Chapter 7

# Cerebrovascular Atherosclerosis: Cognitive Dysfunction Progress and Autophagic Regression

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Additional information is available at the end of the chapter

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**Abstract**

As the aging of society, metabolic disorders have become a major concern and a major cause for cardio- and neurovascular diseases such as atherosclerosis, stroke, and even cognitive decline. This chapter shows the progressive plaque formation mechanisms and regression under autophagic flow in both experimental and clinical side. Atherosclerotic plaque formation is not irrevocable. Clinical and experimental reports accept that atherosclerosis can regress after statin treatment. This chapter focuses on autophagic roles in atherosclerotic plaque formation, progression, and regression. Another focus is on the relationship between atherosclerosis and an increased risk of cognitive decline and further conversion from mild cognitive impairment (MCI) to dementia. There has been broad and strong support on the relationship between atherosclerotic severity and cognitive function. Ultrasound findings such as intima-media thickness (IMT) and plaque numbers could potentially be useful in identifying individuals with a higher risk of progression from cognitive decline according to morphological criteria. This also suggests the possibility as a predictive indicator of MCI and dementia by considering the presence of atherosclerotic changes. Focusing on therapeutics, this chapter provides mechanisms for regressing atherosclerotic plaques. Autophagy suggests therapeutic possibilities for atherosclerosis and it consequently paves the way for preventing cognitive impairment.

**Keywords:** atherosclerosis, autophagy, mild cognitive impairment, dementia, neurovascular

## 1. Pathogenesis of atherosclerosis

Cardio and cerebrovascular disease are leading causes of mortality worldwide. Mainly, they are caused by atherosclerosis, which is a chronic inflammatory disease of blood vessels. Cardiovascular events in the arterial wall result from the interactions between lipoproteins

and macrophages [1]. Accumulated lipoproteins bound to infiltrated macrophages to form a fibrous cap composed mostly of collagen in vascular smooth muscle cells, and this develops into an atherosclerotic plaque [2]. The developed plaque can cause stenosis, which can lead to ischemic conditions in surrounding tissues. When a plaque ruptures, in the worst-case scenario, thrombogenic materials are exposed, platelets are aggregated, and they form a thrombus. The detached thrombus becomes an embolus capable of blocking blood flow. Thus, atherothrombosis has the capability to cause ischemic stroke and myocardial infarction.

Lipoproteins transport cholesterol through the blood. Low-density lipoprotein (LDL) consists of esterified cholesterol and triglycerides that contain phospholipids, free cholesterol, and apolipoprotein B100 (ApoB100) [3]. Cellular LDL uptake is well-regulated. Their feedback mechanisms systemically limit excessive uptake and lipid overload in cells [4]. In contrast, oxidized LDL (oxLDL) mostly bypasses the feedback system, and results in intracellular lipid accumulation as foam cells present in atherosclerotic plaques [5]. Generally, the scavenger receptor-mediated uptake system misses the modified lipoproteins such as oxidized forms of LDL. During oxidation, physico-chemical properties such as lipid charge, size, and content are changed. Because oxidation modifies the LDL particles, oxLDL particles have already undergone physico-chemical changes. The oxLDL is technically different from natural LDL. The components of oxLDL can activate endothelial cells, and also induce the expression of adhesion molecules (E-selectin and VCAM-1) on the endothelial surface of the artery [2]. With endothelial activation by these oxidized lipids, oxLDL help macrophages to infiltrate into tissues and to produce chemokines as well as adhesion molecules [6].

The role of macrophages is critical in the development of atherosclerosis. Macrophages infiltrate to the arterial intima in response to oxLDL in the vessel. Macrophages engulf various lipids containing oxLDL and show a changed phenotype in comparison to lipid-laden foam cells. Spontaneously, they progress to a pro-inflammatory state. This is an early event in forming atherosclerotic lesion plaques. Macrophages secrete pro-inflammatory cytokines and recruit additional macrophages into the artery, and continuously increase atherosclerotic plaque size and complexity [7]. The early events of atherosclerosis induce additional immune cell infiltration and a progressive dysfunction to initiate a cell death pathway [5]. When atherosclerotic lesions develop, apoptotic as well as necrotic cell death occur. Cell debris and cholesterol form a necrotic core in the lesion covered by a fibrous cap of variable thickness [8]. Atherosclerosis forms under chronic exposure to cellular stressors, which promotes accumulated lipid degrading cascades and consequently dysfunction. It has been revealed that macrophage autophagy is linked to lipid metabolism [7]. In atherosclerotic plaques, there is intracellular accumulation of LDL as well as damaged tissue and misfolded/aggregated proteins. Biologically, these extra-accumulating materials are dealt via autophagy. Through the use of adapter proteins, the cells undergo autophagy. The process involves selective events rather than random bulk cleavage [9]. The selective autophagy can be described as: mitophagy, handling mitochondria; pexophagy, charging on peroxisomes; lipophagy, dealing with lipids; aggrephagy, taking care of aggregated proteins; and xenophagy, treating microorganisms. Among them, lipophagy is

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the initiating event of autophagy by mediating a cholesterol efflux [10]. It has slowly become clear that an atherosclerotic macrophage can induce and degrade cargo lipids by selective autophagy. In the case of the chaperone protein p62/SQSTM1 in chaperone-mediated autophagy (CMA), p62/SQSTM1 can hold and transfer poly-ubiquitinated cargo to autophagosomes for degradation. This machinery performs degrading events through the ubiquitin-binding domain (UBD) and the LC3-interacting region (LIR) [11]. The dysregulation of this autophagic pathway results from the markedly elevated p62/SQSTM1 protein levels in macrophages of the atherosclerotic plaque [12].

It is apparent that inflammatory factors and inflammatory reactions play critical roles in atherothrombotic disease. However, this chapter will focus on the autophagic pathway and dysregulated autophagy among contributors (members) to atherothrombosis.

## 2. Autophagic regulation and dysfunction in atherosclerosis

Autophagy literally means "to eat oneself" and originated in Greek. It is an evolutionary conserved mechanism, that is, a catabolic process to degrade cytoplasmic contents such as cellular proteins and organelles through lysosomes for recycling and use in downstream metabolism [13]. Biomolecules degrade and generate free fatty acids, amino acids, and nucleotides, which can be reused by the cell to maintain energy production and protein synthesis [13]. Degradation of intracellular molecules occurs through two distinct systems: the ubiquitin-proteasome system and the lysosome-autophagy system [14, 15]. In mammals, autophagy is the major pathway used to degrade abnormal products besides the ubiquitin-proteasome system. Autophagy is primarily used for the removal of damaged organelles, abnormal proteins, and protein aggregates [16], and this housekeeping function is particularly essential in the heart and brain. When autophagy-specific genes are lacking, a severe cardiomyopathy or neurodegeneration occurs [17].

There are several types of autophagy according to the method of delivery of the cargo to lysosomes: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [18]. Macroautophagy is the predominant mechanism among these three types. Macroautophagy starts with the formation of double-membrane vesicles, autophagosomes. Autophagosomes fuse with lysosomes and finally progress to autolysosomes. Physiological stress conditions such as starvation upregulates autophagy. Identified genes and molecules involved include around 30 genes and they are called autophagy-related genes (ATGs) required for autophagic pathways [19]. Among them, ATG5 and ATG12 are involved in the first step, controlling autophagy with two ubiquitylation-like reactions. ATG12 links to ATG5 requiring ATG7 (serves as an ubiquitin-activating enzyme, E1) and ATGT10 (serves as an ubiquitin-activating enzyme, E2). Then, the ATG5-ATG12 complex is involved in autophagosome formation. Autophagosomes randomly formed in the cytoplasm are trafficked along microtubules to lysosomes in a way that is dynein-dependent. Autophagosomes, then, are fused with lysosomes. The SNARE proteins of yeast are thought to be involved in this fusion [20, 21].

