Minireview *Highlight article*

Recent insights into atherosclerotic plaque cell autophagy

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Impact statement

Atherosclerosis is a chronic vascular lesion that is responsible for most cardiovascular ischemic events. Autophagy is an evolutionarily conserved subcellular mechanism that mediates protein breakdown and organelle damage through lysosomes, thus preserving cellular homeostasis. Importantly, cell autophagy affects plaque stability. In this review, we describe the function of autophagy in various cells present in atherosclerotic plaques, such as monocytes, macrophages, endothelial cells, dendritic cells, and vascular smooth muscle cells, and suggest areas for therapeutic intervention.

Abstract

Cardiovascular and cerebrovascular diseases, such as coronary heart disease and stroke, caused by atherosclerosis have become the "number one killer", seriously endangering human health in developing and developed countries. Atherosclerosis mainly occurs in large and medium-sized arteries and involves intimal thickening, accumulation of foam cells, and formation of atheromatous plaques. Autophagy is a cellular catabolic process that has evolved to defend cells from the turnover of intracellular molecules. Autophagy is thought to play an important role in the development of plaques. This review focuses on studies on autophagy in cells involved in the formation of atherosclerotic plaques, such as monocytes, macrophages, endothelial cells, dendritic cells, and vascular smooth muscle cells, indicating that autophagy plays an important role in plaque development. We mainly discuss the roles of autophagy in these cells in maintaining the stability of atherosclerotic plaques, providing a reference for the next steps to unravel the mechanisms of atherogenesis.

Keywords: Cell, autophagy, cell autophagy, atherosclerosis, atherosclerotic plaque, stability

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Introduction

Atherosclerosis is a chronic inflammatory condition of major and medium-sized arteries that leads to ischemic heart disease, stroke, and peripheral vascular disease, all of which are referred to as cardiovascular disease (CVD) in the medical community.[1] The fundamental pathological changes are the formation of plaques on the intima of an artery, with lipid streaks, fibrous plaques, and atheromatous plaques. Despite advances in understanding in recent years, CVD remains the major cause of mortality and disability globally.[2] Atherosclerotic (AS) plaques typically remain stable for years but rapidly become unstable, rupture, and initiate thrombosis. The progression of the illness results in the formation of AS plaques in arteries, which narrows the lumen of the vessels.[3] These life-threatening clinical outcomes are caused by vulnerable AS plaques.[4] Thus, atherosclerosis is an ancient disease in human history, and research has focused on it over time.

The function of autophagy in atherosclerosis has recently received greater attention.[5] To date, the forms of autophagy are as follows: (1) macroautophagy, (2) chaperone-mediated autophagy, and (3) microautophagy. Because macroautophagy is the most well-studied of the three forms, the term "autophagy" is sometimes interchanged with "macroautophagy".[6,7] Autophagy is a highly conserved process involving degradation of cytoplasmic organelles, proteins and macromolecules, and recycling of the breakdown products.[8] Autophagy has been shown to influence triglyceride and cholesterol breakdown, resulting in hyperlipidemia in atherosclerosis.[9] Thus, autophagy is necessary to regulate proper cardiovascular function. Moreover, in advanced AS plaques, inflammation, metabolic stress conditions, and oxidized lipids have been reported to stimulate autophagy.[10] Inflammation has been considered an essential feature of plaque vulnerability and AS thrombosis.[11] Basal autophagy

is an essential mechanism in cardiac homeostasis, allowing recycling and clearance of damaged proteins and organelles. This normal housekeeping function of autophagy is particularly important in cardiomyocytes because these cells are terminally differentiated and cannot decrease their cellular waste through replication.[12] Berberine has been shown to reduce the inflammatory response of aortic tissue and inhibit the formation of AS plaques in rats with damp heat syndrome.[13]

Further understanding of the relationship between autophagy and AS plaques will provide potential new strategies for preventing and treating heart disease. The function of autophagy in AS plaques will be discussed in this review, with an emphasis on the effect of autophagy on immune cells.

