J, Su L, Zhao B, Zhang Y, Zhang S and Miao J: Phosphatidylethanolamine binding protein 1 in vacular endothelial cell autophagy and atherosclerosis. J Physiol 591: 5005-5015, 2013.
5. Le Guezennec X, Brichkina A, Huang YF, Kostromina E, Han W and Bulavin DV: Wip1-dependent regulation of autophagy, obesity, and atherosclerosis. Cell Metab 16: 68-80, 2012.
6. Ashford TP and Porter KR: Cytoplasmic components in hepatic cell lysosomes. J Cell Biol 12: 198-202, 1962.
7. He C and Klionsky DJ: Regulation Mechanisms and Signaling Pathways of autophagy. Annu Rev Genet 43: 67-93, 2009.
8. Zhang YL, Cao YJ, Zhang X, Liu HH, Tong T, Xiao GD, Yang YP and Liu CF: The autophagy-lysosome pathway: A novel mechanism involved in the processing of oxidized LDL in human vascular endothelial cells. Biochem Biophys Res Commun 394: 377-382, 2010.
9. Schrijvers DM, De Meyer GR, Herman AG and Martinet W: Phagocytosis in atherosclerosis: Molecular mechanisms and implications for plaque progression and stability. Cardiovasc Res 73: 470-480, 2007.
10. Ren J and Taegtmeyer H: Too much or not enough of a good thing-the janus faces of autophagy in cardiac fuel and protein homeostasis. J Mol Cell Cardiol 84: 223-226, 2015.
11. Xie Y, You SJ, Zhang YL, Han Q, Cao YJ, Xu XS, Yang YP, Li J and Liu CF: Protective role of autophagy in AGE-induced early injury of human vascular endothelial cells. Mol Med Rep 4: 459-464, 2011.
12. Vindis C: Autophagy: An emerging therapeutic target in vascular diseases. Br J Pharmacol 172: 2167-2178, 2015.
13. Sato M, Seki T, Konno A, Hirai H, Kurauchi Y, Hisatsune A and Katsuki H: Fluorescent-based evaluation of chaperone-mediated autophagy and microautophagy activities in cultured cells. Genes Cells 21: 861-873, 2016.
14. Bonhoure A, Vallentin A, Martin M, Senff-Ribeiro A, Amson R, Telerman A and Vidal M: Acetylation of translationally controlled tumor protein promotes its degradation through chaperone-mediated autophagy. Eur J Cell Biol pii: S0171-9335, 2017 (Epub ahead of print).
15. Nussenzweig SC, Verma S and Finkel T: The role of autophagy in vascular biology. Circ Res 116: 480-488, 2015.
16. Mizushima N: Autophagy: Process and function. Genes Dev 21: 2861-2873, 2007.
17. Tang TX, Jo A, Deng J, Ellena JF, Lazar IM, Davis RM and Capelluto DG: Structural, thermodynamic, and phosphatidylinositol 3-phosphate binding properties of Phafin2. Protein Sci 2017 (Epub ahead of print).
18. Gatica D, Chiong M, Lavandero S and Klionsky DJ: Molecular mechanisms of autophagy in the cardiovascular system. Circ Res 116: 456-467, 2015.
19. Matsunaga K, Saitoh T, Tabata K, Omori H, Satoh T, Kurotori N, Maejima I, Shirahama-Noda K, Ichimura T, Isobe T, *et al*: Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. Nat Cell Biol 11: 385-396, 2009.
20. De Meyer GR, Grootaert MO, Michiels CF, Kurdi A, Schrijvers DM and Martinet W: Autophagy in vascular disease. Circ Res 116: 468-479, 2015.
21. Lavandero S, Troncoso R, Rothermel BA, Martinet W, Sadoshima J and Hill JA: Cardiovascular autophagy: Concepts, controversies, and perspectives. Autophagy 9: 1455-1466, 2013.
22. Perrotta I and Aquila S: The role of oxidative stress and autophagy in atherosclerosis. Oxid Med Cell Longev 2015: 130315, 2015.
23. Ding Z, Wang X, Khaidakov M, Liu S, Dai Y and Mehta JL: Degradation of heparan sulfate proteoglycans enhances oxidized-LDL-mediated autophagy and apoptosis in human endothelial cells. Biochem Biophys Res Commun 426: 106-111, 2012.
24. Ramakrishnan S, Nguyen TM, Subramanian IV and Kelekar A: Autophagy and angiogenesis inhibition. Autophagy 3: 512-515, 2007.
25. Kockx MM, De Meyer GR, Buyssens N, Knaapen MW, Bult H and Herman AG: Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. Circ Res 83: 378-387, 1998.
26. Salabei JK and Hill BG: Autophagic regulation of smooth muscle cell biology. Redox Biol 4: 97-1033, 2015.
27. Jia G, Cheng G, Gangahar DM and Agrawal DK: Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. Immunol Cell Biol 84: 448-454, 2006.