Microautophagy raises the possibility of direct cytoplasmic engulfment by the lysosome in mammals or the vacuole in plant and fungi [22]. In macroautophagy, a double- or multi-membrane-surrounded autophagosome forms, which fuse with lysosomes in a non-specific way for degradation [22]. In contrast to macroautophagy, in microautophagy, the lysosomal/vacuolar membrane is randomly engulfed and differentiates into the autophagic tube enclosing the cytosolic portion [23]. Microautophagy starts with making membrane knobs into the surface of the lysosome, and constructs small smooth areas that are able to degrade. The invaginations move laterally and also can shrink, which are specified into particular tubular shape "autophagic tubes" [23]. This characteristic is unique and gives them an autophagic function. After that, a dramatic decrease occurs along with the autophagic tube intramembranous proteins toward the top of the tube. Collectively, microautophagy performs degradation of cargo lipids and proteins in the following order: vesicle formation, vesicle expansion, vesicle scission, and eventual vesicle degradation and recycling [22].

Another case of autophagy is chaperone-mediated autophagy (CMA). Cargo recognition in macroautophagy has a non-selective process, because soluble cytosolic proteins cannot be selected as single protein molecules and are targeted for degradation through this pathway [24]. CMA targets only single proteins. In CMA, proteins are identified one by one, and the identified proteins are degraded by using a cytosolic chaperone system that delivers them to the surface of the lysosomes [25]. Selectivity in CMA uses a pentapeptide amino acid sequence motif in the substrate proteins. When the substrate proteins are recognized by a cytosolic chaperone, it results in targeting substrates to lysosomes [26]. CMA proceeds in sequential multi-steps: (i) recognition of substrate proteins; (ii) binding and unfolding of substrates; (iii) translocation of substrates inside the lysosomes; and (iv) degradation of substrates in the lysosomal lumen through its cellular functions [27].

CMA-targeting motifs are generated through posttranslational modifications with KFERQ on the targets. This pentapeptide was first reported to be critical in the degradation of RNase A [28], and it is shared by all identified substrate proteins to date [24]. The proteins carrying the KFERQ motif are targeted by a constitutive chaperone, the heat shock-cognate protein of 70 kDa (Hsc70). Hsc70 is the only chaperone to interact with the substrate via regulated ATP/ADP binding cycles [29]. The chaperone Hsc70 combines the proteins with a KFERQ motif, and binds with the cytosolic tail of the single-span membrane protein lysosome-associated membrane protein type 2A (LAMP-2A19, LAMP-2A), which shuttles the chaperone complex and the targeted protein into the lysosomal lumen [29]. Hsc70 also interacts with protein aggregates, and has mediated their degradation by macroautophagy, which is called a chaperone-assisted selective autophagy (CASA) [30]. This reaction works the same way on a responsible disassembly of clathrin from coated vesicles, and is needed to fold the unfolded cytosolic proteins upon recognition of exposed hydrophobic regions [28]. Once the substrate is translocated into the lysosomal lumen, LAMP-2A is rapidly dissembled from the complex into monomers, which endows LAMP-2A to bind with other substrates again [27]. LAMP-2A is one of the three splice variants of the *lamp2* gene [9], and is a single-span membrane protein. There is a very heavily glycosylated luminal region and a short (12-amino acid) C-terminus

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tail in LAMP-2A. When they are exposed on the surface of the lysosomes, substrate proteins bind to them [25].

### 2.1. Autophagy in VSMC of atherosclerotic plaques

The general architecture and cellular composition of blood vessels have basic components in the wall of blood vessels: endothelial cells (EC), vascular smooth muscle cells (VSMC), and extracellular matrix (ECM), including elastin, collagen, and glycosoaminoglycans. Each vessel is composed of the three concentric layers intima, media, and adventitia [31]. In normal mature blood vessels, VSMC predominantly exist in a contractile or differentiated phenotype that regulates blood flow and blood vessel diameter with vasodilation and vasoconstriction. The contractile VSMCs are surrounded by their own basement membrane, some macrophages, and fibroblasts. When damaged, VSMC generates intimal vascular lesions. The VSMC layers of the nearest vessel lumen receive oxygen as well as nutrients by direct diffusion from the vessel lumen.

VSMC in atherosclerosis consists of aberrantly proliferated VSMC to promote plaque formation. However, VSMC in advanced plaques is involved in preventing rupture of the fibrous cap [32]. The tensile strength of the protective cap relies on structural properties that are determined by the number of VSMCs and the collagen [33]. This is the reason why loss of VSMC leads to plaque destabilization and rupture. In advanced plaques, disintegrating VSMCs in the fibrous cap undergo programmed cell death, which is not apoptosis but autophagy. This has been shown in electron-microscopy imaging as formation of myelin figures [34], accumulation of ubiquitinated inclusions in the cytosol [13], and severe vacuolization [34]. In this stage, macrophages actively induce SMC death [13].

VSMCs loading a lot of cholesterol activate multiple pro-inflammatory genes and are altered to form macrophage-like cells driven by lipid accumulation in the plaque, and are then induced to perform phagocytic activity [32]. In the fibrous cap of advanced human plaques, VSMCs die showing ubiquitinated inclusions indicating they are undergoing autophagic death [13, 19]. Actually, the fibrous cap is a thick layer of basal lamina. It may be easier to let these "caged" cells undergo autophagy. Also, it has been suggested in *in vitro* studies that caged cells can trigger autophagy in atherosclerotic plaques. It has been reported in human plaques that lipid-laden VSMCs increase the expression of death-associated protein (DAP) kinase, a pro-apoptotic mediator, and regulate the formation of autophagic vesicles [35].

Generally, autophagy is well-recognized as a survival mechanism under starvation and not as a death pathway [36]. In atherosclerotic plaques, autophagy most likely plays a safeguarding role for plaque cells against oxidative stress, a hallmark of advanced atherosclerotic lesions. The successful autophagy in the atherosclerotic plaque is anti-apoptotic and eventually contributes to cellular recovery. However, it becomes another story under acute or persistent oxidative stress. In this case, over-produced intracellular ROS can be harmful to the lysosomal membrane. When autophagy does not work in the oxidative stress response in atherosclerotic plaques, or when oxidative injury overwhelms the cellular defenses, cells might undergo apoptosis.

### 2.2. Autophagy in macrophages of atherosclerotic plaques

Macrophages are immune cells having a strong phagocytic potential. They migrate into tissues derived from the differentiation of monocyte precursors in blood [7]. They are primarily involved in the phagocytosis against extracellular pathogens. They are also responsible for treating cellular debris, antigen presentation, and activation of the adaptive immune system. Macrophages secrete either pro- or anti-inflammatory cytokines according to their activation state [8]. Monocytes are recruited to the vessel intima, and they are initiated by chemokines secreted from endothelial cells, which are activated by excess lipoprotein accumulation [21]. These events show a profound effect on the reduction of atherosclerotic plaque burden through a lower number of circulating monocytes or to prevent their interactions with the endothelium via chemokine/chemokine receptor blockage [37].