Atherosclerotic plaques

Stable and unstable atherosclerotic plaques

AS plaques have been reported to be markers of AS lesions. AS plaques consist of extracellular lipid particles, foam cells, and debris accumulated in the arterial wall intima with a lipid or necrotic core. The core is surrounded by a layer of collagen-rich matrix and smooth muscle cells (SMCs) and is covered by endothelial cells (ECs), known as the fibrous cap.[14]

AS plaques are kept stable by the development of a thick fibrous cap over a massive necrotic core of oxidized lipids and necrotic debris by vascular smooth muscle cells (VSMCs) and macrophages. Numerous VSMCs produce vast quantities of extracellular matrix (ECM), including collagen, elastin, and proteoglycans, to create the thick fibrous cap.[15] However, unstable plaques multiply via short lipid deposition; these plaques possess thin fibrous caps and edges and contain T helper 1 cells, macrophages, and a few natural killer cells, with a core consisting of lipids and necrotic material.[16] Unstable plaques are more common in uneven shear stress areas, such as around the carotid artery or coronary artery bifurcation.[17] Vulnerable AS plaques eventually become life-threatening.[4]

The main features of unstable plaques are as follows: (1) a large lipid core containing large quantities of low-density lipoproteins (LDLs) and foam cells, causing thrombosis; (2) large eccentric, irregular plaque lesions; and (3) a thin fibrous cap covering the surface of the lipid core (a vital role of the fibrous cap is to stabilize the plaque).

Factors affecting plaque stability

Plaque formation is a worldwide phenomenon that affects people of all races, ethnicities, genders, and geographical locations. However, *in vitro* studies have reported that many factors affect the stability of plaques. Patients with risk factors, such as smoking, diabetes, and genetic factors, develop plaques faster than those without these risk factors.[18] Moreover, elevated lipoprotein (a) levels may promote atherosclerosis through the incorporation of lipoprotein (a)-derived cholesterol in the intima, inflammatory cell recruitment, and proinflammatory oxidized phospholipids.[19] A thin fibrous cap covers high-risk plaques, which are infiltrated by inflammatory cells and exhibit widespread calcification. There is a lipid-rich necrotic core in the middle. Expansion of the vulnerable intima and development of vascular leakage are responsible for expanding the necrotic core and increasing plaque vulnerability, in addition to biomechanical, hemodynamic, and physical factors contributing to plaque instability.[20]

In particular, the presence of a high number of inflammatory cells in plaques is critical for plaque formation. Inflammatory tissue factor is the essential factor in plaque thrombosis.[19] The impact and duration of the first inflammatory activation are aided by AS plaques. ECs, activated macrophages, and SMCs all generate chemokines, which attract macrophages and neutrophils to AS plaques.[21] Autophagy has recently been linked to inflammation. Both impaired and inhibited autophagic flux may promote the oxLDL-induced inflammatory response in SMCs and ECs.[22] In molecular pathology, proteomics, and genomics, the mechanisms behind these AS risk factors are being studied in detail with the goal of identifying the exact molecular biological processes involved in the disease's progression.[18]

Autophagy is associated with atherosclerotic plaques

Autophagy is a continual process in which damaged organelle proteins and cellular material are destroyed within autophagosomes, which are double-membrane vesicles. These autophagosomes fuse with lysosomes to form autophagolysosomes, in which a series of degradations occurs to maintain the normal metabolism of the cell.[23] In recent years, a growing number of studies have provided evidence that autophagy occurs in atherosclerosis.[24]

Using transmission electron microscopy, we observed that autophagy occurs in primary cell types (i.e. macrophages, VSMCs, and ECs) in human AS plaques.[25] Western blotting is often utilized to identify microtubule-associated protein light chain 3 (LC3)-II, a commonly used autophagy marker, in unstable AS plaques. When compared to normal controls, the LC3-II protein level in AS plaques is higher.[5] Double immunofluorescence labeling has been used to detect the expression of microtubule-associated protein 1 light chain 3 (MAP1-LC3) in various cells in unstable AS plaques. MAP1-LC3 is a relatively specific autophagy marker. Similarly, MAP1-LC3 expression is present in both ECs and SMCs.[5] In macrophages, we found that many macrophages expressing MAP1-LC3 accumulate in the fibrous caps and shoulders of plaques.

These findings indicate that autophagy occurs in AS plaques. As a result, the potential impact of autophagy in these cells on maintaining AS plaque stability was investigated (Figure 1).