28. KIFFIN R, Bandyopadhyay U and Cuervo M: Oxidative stress and autophagy. Antioxidants Redox Signaling 8: 152-162, 2006.

29. Bao L, Li Y, Deng SX, Landry D and Tabas I: Sitosterol-containing lipoproteins trigger free sterol-induced caspase-independent death in ACAT-competent macrophages. J Biol Chem 281: 33635-33649, 2006.
30. Moghadasian MH, Alsaif M, Le K, Gangadaran S, Masisi K, Beta T and Shen GX: Combination effects of wild rice and phytosterols on prevention of atherosclerosis in LDL receptor knockout mice. J Nutr Biochem 33: 128-135, 2016.
31. Kostin S, Pool L, Elsässer A, Hein S, Drexler HC, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, Klövekorn WP and Schaper J: Myocytes die by multiple mechanisms in failing human hearts. Circ Res 92: 715-724, 2003.
32. Deng M, Huang L, Ning B, Wang N, Zhang Q, Zhu C and Fang Y: β-asarone improves learning and memory and reduces Acetyl Cholinesterase and Beta-amyloid 42 levels in APP/PS1 transgenic mice by regulating Beclin-1-dependent autophagy. Brain Res 1652: 188-194, 2016.
33. Lin HH: In Vitro and in Vivo Atheroprotective Effects of Gossypetin against Endothelial Cell Injury by Induction of Autophagy. Chem Res Toxicol 28: 202-215, 2015.
34. Martinet W and De Meyer GR: Autophagy in atherosclerosis. Curr Atheroscler Rep 10: 216-223, 2008.
35. Torii S, Yoshida T, Arakawa S, Honda S, Nakanishi A and Shimizu S: Identification of PPM1D as an essential Ulk1 phosphatase for genotoxic stress-induced autophagy. EMBO Rep 17: 1552-1564, 2016.
36. Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, Ting JP, Virgin HW, Kastan MB and Semenkovich CF: Autophagy links inflammasomes to atherosclerotic progression. Cell Metab 15: 534-544, 2012.
37. Brichkina A and Bulavin DV: WIP-ing out atherosclerosis with autophagy. Autophagy 8: 1545-1557, 2012.
38. Peng N, Meng N, Wang S, Zhao F, Zhao J, Su L, Zhang S, Zhang Y, Zhao B and Miao J: An activator of mTOR inhibits oxLDL-induced autophagy and apoptosis in vascular endothelial cells and restricts atherosclerosis in apolipoprotein $E^{-/-}$ mice. Sci Rep 4: 5519, 2014.
39. Swaminathan B, Goikuria H, Vega R, Rodríguez-Antigüedad A, López Medina A, Freijo Mdel M, Vandenbroeck K and Alloza I: Autophagic marker MAP1LC3B expression levels are associated with carotid atherosclerosis symptomatology. PLoS One 9: e115176, 2014.
40. Mollace V, Gliozzi M, Musolino V, Carresi C, Muscoli S, Mollace R, Tavernese A, Gratteri S, Palma E, Morabito C, *et al*: Oxidized LDL attenuates protective autophagy and induces apoptotic cell death of endothelial cells: Role of oxidative stress and LOX-1 receptor expression. Int J Cardiol 184: 152-158, 2015.
41. Han J, Pan XY, Xu Y, Xiao Y, An Y, Tie L, Pan Y and Li XJ: Curcumin induces autophagy to protect vascular endothelial cell survival from oxidative stress damage. Autophagy 8: 812-825, 2012.
42. Ouimet M: Autophagy in obesity and atherosclerosis: Interrelationships between cholesterol homeostasis, lipoprotein metabolism and autophagy in macrophages and other systems. Biochim Biophys Acta 1831: 1124-1133, 2013.
43. Li BH, Yin YW, Liu Y, Pi Y, Guo L, Cao XJ, Gao CY, Zhang LL and Li JC: TRPV1 activation impedes foam cell formation by inducing autophagy in oxLDL-treated vascular smooth muscle cells. Cell Death Dis 5: e1182, 2014.
44. Arora M and Kaul D: Coronary atherosclerosis: Significance of autophagic armour. World J Cardiol 4: 271-274, 2012.
45. Chew LH, Lu S, Liu X, Li FK, Yu AY, Klionsky DJ, Dong MQ and Yip CK: Molecular interactions of the Saccharomyces cerevisiae Atg1 complex provide insights into assembly and regulatory mechanisms. Autophagy 11: 891-905, 2015.
46. Martinet W, Verheye S and De Meyer GR: Everolimus-induced mTOR inhibition selectively depletes macrophages in atherosclerotic plaques by autophagy. Autophagy 3: 241-244, 2007.
47. Panda PK, Mukhopadhyay S, Das DN, Sinha N, Naik PP and Bhutia SK: Mechanism of autophagic regulation in carcinogenesis and cancer therapeutics. Semin Cell Dev Biol 39: 43-55, 2015.