In atherosclerotic plaques, macrophages contribute to cytokine production, the maintenance of vessel wall inflammation, and finally atherosclerotic progression [38]. Inflammatory signaling is a general and major event in atherosclerosis. Several other pathways, besides inflammation signals, are triggered by macrophages involved in wreaking havoc on the plaque [7]. They are over-expressed reactive oxygen intermediates containing myeloperoxidase-induced reactive nitrogen species. Under oxidative stress, secreted cathepsins and matrix metalloproteinases (MMPs) worsen the sub-endothelial environment. This toxic environment results in a vicious cycle of lipoprotein oxidation, enhanced lipoprotein uptake, and increased inflammatory signaling [7]. When the membrane cholesterol content of macrophages exceeds their handling capacity, a lipid droplet is formed. Cells with lipid droplets are defined as foam cells in the atherosclerotic lesion. A primary response to such lipid overload is the efflux of excess cholesterol out of macrophages with the help of high-density lipoproteins (HDLs). This process occurs in the cytoplasm where a family of cholesteryl ester hydrolases releases cholesterol from macrophage lipid droplets. This is followed by ATP-binding cassette transporter ABCA1 (ABCA-1), known as the cholesterol efflux regulatory protein (CERP). Finally, exogenously derived cholesteryl esters are hydrolyzed in lysosomes [7]. After that, the free cholesterols are distributed to different cellular membrane compartments. In addition to cholesterol, the focal lipid substrate and other lipid species may affect macrophage lysosomal function [7].

Under physiological states in contrast to pathological states, most cells turn on compensatory mechanisms for handling such insults. Autophagy is one of the responses to toxic intermediates found in the atherosclerotic plaque, and autophagic processes concomitantly increase in macrophages [14]. Lipophagy was first discovered in the liver where a specific mechanism handles lipids [39]. It has also been evaluated in foam cell macrophages. Autophagic uptake of lipid droplets is subsequently subjected to lysosomal acid lipase (LAL)-dependent degradation of cholesteryl esters in lysosomes. This is also an alternative mechanism of generating free cholesterol for ABCA1-mediated efflux to HDL [14]. It can be concluded that autophagy deficiency in macrophages increases macrophages' susceptibility to foam cell formation. It is undoubtedly true that macrophage autophagy has an essential role in the atherosclerotic process. In mice lacking *Atg*5, atherosclerotic plaques are enlarged and overloaded with lipids, there is extensive pro-inflammation, and the atherosclerotic core is filled with apoptotic and necrotic cells [7]. Recently, autophagy has been implicated in regulating cholesterol

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efflux, suppressing inflammasome activation, and improving apoptosis in atherosclerotic macrophages.

### 2.3. Autophagy in vascular endothelial cells

Endothelial cells are arranged in many layers in large blood vessels in which they form a tough wall as connective tissue. The endothelial cells in mature vessels send signals to the surrounding connective tissue, and take on an important part in regulating the vessel's function and structure [40]. For regulating roles, the endothelial cells mediate fluid filtration, hormone trafficking, neutrophil recruitment, and finally maintaining hemostasis [41]. Endothelial cell dysfunction (ECD) in the artery is the first detectable change of a forming atherosclerotic lesion [42]. The changes in the sub-endothelial area contain: the focal permeation and trapping, and the physiological and chemical modification of circulating lipoprotein particles [43].

The term endothelial dysfunction has already entered the lexicon of modern cardiovascular medicine [40]. However, the concept has not been developed to our present understanding of the cellular and molecular mechanisms of atherosclerosis. Under atherogenesis, the earlier characterization of endothelial dysfunction was focused on whether anatomical integrity of the intima was intact. The simplest definition of endothelial dysfunction is a lack of nitro oxide (NO), which is involved in various disease states: atherosclerosis, diabetes mellitus, coronary artery disease, hypertension, and hypercholesterolemia [41]. Endothelium-derived NO can modulate leukocyte adhesion. Endothelium-derived NO prevents leukocyte recruitment to the vascular wall via the anti-inflammatory effects of NO. Endothelium-derived NO suppresses the expression level of VCAM-1, ICAM-1, and E-selectin, which respond to pro-inflammatory cytokines. The cellular adhesion molecules mediate activation of the transcription factor NF-κB, and NF-κB inhibited by endothelial NO prevents endothelial cell activation [44]. It has been found that inhibition of basal eNOS activity rapidly induces VCAM-1 and also increases monocyte adhesion [45]. This linkage could induce or enhance endothelial cell activation. The mediators of endothelial dysfunction such as hypercholesterolemia or oxidative stress can lead to increasing vasoconstriction, smooth muscle proliferation, platelet aggregation, leukocyte adhesion, LDL oxidation, and MMP activation. In the vessel wall when there is turbulent flow, endothelial cell activation and atherosclerosis may occur more readily because there is less endothelium-derived NO. With support, atherosclerotic lesions develop more frequently at vascular branching sites when exposed to turbulent flow rather than laminar flow. In animal studies, eNOS deleted mice develop increased atherosclerosis and vascular inflammation [46].

In contrast to endothelial dysfunction, endothelial cell activation is defined by the endothelial cell surface adhesion molecules, such as VCAM-1, ICAM-1, and endothelial leukocyte adhesion molecule (E-selectin) [41]. Endothelial cell activation is typically induced by pro-inflammatory cytokines, such as TNF-$\alpha$ and IL-6. When endothelial cells are activated, they facilitate the recruitment and attachment of circulating leukocytes to the vessel wall. Progressive structural remodeling of developing lesions starts with the formation of a fibrous cap. The lateral edges of these complicated atherosclerotic plaques contain a rich population of inflammatory cells such as activated macrophages, T-cells, natural killer T-cells (NK T-cell), and dendritic

cells. These inflammatory cells further modulate the endothelial cells into a pro-inflammatory phenotype, and have the endothelial cells work on structural instability of the plaque by modifying the proteolytic activity of extracellular matrix components [47].

Because of the above-mentioned characteristics, exogenous NO has been implicated as a therapeutic target. NO has benefits in vascular inflammatory diseases, and some researchers have tried to ameliorate atherosclerosis and other vascular diseases with NO donor therapy [41]. The therapeutic use of NO therapy has been reported to ameliorate atherosclerosis [48]. It is an important aspect of therapy whether atherogenesis initiates the formation of endothelial dysfunction or activation. Although it is unclear how endothelial cells recruit inflammatory cells, it is clear that inflammatory cytokines secretion of endothelial cells is tightly linked to eNOS expression. This relationship gives us hints for therapy. Also, vascular endothelium-derived NO has a protective role extending to endothelial-leukocyte interactions, leukocyte trafficking to hinder platelet activation, and smooth muscle contraction and proliferation. Statins (HMG-CoA reductase inhibitors) restore endothelial function, and protect vessels by boosting endothelium-derived NO. Endostatin has been reported to induce autophagic cell death in human endothelial cells (EA.hy926) [49]. When human endothelial cells are exposed to oxLDL, autophagy in the cells is increased to deal with plaque components. It is accepted that endostatin induces damaged endothelial cells by overloaded lipid through autophagic cell death pathways [50].

## 3. Atherothrombosis stabilization and regression mechanism

Atherosclerotic plaque lesions are generally asymptomatic for years with slowly evolving in restricting blood flow around lesion [51]. The transition between stable and unstable is decided by the development of a large necrotic core resulting from cell death within the plaque and failure to clearing dead cells. Macrophages are key players in the transition from stable to unstable lesions [52].

Primarily, autophagy is recognized as a survival mechanism and not as one of the cell death pathways. This renders the role of autophagy in atherosclerosis to be equivocal [53]. Successful autophagy generally contributes to cellular survival by acting anti-apoptosis and cellular recovery by supplying biomaterials. Autophagy serves as safeguards for atherosclerotic plaque cells against cellular oxidative stress by polarizing mitochondria not to release cytochrome c [54]. In this reason, autophagy of VSMCs of the fibrous cap in advanced atherosclerotic lesion is important to plaque stabilization. Autophagic death in VSMCs results from excessively stimulated autophagy, and results in plaque destabilization [53]. Autophagic death in endothelial cells affects to maintain the structure of the thrombotic plaques. In view of stabilizing plaque on the rupture-prone lesion, induction of autophagic macrophages might be a promising strategic role in plaque which is not obstructive into lumen but prone to rupture [55]. Dysfunctional autophagy stimulates accumulation of damaged mitochondria, ROS over-expression, and ceroid in human plaque. Continuously and excessively stimulated autophagy can initiate autophagic VSMCs death resulting in plaque destabilization because

collagen synthesis in reduced and also the fibrous plaque cap gets thinning. Of course, autophagic cell death is triggered in endothelial cells, which is detrimental role in the sustaining structure of the atherosclerotic plaque. It is an acute clinical event promoting thrombosis on the atherosclerotic lesion.