Monocyte autophagy increases the stability of atherosclerotic plaques

Monocytes are circulating white blood cells that are essential in both innate immunity and acquired immunity. Monocytes mainly play a role in immune defense, inflammation, and tissue remodeling.[26] In the 1970s, researchers found that monocytes participate in the formation of AS

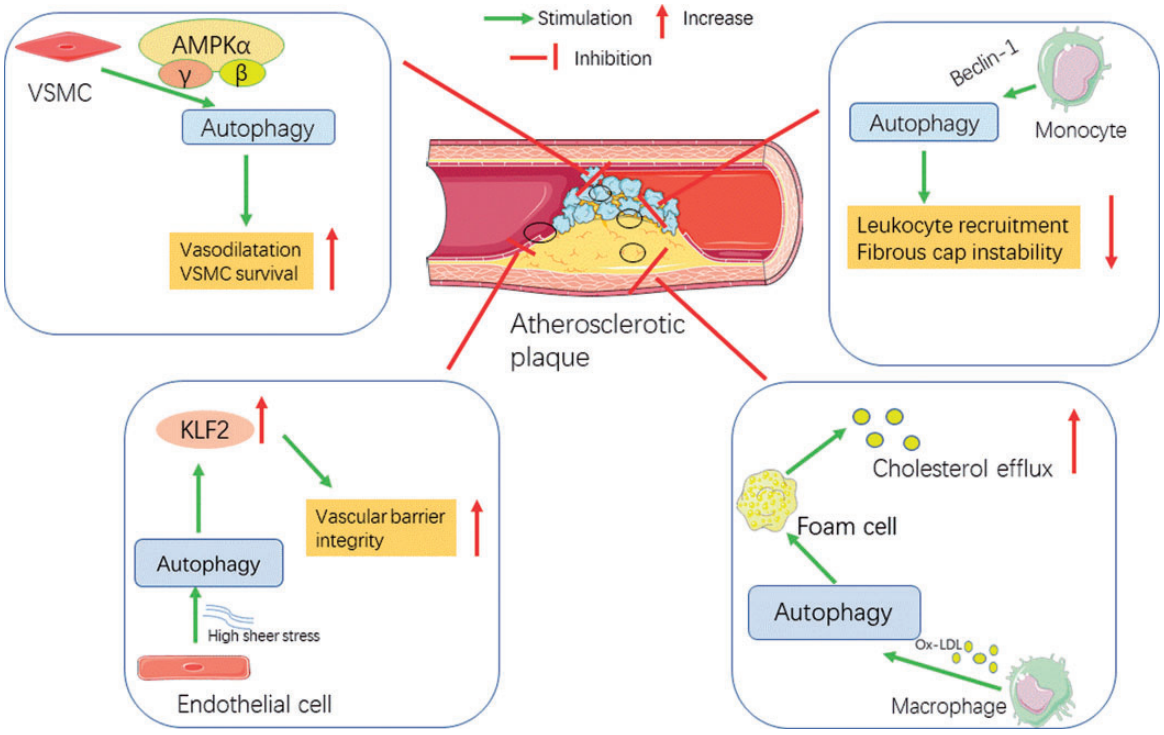

Figure 1. The effect of autophagy on monocytes, macrophages, vascular smooth muscle cells, and endothelial cells in the process of inhibiting the formation of atherosclerotic plaques. Monocytes undergo autophagy under Beclin-1 stimulation to reduce leukocyte aggregation and fibrous cap instability. Macrophages induce autophagy under the stimulation of LDLs, which enhances the outflow of cholesterol from foam cells. AMPK activation regulates the contraction and migration of VSMCs as well as the expansion of blood vessels through autophagy to exert an antiatherosclerotic effect. Autophagy of endothelial cells reduces the level of cholesterol in the blood, which enhances the expression of KLF2, thereby maintaining the integrity of the vascular barrier and preventing plaque rupture. (A color version of this figure is available in the online journal.)

plaques and accumulate in porcine AS lesions.[27] Monocytes are recruited to AS plaques throughout the different stages of lesion progression.[16] Monocytes promote atherosclerosis formation by promoting leukocyte recruitment to the plaque and by promoting instability of the fibrous cap, leading to plaque rupture.[28] BECLIN-1 is a highly conserved protein in eukaryotes encoded by the BECN1 gene, also known as autophagy-related gene (ATG) 6, and is essential to initiate the formation of autophagosomes during autophagy.[29]

BECLIN-1 expression in peripheral blood mononuclear cells of patients with acute myocardial infarction is substantially lower than that in individuals with unstable angina, according to experiments.[30] Autophagy in monocytes in the peripheral circulation plays a role in plaque susceptibility and rupture. Enhancing autophagy in macrophages in the peripheral circulation may be a novel therapeutic target for AS plaque stabilization.[30]