Lipid modification such as LDL oxidation brings about a range of modifications with various physiological and biochemical properties [8]. Modified lipids in macrophage cells are able to induce lysosomal dysfunction which can result in the accumulation of intra-lysosomal cholesteryl esters [56]. A number studies have shown that uptake of modified lipids induces a lysosomal lipid storage disease-like condition [5]. Accumulated lipids in lysosomes cause lysosomal dysfunction and affects the intracellular transport machinery. When macrophages are exposed to oxLDL and cholesterol, so-called atherogenic or modified lipids, lysosomal dysfunction occurs [16]. The oxLDL-derived cholesteryl esters form cholesterol crystal when oxLDL-derived cholesteryl esters are inefficiently hydrolyzed and transported in lysosomes [57]. Through CD36-dependent mechanisms, oxLDL is moved to macrophage lysosomes; cholesterol crystals accumulate in the lysosomes. Cholesterol crystals beyond the dealing range initiate lysosomal damage and result in leaking lysosomes [57]. As an example, phagocytosis of apoptotic cells (efferocytosis) is detected in plaque progression and is regarded as a critical feature of increasing plaque complexity [5]. PRPs, cell surface receptors and also scavenger receptors, recognize modified lipids (oxLDL) and pathogens. Plasma levels of soluble CD36, one of scavenger receptors, are higher in the context of risk factors for the development of atherosclerosis such as diabetes [58]. The altered "eat-me" signals can also affect efferocytosis and the targets of apoptotic cells. For example, mice lacking complement factor C1q exhibited efferocytosis dysfunction and atherosclerotic plaque burden [59]. In human atherosclerotic plaques, efferocytosis is impaired and also shaded phagocytic receptors, which impedes phagocytic capacity of macrophages and involves activation of the inflammatory response [60]. The LDLR-related protein 1 (LRP1) is one of the important receptors interacting with C1q for opsonizing.

Prolonged oxidative damage induces protein misfolding and the accumulation of dysfunctional proteins to be degraded [61]. Large protein aggregates are ubiquitinated, and the poly-ubiquitinated protein aggregates are shuttled to the autophagosome. This is generally performed via chaperone proteins such as p62/SQSTM1 [11]. The reason for inflammasome activation in the plaque is not currently unclear, but two mechanisms have been suggested. One is that inefficient mitophagy clearing of damaged mitochondria results in increasing reactive oxygen species (ROS), which induces inflammasome activation. However, the level of protein oxidation and superoxides are augmented in autophagy-deficient macrophages and atherosclerotic plaques [12]. The other mechanism is that overloaded oxLDLs and cholesterol crystals destabilize the lysosomal membrane, resulting in inflammasome activation by producing IL-1β [7]. In the atherosclerotic context, it has been shown that aggregated proteins activate inflammasomes and aggravate atherosclerosis in autophagy-deficient systems [12].

Atherosclerosis progression presents the features of impaired autophagy. Autophagy is sequential events called as autophagic flux (autophagosome formation, cargo sequestration, and autolysosomal fusion), and unfortunately, hard to assess the flux in vivo. When p62/SQSTM1, a

chaperone shuttling protein aggregates from cytosol to autophagosomes, is combined to protein aggregates and degraded, increased level of p62/SQSTM1 indicate defective in autophagic flux autophagy [62]. Correspondingly, deficient autophagy of macrophage can facilitate atherosclerotic plaque progression. *Atg*5 knock-out mice with ApoE-null background showed that western diet for 2 months increased the level of p62/SQSTM1 in the vessel with similar level of control mice whereas atherosclerotic lesion was bigger than control both in aortic root and whole aorta [62]. Using animals with experimental atherosclerosis, ApoE-null mice, recent study proposed that plaque formation expands when macrophagic autophagy is completely disrupted and not partially disrupted. Partially disrupted autophagic condition induces rather macrophagy inflammation and excess IL-1beta, because cholesterol crystal of atherosclerotic plaques is potent stimuli to activate inflammasome [62].

Cholesterol efflux is induced to balance the level of macrophage storing lipid by transferring increased cholesterol from peripheral tissues to the liver. The primary cholesterol efflux mechanism has been thought that cholesterols are hydrolyzed cholesteryl esters cytosolic hydrolases; free cholesterols are moved to the plasma membrane; finally free cholesterols are delivered to the periphery by ATP-binding cassette transporters (ABCA1 or ABCG1) [63]. Autophagic malfunction of macrophages abrogates this cholesterol efflux when macrophages are faced to hinder autophage by chemically (chloroquine) or genetically (*Atg*5-deficiency). Furthermore, inhibitors of lysosomal acid lipase also diminish cholesterol efflux. These showed that cholesterol hydrolysis as well as autophagic delivery is a critical step in atherosclerotic plaque progression and regression. Although lipid-laden macrophages induce lipophagy and also trigger a counter regulatory mechanism are unclear, it is clear that lipophagy-mediated efflux plays an important role in cholesterol transport in vivo [7]. Therefore, efficient cholesterol metabolism and efflux considered athero-protective mechanisms against accumulated lipid-laden atherogenic condition [64].

## 4. Cognitive impairment after atherosclerosis

Aging is a major risk factor for neurodegenerative disease associated with atherosclerosis [65]. Previous studies have demonstrated a strong association between aging and vascular diseases. Recent clinical investigations have focused on the relationship between levels of circulating adhesion factors in peripheral blood and cerebrovascular diseases [66]. Platelets and leukocytes play a major role in atherothrombosis, aggregates of which result in the formation of atherosclerotic plaques [67]. Although other factors associated with vascular disease can influence the cognitive state, few studies have utilized flow cytometry to investigate platelet and leukocyte markers in older adults with cognitive decline. Research has demonstrated a correlation between circulating adhesion molecules in patients with atherosclerosis and atherosclerosis factors such as intima-media thickness (IMT) and the number of plaques, which may assist in determining the presence and/or extent of cognitive decline [68]. To determine the potential usefulness of this correlation for determining diagnoses/prognoses, blood factor analysis is required. Based on the pathophysiological mechanism underlying dementia, most relevant studies have aimed to identify molecular markers based on drug responses [69]. As

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such, little is known regarding the potential role of circulating adhesion molecules in patients with vascular diseases during the early and later stages of cognitive dysfunction.

Many definitions have been proposed for the transition point when healthy aging with a slight cognitive decline progresses to dementia [70]. Mild cognitive impairment (MCI), which was first proposed by a group of investigators from the Mayo Clinic in the late 1990s [70], was defined to be based on a memory problem. This section provides our results about assessing the relationship between changes in blood factors and ultrasound findings in patients with MCI and dementia who were also exhibiting signs of atherosclerosis.

### 4.1. Atherosclerosis and dementia

Carotid atherosclerosis severity is assessed by considering the plaque number, proportions, and location as well as the presence of carotid stenosis that is caused by plaques. Additionally, the severity of carotid stenosis is determined according to the blood flow velocities, residual rumen diameter, and carotid artery flow velocities ratio to internal carotid artery versus the common carotid artery [71]. For AD, it is generally accepted that vascular risk factors have an epidemiological effect on dementia [72]. It has been reported that a narrowed carotid lumen is a risk factor for cognitive impairment in steno-occlusive carotid artery disease patients [72]. Revascularization procedures may have some benefit in the alleviation of dementia, but not for all of these patients [72]. In cases of mild AD with severe asymptomatic intra-carotid artery (ICA) stenosis, cognitive decline progressed even though they have not experienced cerebral ischemia [72]. One possible explanation of this relationship is that insufficient cerebrovascular flow causes cerebral atrophy. Another one is vascular factors that are promoting the degenerative changes of AD [72].

The available studies have identified factors associated with aging and vascular dysfunction that exhibit a cross-sectional relationship with mental status based on the Mini-Mental State Examination (MMSE) score. Recent studies have reported that carotid artery atherosclerosis is associated with a subsequent risk of new or recurrent cerebrovascular diseases, such as stroke, post-stroke vascular dementia, and MCI [66, 73, 74]. Furthermore, chronic hypoperfusion caused by carotid stenosis has been reported to play a role in cognitive decline [75]. Dementia represents a major public health concern [68], as accumulating evidence has demonstrated that the incidence and prevalence of dementia increases rapidly with advancing age. Although it has been difficult to investigate changes in the incidence and prevalence of dementia due to variations in diagnostic criteria and methods, a recent epidemiological study indicated that the dementia prevalence and incidence have decreased in some countries. Moreover, the number of patients with dementia has remained stable in the aging population of these countries [76]. Some evidence has suggested that vascular risk factors are associated with the onset and progression of AD [77]. There are increasing concerns that microvascular disease and tau deposition are found concomitantly and it is thought that treating vascular risk factors is as important as preventing cognitive decline [66]. Although the association between anterior cerebral artery (ACA) plaques and dementia has not been fully determined for the number and the location of plaques, it can be used as a better indicator of disease progression and severity.

In addition, increased cerebrovascular risk has been associated with more severe dementia and a higher MCI incidence [78]. Considering the role of vascular blood factors in patients with MCI, such factors may also influence the progression of cognitive decline [79]. However, there are currently no markers for the prediction of prognosis or the risk of conversion from MCI to dementia. Therefore, it is necessary to develop noninvasive diagnostic methods for the assessment of vascular status [80]. This aspect is discussed in more detail in the next subsection with my results.

### 4.2. Neurosonological findings of atherosclerosis and dementia

In our recent study, we demonstrated that alterations in IMT and plaque number are associated with an increased risk of cognitive decline as well as a risk of dementia. Our results suggest that ultrasound findings may aid in identifying older individuals at increased risk for the progression of cognitive decline when morphological impairment of cerebrovascular structures has been identified. Moreover, our findings suggest that the presence of atherosclerotic changes and changes in blood factors such as p-selectin glycoprotein ligand (PSGL, CD162), platelet-leukocyte aggregation (PLA), and platelet-monocyte aggregation (PMA) can be used to predict MCI and dementia.

Our study showed that levels of p-selectin in circulating platelets, PSGL, and circulating platelet-monocyte aggregates were significantly increased in patients with MCI relative to controls. The changes in circulating blood factors have been reported to relate with vascular diseases such as ischemic stroke or atherosclerosis [81]. Based on this association, several noninvasive measures for evaluating subclinical atherosclerosis have received intense attention in clinical and research settings for the predictive diagnosis of cerebrovascular diseases. Researchers have suggested a relationship between atherosclerotic severity and circulating adhesion blood factors and atherosclerotic severity and cognitive decline in the above-mentioned reports. With one step further linked between them, our findings provide insight into the use of blood factor analysis (using FACS) as well as ultrasonographic evaluation of vessel status in both clinical and research settings. Changes in platelet activation and monocyte distribution are observed in the early stages of atherosclerosis. Such changes are strongly associated with stroke onset, as demonstrated by various studies [82]. The monocyte receptor CD14 and leukocyte antigen CD45 are best known for their crucial role in immunity. In addition, CD14 and CD16 are well-known biomarkers for atherosclerotic disease progression [67]. Research has also suggested that PSGL is a pro-atherogenic marker of vascular disease progression [67].

The present study shows that increased IMT was more frequently observed in patients with MCI, whereas increased numbers of carotid plaques were more frequently observed in patients with dementia. The patients with MCI in our study comprise 32% of all patients with atherosclerosis, and all patients of the MCI group in the present study had been diagnosed with carotid vascular stenosis or atherosclerosis. These findings suppose that vessel damage is followed by MCI. A lot of findings in previous studies suggest that greater degrees of carotid atherosclerosis are associated with the progression from MCI to dementia [66, 68, 78]. A recent study reported that up to 50% of patients develop vascular stenosis, and that ACA

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plaques are associated with dementia even after controlling for vascular risk factors [66]. Other researchers have suggested that atherosclerosis plays a role in cognitive impairment, particularly in older adults [83]. Such research has further demonstrated a converging relationship between degenerative vascular dysfunction and cognitive dysfunction. In our study, most patients with MCI exhibit atherosclerotic vessel abnormalities, such as increased IMT and plaque numbers, increasing the risk for progression to dementia. An estimated 15–42% of people over the age of 65 years exhibit some form of MCI, and approximately 5–15% of patients with MCI go on to develop dementia [70]. Recent evidence has revealed that vessel dysfunction contributes to AD as well as vascular dementia [84]. In this previous study, the authors reported an IMT cutoff value of 0.805 for the prediction of MCI development (baseline: 0.825 mm) [84]. Diagnosis of dementia in such patients is required in order to ensure the appropriate therapeutic guidelines and treatments are utilized [76].

Our results indicated that intima thickness and plaque number are associated with higher levels of p-selectin, supporting the evidence that platelets are engaged in the formation of PLAs [85]. In the dementia group of the present study, which included individuals with dementia, plaque numbers corresponded strongly with levels of PSGL-positive platelets. Control of plaque numbers with appropriate therapy such as statin treatment may thus delay or prevent the progression of cognitive decline to dementia. Our findings also indicated that carotid atherosclerosis correlates with MCI as well as increased numbers of PSGL-expressing platelets. Analysis of blood factors using ultrasonography may aid clinicians in determining the most appropriate treatment strategy for patients with cognitive decline with vessel disease. Our simple assessment of vascular risk factors does not seem to be a fully satisfactory approach for adequately counteracting the risk of developing dementia, when compared to other large-scale studies [86]. Nevertheless, we suggest that analysis of circulating adhesion factors may aid in predicting the risk of progressive cognitive impairment. Additionally, aggressive treatments for vascular disease should be considered for individuals with a predisposition toward dementia. Despite these limitations, our findings provide a basis for a future study regarding biomarkers of both cerebrovascular disease and cognitive dysfunction.

In conclusion, our findings demonstrate that circulating adhesion molecule levels and interaction between factors present significant differences in patient with MCI or dementia. Alterations in IMT and plaque number are associated with an increased risk of cognitive decline as well as conversion from MCI to dementia. These results suggest that ultrasound findings may aid in identifying older individuals at increased risk for the progression of cognitive decline when there is cerebrovascular damage. Moreover, our findings suggest that the presence of atherosclerotic changes and changes in blood factors such as p-selectin, PSGL, PLA, and PMA can be used to predict the progression of MCI and dementia.