Macrophage autophagy increases the efflux of cholesterol from foam cells to increase the stability of atherosclerotic plaques

Macrophages are critical in unstable AS plaque rupture.[31] The differentiation of monocytes into macrophages may be triggered by physiological or atherogenic stimuli and is considered to be the most important step in atherosclerosis progression. Angiogenesis is the process by which monocytes attach to ECs and move to the underlying endothelial areas, where they develop into macrophages and macrophage-derived foam cells, resulting in AS plaques.[32] Atherogenesis occurs when foam cells produced from macrophages form a cluster and ultimately develop into a necrotic core.[33] The immunological response is mediated by the migration of monocyte-derived cells into the subendothelial region, where they develop into mononuclear phagocytes, which then change into cholesterol-laden "foam cells". In plaques, foam cells, which are usually categorized as macrophages, promote disease progression.[34]

Macrophage infiltration has widely been established to lead to AS plaque instability, while macrophage autophagy promotes stabilization of susceptible AS plaques.[35] Blocking autophagy made macrophages more vulnerable to death, made efferocytosis detection and clearance of dying cells worse, and increased plaque necrosis in a mouse model of advanced atherosclerosis.[36] Autophagy has been shown to increase cholesterol efflux from macrophages and reverse cholesterol transport (RCT), suggesting therapeutic potential.[37] Macrophage autophagy involves the induction of oxidative stresses or modifiable LDLs (e.g. oxLDLs), as a cellular safeguard, by eliminating damaged organs or deflated proteins, protecting apoptotic cells, maintaining macrophage epherozytesis, and increasing macrophage-derived cholesterol efflux in different AS stages.[38]

VSMC autophagy is a potentially vital mechanism to maintain the stability of advanced plaques

VSMCs are a significant cell type present during AS plaque stages. In healthy arteries, VSMCs reside in the medial

layer. VSMCs are involved in arterial contraction, ECM formation, artery compliance, and elastic recoil in response to changing hemodynamic circumstances.[39] The ultrastructural characteristics of autophagy, including vacuolation and the development of myelin patterns, have been observed using transmission electron microscopy of SMCs in the fibrous caps of advanced plaques. The autophagic breakdown of cell membrane components is represented by the later structure, which is made up of phospholipids and membrane fragments.[40]

Widespread expression of adenosine monophosphate-activated protein kinase (AMPK), a metabolic and redox status sensor, may be found in a variety of tissues and organs, including skeletal muscle, the liver, and blood vessels. One catalytic subunit (α-subunit) and two regulatory subunits (β- and γ-subunits) make up mammalian AMP.[41] AMPK activation has been found to control the contraction and migration of VSMCs as well as the expansion of blood vessels through autophagy to exert an antiatherosclerotic effect, ultimately maintaining the stability of plaques and delaying the progression of atherosclerosis.[42]

Diet-induced atherosclerosis is promoted by defective autophagy in VSMCs, which accelerates aging and increases the production of metalloproteinases and chemokines.[43] Moreover, defects in SMC autophagy are related to AS plaque hemorrhage visible to the naked eye. In addition, SMC autophagy defects promote atherosclerosis progression and enhance plaque rupture and thrombosis. Studies have shown that the death of SMCs caused by autophagy defects is related to plaque growth.[24,44] Autophagy has been demonstrated in a number of studies to be a protective mechanism that promotes cell survival rather than cell death, indicating that autophagy induced in SMCs in advanced plaques may be a potentially important strategy for preserving plaque stability.[5] Stimulating the autophagy response may be an essential part of the stress response of VSMCs and may also be an essential determinant of intimal proliferation or AS plaque stability.[45] Because of the critical function of VSMC autophagy in AS plaque development, medicines that directly target VSMC autophagy are urgently needed.[46]

Sufficient endothelial autophagy limits the formation of atherosclerotic plaques

In both homeostasis and disease, vascular ECs play a crucial function.[47] ECs with defective autophagy experience oxidative stress and reduced nitric oxide (NO) bioavailability.[48] The decrease in endothelial NO activity may be one of the potential spastic coronary intimal thickening mechanisms. Furthermore, coronary vasospasm may produce endothelial damage, which can lead to further coronary vasospasm. This vicious loop may accelerate the progression of intimal hyperplasia and atherosclerosis, demonstrating the importance of vasospasm in the development of atherosclerosis.[49]