### 4.3. Prevalence and incidence of MCI and dementia in atherosclerosis

The World Alzheimer Report 2015 announced the estimate that 46.8 million people worldwide have dementia, and this number is expected to increase to 74.7 million by 2030 and 131.5 million by 2050 [76]. Accordingly, due to concerns about the increasing incidence of dementia, dementia is predicted to be 'epidemic' and a consequent economic burden. The G8 dementia

summit in 2013 and the WHO Ministerial Conference in 2015 decided to engage in a global action against dementia. The Atherosclerosis Risk in Communities (ARIC) study performed in 1987–1989 enrolled 15,792 individuals: they were a bi-racial group, with an age range from 45 to 64 years, from 4 US communities. Cognitive assessments were performed in the second ARIC examination in 1990–1992 [74]. A comprehensive dementia study, Atherosclerosis Risk in Communities Neurocognitive Study (ARIC-NCS), was used as the fifth ARIC examination in 2011–2013. They evaluated what happens to participants with extensive cardiovascular disease and cognitive dysfunction after a long history of ARIC and for the participants who died. This is a longitudinal cohort depicting the association of cognitive function, cardiovascular condition, cerebrovascular condition, and mortality. In ARIC-NCS, they reported that the overall prevalence of dementia in living ARIC participants is similar to the estimate of the World Alzheimer Report 2015. Although the prevalence of MCI in ARIC-NCS and the prevalence of MCI have been reporting to be at a similar level, there is a variation in MCI prevalence because of different MCI definitions [74]. Therefore, longitudinal studies of incident dementia in this cohort are required for validation of the MCI definition.

From some studies, it is obvious that the prevalence of dementia is related to stroke, heart disease, hypertension, and diabetes. These results are limited because many medications used to treat cardiovascular disease and other vascular diseases have been observed to have an effect on dementia prevalence and incidence. This is why no single factor has been identified to fully explain the changes in dementia prevalence and incidence. However, it is important to identify multiple risk factors and protective factors throughout a personal whole life-course relating to physical, mental, and cognitive health. In particular, atherosclerotic and vascular risk factors need to be well-controlled for reducing the risk of dementia in later life.

## 5. Prospects of therapy

For developing new therapeutic strategies for atherosclerosis, it is important to understand the cause of the disease pathogenesis and its progression [33]. A number of studies have shown that cell deaths produce different patterns depending on the stage of the plaque and types of cells involved in cell death. In summary, atherosclerosis is an inflammation-related arterial intima disease. A number of pharmacologic approaches have developed the way to stabilize rupture-prone atherosclerotic lesion by applying macrophage autophagic death. For therapeutic approach in atherosclerosis, it should be implicated for plaque stability and selective depletion of macrophages by modulating sterols. This section will introduce them.

### 5.1. Harnessing macrophagic autophagy as a therapy for atherosclerosis

One such pathway regulating the initiating autophagy involves mammalian target of rapamycin (mTOR). Blocking mTOR using rapamycin has an effect on cell proliferation both *in vitro* and *in vivo* [87]. Inhibition of mTOR leads to autophagic cell death through some ATG protein pathways [88]. ATG13 is rapidly dephosphorylated when the mTOR pathway is inhibited. By stimulating affinity to ATG1, the ATG1-ATG13 complex is involved in autophagosome

formation. When the rapamycin derivative everolimus is delivered from a stent in atherosclerotic plaques, in the lesion site of cholesterol-fed rabbits, autophagic macrophage death occurred due to macrophage reduction. In contrast, the amounts of VSMCs were sustained without change [89]. An mTOR-mediated pathway induces dephosphorylation of p70 S6 kinase, which is responsible for selective induction of macrophage death. On the other hand, the protein synthesis inhibitor cycloheximide induced selective macrophage death in plaques of cholesterol-fed rabbits. In this case, apoptosis occurred not only via autophagy, because plaque macrophages might be highly metabolic active and vulnerable to protein synthesis inhibitors relative to SMCs [90].

As another therapeutic case of rapamycin, inhibited translation of VSMCs leads to upregulation of smooth muscle B-actin, calponin, and myosin heavy chain, which modulates VSMCs to be differentiated, quiescent, and have a contractile phenotype. As a result, VSMCs undergo cell death. It has been suggested that restricted protein translation might selectively induce macrophage cell death rather than cell death protein expression. mTOR gene silencing is another therapeutic approach. Transfection of mTOR-specific small interfering RNA selectively induces macrophage cell death [89]. The recent evidence shows that the transcription factor TFEB works as a transcriptional activator for a network of autophagy and lysosomal genes [91]. The studies show that macrophage specific gene activator of TFEB has the ability of lysosomal biogenesis, recovering lysosomal dysfunction via atherogenic lipids. It allows some functions such as increasing cholesterol efflux, inhibiting inflammation activation, and clearing abnormal protein aggregate [16]. This study provides the possibility that overexpressed TFEB in macrophages alleviates atherosclerosis [16].

Lipid reduction is the one of the well-known ways to eradicate macrophages from atherosclerotic plaques [36]. One of recent studies using a rabbit atherosclerosis model suggested that the lower levels of lipid led not to macrophage apoptosis but instead monocyte recruiting impairment because of a decrease in macrophage replication. Statins are generally used in myocardial infarction patients. In SMCs treated with the autophagy inducer 7-ketocholesterol, fluvastatin failed to activate caspases. It has been suggested there is a possibility that activation of autophagy interferes with the statin-induced apoptotic pathway [36]. Another suggestion that has been proposed is that defective mitochondria are engulfed by autophagosomes, which limits the relocation of pro-apoptotic molecules from mitochondria into the cytosol or nucleus [36].

### 5.2. Treating atherosclerosis as a therapy for cognitive impairment

Currently, statin drugs are major therapeutics to prevent acute coronary events. Statins inhibit cholesterol biosynthesis, reduce LDL receptors (LDLRs), and consequently trigger a reduction in blood cholesterol levels [92]. Statins work at multiple stages in atherosclerotic plaque formation. Among these stages, statins have effects at earlier atherosclerosis development stages because they hinder cholesterol accumulation, monocyte infiltration, and inflammation in arteries [92]. Carotid atherosclerosis is measured by two distinct characteristics: carotid intima-media thickness (cIMT) and carotid plaque burden quantified by plaque presence or localization. A recent study suggests that plaque burden may act as a predictor of cardiovascular

disease other than cIMT, although cIMT has been better represented in preventative measures [68]. It has been suggested that plaque numbers and cIMT may be involved in cognitive impairment and dementia [66]. These studies report early intervention of atherosclerosis to prevent cognitive impairment [72]. It is obvious that carotid atherosclerosis can be a potential target for early intervention and risk management for those at risk for cognitive decline. A recent study was performed under the hypothesis that cognitive performance in the dominantly affected domain would be related to carotid plaque burden and cIMT, and cognitive decline would be shown differently in the racial and ethnical diverse Northern Manhattan Study (NOMAS) [93]. They indicated that elderly individuals with a larger cIMT have a higher future risk of progression to MCI or dementia. It is required to monitor patients for earlier detection of cognitive dysfunction [93]. Additionally, they identified a cutoff value for predicting cognitive impairment progression (0.825 mm of cIMT), which corresponds to the cutoff values for predicting stroke and CVD in the previous report [93].

Considering the role of vascular blood factors in patients with MCI, some blood factors suggested in our previous study may also influence the progression of cognitive decline [94]. However, there are currently no markers for prognostication or the risk of conversion from MCI to dementia. Therefore, it is necessary to develop noninvasive diagnostic methods for the assessment of vascular status [80]. This aspect is discussed further in the next section along with my results. Our results may provide a route for determining the most appropriate treatment strategy for patients with MCI or multiple diagnoses.

According to recent research, trends of dementia prevalence and incidence have been reported, which are based on healthcare and insurance databases, clinical records, and meta-analysis. These studies have not currently provided how to control the recent trends of cognitive function about diagnosis, clinical details, or public awareness for it. Nonetheless, researchers and clinicians are agreeing that long-term determinants are needed for both healthy and unhealthy aging in the most of society. Furthermore, it goes on the efforts to reduce risk of dementia by maintaining health with age.

## 6. Conclusions

Clinically and pathologically, atherosclerosis is an important disease in a worldwide aging society. It has been shown that innate immune factors and adaptive immune factors are associated with the atherosclerotic process since inflammatory mechanisms are identified as major causes in patients. A number of studies have identified several potential targets for therapy. Unfortunately, however, inflammation is an independent risk factor for atherosclerosis progression in humans. Researchers have tried to evaluate the mechanism of immune-related therapies in atherosclerotic cardiovascular disease. Along with inflammation-related mechanisms, autophagy in atherosclerosis is also responsible for the foam cell formation and insoluble oxLDL uptake and clearance in human atherosclerotic lesions. Autophagic macrophages produce pro-inflammatory cytokines such as TNF-beta and interleukin-6, and these cytokines are not immunologically silenced during the autophagic process. Lipid droplets

are spilled from lipid-overloaded macrophages in the plaques. Based on previous research, future studies are focusing on therapeutic advantages of autophagic microphages in unstable atherosclerotic plaques.

Despite efforts to develop strong therapeutic targets, it is not feasible to establish respective contributors to degeneration and vascular disease onset and progression in each patient. Today, in our aging society, dementia has becoming an important issue in the public heath, economics, and social aspects, and also in political fields. With careful converging and treating on vascular risk factors containing atherosclerosis, it would get available therapeutic strategies for prognosis and diagnosis in patients with progressive dementia.

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## References

[1] Fisher EA. Regression of atherosclerosis: The journey from the liver to the plaque and back. Arteriosclerosis, Thrombosis, and Vascular Biology. 2016;**36**(2):226-235

[2] Hansson GK, Hermansson A. The immune system in atherosclerosis. Nature Immunology. 2011;**12**(3):204-212

[3] Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, et al. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature. 2002;**417**(6890):750-754

[4] Goldstein JL, Brown MSA. Century of cholesterol and coronaries: From plaques to genes to statins. Cell. 2015;**161**(1):161-172

[5] Sergin I, Evans TD, Razani B. Degradation and beyond: The macrophage lysosome as a nexus for nutrient sensing and processing in atherosclerosis. Current Opinion in Lipidology. 2015;**26**(5):394-404

[6] Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. The New England Journal of Medicine. 2005;**352**(16):1685-1695

[7] Sergin I, Razani B. Self-eating in the plaque: What macrophage autophagy reveals about atherosclerosis. Trends in Endocrinology and Metabolism: TEM. 2014;**25**(5):225-234

[8] Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. Nature Reviews Immunology. 2011;**11**(11):723-737

[9] Kraft C, Peter M, Hofmann K. Selective autophagy: Ubiquitin-mediated recognition and beyond. Nature Cell Biology. 2010;**12**(9):836-841

[10] Liu K, Czaja MJ. Regulation of lipid stores and metabolism by lipophagy. Cell Death and Differentiation. 2013;**20**(1):3-11

[11] Komatsu M, Kageyama S, Ichimura Y. p62/SQSTM1/A170: Physiology and pathology. Pharmacological Research. 2012;**66**(6):457-462

[12] Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, et al. Autophagy links inflammasomes to atherosclerotic progression. Cell Metabolism. 2012;**15**(4):534-544

[13] Martinet W, De Meyer GR. Autophagy in atherosclerosis. Current Atherosclerosis Reports. 2008;**10**(3):216-223

[14] Kroemer G, Marino G, Levine B. Autophagy and the integrated stress response. Molecular Cell. 2010;**40**(2):280-293

[15] Zhang YL, Cao YJ, Zhang X, Liu HH, Tong T, Xiao GD, et al. The autophagy-lysosome pathway: A novel mechanism involved in the processing of oxidized LDL in human vascular endothelial cells. Biochemical and Biophysical Research Communications. 2010;**394**(2): 377-382

[16] Emanuel R, Sergin I, Bhattacharya S, Turner J, Epelman S, Settembre C, et al. Induction of lysosomal biogenesis in atherosclerotic macrophages can rescue lipid-induced lysosomal dysfunction and downstream sequelae. Arteriosclerosis, Thrombosis, and Vascular Biology. 2014;**34**(9):1942-1952

[17] Nakai A, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, et al. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. Nature Medicine. 2007;**13**(5):619-624

[18] Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. Current Opinion in Cell Biology. 2010;**22**(2):124-131

[19] Feng Y, He D, Yao Z, Klionsky DJ. The machinery of macroautophagy. Cell Research. 2014;**24**(1):24-41

[20] Villanueva MT. Autophagy: Exploring the anticancer effects of autophagy inhibition. Nature Reviews Cancer. 2015;**15**(9):512-513

[21] Glass CK, Witztum JL. Atherosclerosis. The road ahead. Cell. 2001;**104**(4):503-516

[22] Li WW, Li J, Bao JK. Microautophagy: Lesser-known self-eating. Cellular and Molecular Life Sciences: CMLS. 2012;**69**(7):1125-1136

[23] Mijaljica D, Prescott M, Devenish RJ. Microautophagy in mammalian cells: Revisiting a 40-year-old conundrum. Autophagy. 2011;**7**(7):673-682

[24] Cuervo AM. Chaperone-mediated autophagy: Selectivity pays off. Trends in Endocrinology and Metabolism: TEM. 2010;**21**(3):142-150

[25] Kaushik S, Arias E, Kwon H, Lopez NM, Athonvarangkul D, Sahu S, et al. Loss of autophagy in hypothalamic POMC neurons impairs lipolysis. EMBO Reports. 2012;**13**(3):258-265

[26] Orenstein SJ, Cuervo AM. Chaperone-mediated autophagy: Molecular mechanisms and physiological relevance. Seminars in Cell & Developmental Biology. 2010;**21**(7):719-726

[27] Quintavalle C, Di Costanzo S, Zanca C, Tasset I, Fraldi A, Incoronato M, et al. Phosphorylation-regulated degradation of the tumor-suppressor form of PED by chaperone-mediated autophagy in lung cancer cells. Journal of Cellular Physiology. 2014;**229**(10): 1359-1368

[28] Bejarano E, Cuervo AM. Chaperone-mediated autophagy. Proceedings of the American Thoracic Society. 2010;**7**(1):29-39

[29] Kaushik S, Cuervo AM. Chaperones in autophagy. Pharmacological Research. 2012;**66**(6):484-493

[30] Kaushik S, Cuervo AM. Chaperone-mediated autophagy: A unique way to enter the lysosome world. Trends in Cell Biology. 2012;**22**(8):407-417

[31] Rudijanto A. The role of vascular smooth muscle cells on the pathogenesis of atherosclerosis. Acta Medica Indonesiana. 2007;**39**(2):86-93

[32] Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circulation Research. 2016;**118**(4):692-702

[33] Clarke MC, Figg N, Maguire JJ, Davenport AP, Goddard M, Littlewood TD, et al. Apoptosis of vascular smooth muscle cells induces features of plaque vulnerability in atherosclerosis. Nature Medicine. 2006;**12**(9):1075-1080

[34] Kockx MM, De Meyer GR, Buyssens N, Knaapen MW, Bult H, Herman AG. Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal. Circulation Research. 1998;**83**(4):378-387

[35] Inbal B, Bialik S, Sabanay I, Shani G, Kimchi ADAP. Kinase and DRP-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death. The Journal of Cell Biology. 2002;**157**(3):455-468

[36] Martinet W, De Meyer GR. Autophagy in atherosclerosis: A cell survival and death phenomenon with therapeutic potential. Circulation Research. 2009;**104**(3):304-317

[37] Koenen RR, Weber C. Therapeutic targeting of chemokine interactions in atherosclerosis. Nature Reviews Drug Discovery. 2010;**9**(2):141-153

[38] Moore KJ, Sheedy FJ, Fisher EA. Macrophages in atherosclerosis: A dynamic balance. Nature Reviews Immunology. 2013;**13**(10):709-721

[39] Newby AC. Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability. Arteriosclerosis, Thrombosis, and Vascular Biology. 2008;**28**(12):2108-2114

[40] Gimbrone MA Jr, Garcia-Cardena G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circulation Research. 2016;**118**(4):620-636