Despite the fact that atherosclerosis is caused by systemic risk factors, AS plaques grow in specific locations where blood flow is interrupted and shear stress is low, such as the interior of arterial bifurcation bends. In areas that are generally resistant to atherosclerosis, the increase in plaque burden indicates that endothelial autophagy defects play a role in the damage of blood flow-dependent AS protective mechanisms. Our findings indicate that sufficient endothelial autophagy flux limits the formation of AS plaques by preventing EC apoptosis, aging, and inflammation.[43]

Endothelial caveolin-1 (Cav-1) expression plays a relevant role during atherogenesis by controlling NO production, vascular inflammation, LDL transcytosis, and ECM remodeling. Previous studies have shown that the absence of Cav-1 promotes autophagy activation in the aortic endothelium, which attenuates vascular inflammation and atherosclerosis initiation.[50]

The Krüppel-like family of transcription factors (KLFs) are important regulators of autophagy.[51] Studies have shown that KLF2 has an important effect on the dynamic balance of vascular ECs. In addition, autophagy in ECs reduces the cholesterol level in the blood, which enhances the expression of KLF2, thereby maintaining the integrity of the vascular barrier and preventing plaque rupture.[52]

The role of dendritic cell autophagy in atherosclerotic plaque stability remains controversial

Dendritic cells (DCs) are the body's most powerful antigen-presenting cells, with a unique ability to activate the immune system. DCs are derived from bone marrow progenitor cells and circulate in the bloodstream, penetrating into the surrounding tissues.[53] Various stimuli induce DCs to mature, and they experience two states of phenotypic maturity and functional maturity in morphology.[54] Vascular DCs are irregularly distributed in AS lesions.[55] Furthermore, new research has shown that DCs form a network in the normal intima, particularly in the region of blood vessels exposed to hemodynamic pressure, suggesting that DCs have a role in plaque development.[56] Advancement of AS plaques may result in the appearance of stained DCs. Regardless of whether the plaques are stable or fragile, DCs are mostly found in the plaque shoulder and on the border of the plaque core.[57]

To date, studies have detected autophagy in monocyte-derived DCs produced by bone marrow precursor cells in culture.[58] Autophagic destruction of DCs induces an anti-regulatory response to maintain immune homeostasis in $LDLR^{-/-}$ mice fed a high-fat diet, thus limiting the occurrence of atherosclerosis. Selective regulation of DC autophagy may become an interesting therapeutic target for atherosclerosis. Surprisingly, little research has been done on the function of autophagy in DCs during atherosclerosis progression and how it impacts disease progression.[59]

Conclusion and future perspectives

This review discusses the mechanisms of autophagy and AS plaque formation. Studies have shown that autophagy occurs in all cell types and has a role in the maintenance of cardiovascular homeostasis as well as in the development of CVDs. As a result, a thorough knowledge of autophagy in the cell types that are involved in the development of AS

plaques is beneficial in the diagnosis and treatment of this deadly disease.

Nevertheless, several questions remain unanswered. For example, the extent to which autophagic flux contributes to the stability of plaques without harmful consequences to cells remains unknown. In addition, whether autophagy affects other cell death pathways in plaques is unknown. Using animal or cell experiments to observe the stability of AS plaques after intervening in autophagy will undoubtedly help to answer these questions and may further clarify the potential role of autophagy in AS plaques. Therefore, further development of effective treatment methods is necessary.

A substantial amount of data indicate that the fundamental process of autophagy shields plaque cells from oxidative stress by digesting damaged intracellular components and improves cell survival. Overstimulation of autophagy in SMCs and/or ECs, in contrast to basic autophagy, may result in autophagic cell death, leading to reduced collagen synthesis, fibrous cap thinning, plaque instability, harmful thrombosis, and acute clinical events.[60,61] Therefore, controlling (moderating) the induction of autophagy, rather than overinducing or inhibiting autophagy, is a promising strategy for stabilizing AS plaques.[62]

However, there is still much to learn about autophagy and the mechanism by which it affects AS plaque stability. As a result, controlling autophagy may be a way to prevent or postpone atherosclerosis, and future preventive and/or treatment approaches should concentrate on these cellular processes. Based on these new insights, while treatments that stimulate autophagy are promising for atherosclerosis treatment, they are also of potential use in treating cardiovascular illnesses caused by other contributing variables (such as age).

AUTHORS' CONTRIBUTIONS

All authors contributed to the planning and writing of the manuscript.

DECLARATION OF CONFLICTING INTERESTS

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