[41] Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, et al. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. The Journal of Clinical Investigation. 2011;**121**(7):2921-2931

[42] Stary HC. Natural history and histological classification of atherosclerotic lesions: An update. Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;**20**(5):1177-1178

[43] Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M. Prelesional events in atherogenesis. Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. The American Journal of Pathology. 1986;**123**(1):109-125

[44] Plump AS, Scott CJ, Breslow JL. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. Proceedings of the National Academy of Sciences of the United States of America. 1994;**91**(20):9607-9611

[45] Castelli WP, Doyle JT, Gordon T, Hames CG, Hjortland MC, Hulley SB, et al. HDL cholesterol and other lipids in coronary heart disease. The cooperative lipoprotein phenotyping study. Circulation. 1977;**55**(5):767-772

[46] Brooks-Wilson A, Marcil M, Clee SM, Zhang LH, Roomp K, van Dam M, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nature Genetics. 1999;**22**(4):336-345

[47] Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metabolism. 2005;**1**(2):121-131

[48] Rong JX, Li J, Reis ED, Choudhury RP, Dansky HM, Elmalem VI, et al. Elevating high-density lipoprotein cholesterol in apolipoprotein E-deficient mice remodels advanced atherosclerotic lesions by decreasing macrophage and increasing smooth muscle cell content. Circulation. 2001;**104**(20):2447-2452

[49] Chau YP, Lin SY, Chen JH, Tai MH. Endostatin induces autophagic cell death in EAhy926 human endothelial cells. Histology and Histopathology. 2003;**18**(3):715-726

[50] Nowicki M, Zabirnyk O, Duerrschmidt N, Borlak J, Spanel-Borowski K. No upregulation of lectin-like oxidized low-density lipoprotein receptor-1 in serum-deprived EA.hy926 endothelial cells under oxLDL exposure, but increase in autophagy. European Journal of Cell Biology. 2007;**86**(10):605-616

[51] Gonzalez L, Trigatti BL. Macrophage apoptosis and necrotic core development in atherosclerosis: A rapidly advancing field with clinical relevance to imaging and therapy. The Canadian Journal of Cardiology. 2017;**33**(3):303-312

[52] Chinetti-Gbaguidi G, Colin S, Staels B. Macrophage subsets in atherosclerosis. Nature Reviews. Cardiology. 2015;**12**(1):10-17

[53] Levine B, Yuan J. Autophagy in cell death: An innocent convict? The Journal of Clinical Investigation. 2005;**115**(10):2679-2688

[54] Kiffin R, Bandyopadhyay U, Cuervo AM. Oxidative stress and autophagy. Antioxidants & Redox Signaling. 2006;**8**(1-2):152-162

[55] Stoneman V, Braganza D, Figg N, Mercer J, Lang R, Goddard M, et al. Monocyte/macrophage suppression in CD11b diphtheria toxin receptor transgenic mice differentially affects atherogenesis and established plaques. Circulation Research. 2007;**100**(6):884-893

[56] Jerome WG. Lysosomes, cholesterol and atherosclerosis. Clinical Lipidology. 2010;**5**(6): 853-865

[57] Sheedy FJ, Grebe A, Rayner KJ, Kalantari P, Ramkhelawon B, Carpenter SB, et al. CD36 coordinates NLRP3 inflammasome activation by facilitating intracellular nucleation of soluble ligands into particulate ligands in sterile inflammation. Nature Immunology. 2013;**14**(8):812-820

[58] Handberg A, Norberg M, Stenlund H, Hallmans G, Attermann J, Eriksson JW. Soluble CD36 (sCD36) clusters with markers of insulin resistance, and high sCD36 is associated with increased type 2 diabetes risk. The Journal of Clinical Endocrinology and Metabolism. 2010;**95**(4):1939-1946

[59] Lewis MJ, Malik TH, Ehrenstein MR, Boyle JJ, Botto M, Haskard DO, Immunoglobulin M. Is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation. 2009;**120**(5):417-426

[60] Novak ML, Thorp EB. Shedding light on impaired efferocytosis and nonresolving inflammation. Circulation Research. 2013;**113**(1):9-12

[61] Doyle SM, Genest O, Wickner S. Protein rescue from aggregates by powerful molecular chaperone machines. Nature Reviews Molecular Cell Biology. 2013;**14**(10):617-629

[62] Liao X, Sluimer JC, Wang Y, Subramanian M, Brown K, Pattison JS, et al. Macrophage autophagy plays a protective role in advanced atherosclerosis. Cell Metabolism. 2012;**15**(4):545-553

[63] Ghosh S, Zhao B, Bie J, Song J. Macrophage cholesteryl ester mobilization and atherosclerosis. Vascular Pharmacology. 2010;**52**(1-2):1-10

[64] Ouimet M, Marcel YL. Regulation of lipid droplet cholesterol efflux from macrophage foam cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2012;**32**(3):575-581

[65] Coutu JP, Goldblatt A, Rosas HD, Salat DH. Alzheimer's disease neuroimaging I. White matter changes are associated with ventricular expansion in aging, mild cognitive impairment, and Alzheimer's disease. Journal of Alzheimer's Disease: JAD. 2016;**49**(2):329-342

[66] Dearborn JL, Zhang Y, Qiao Y, Suri MFK, Liu L, Gottesman RF, et al. Intracranial atherosclerosis and dementia: The atherosclerosis risk in communities (ARIC) study. Neurology. 2017;**88**(16):1556-1563

[67] Folsom AR, Aleksic N, Sanhueza A, Boerwinkle E. Risk factor correlates of platelet and leukocyte markers assessed by flow cytometry in a population-based sample. Atherosclerosis. 2009;**205**(1):272-278

[68] Moon JH, Lim S, Han JW, Kim KM, Choi SH, Park KS, et al. Carotid intima-media thickness is associated with the progression of cognitive impairment in older adults. Stroke. 2015;**46**(4):1024-1030

[69] Silvestrini M, Gobbi B, Pasqualetti P, Bartolini M, Baruffaldi R, Lanciotti C, et al. Carotid atherosclerosis and cognitive decline in patients with Alzheimer's disease. Neurobiology of Aging. 2009;**30**(8):1177-1183

[70] Petersen RC, Caracciolo B, Brayne C, Gauthier S, Jelic V, Fratiglioni L. Mild cognitive impairment: A concept in evolution. Journal of Internal Medicine. 2014;**275**(3):214-228

[71] Urbanova B, Tomek A, Mikulik R, Magerova H, Horinek D, Hort J. Neurosonological examination: A non-invasive approach for the detection of cerebrovascular impairment in AD. Frontiers in Behavioral Neuroscience. 2014;**8**:4

[72] Silvestrini M, Viticchi G, Falsetti L, Balucani C, Vernieri F, Cerqua R, et al. The role of carotid atherosclerosis in Alzheimer's disease progression. Journal of Alzheimer's Disease: JAD. 2011;**25**(4):719-726

[73] Kurdi A, Martinet W, De Meyer GR. mTOR inhibition & cardiovascular diseases: Dyslipidemia and atherosclerosis. Transplantation. 2017

[74] Knopman DS, Gottesman RF, Sharrett AR, Wruck LM, Windham BG, Coker L, et al. Mild cognitive impairment and dementia prevalence: The atherosclerosis risk in communities neurocognitive study (ARIC-NCS). Alzheimer's & Dementia. 2016;**2**:1-11

[75] Pathan SS, Gottesman RF, Mosley TH, Knopman DS, Sharrett AR, Alonso A. Association of lung function with cognitive decline and dementia: The atherosclerosis risk in communities (ARIC) study. European Journal of Neurology. 2011;**18**(6):888-898

[76] Wu YT, Beiser AS, Breteler MMB, Fratiglioni L, Helmer C, Hendrie HC, et al. The changing prevalence and incidence of dementia over time-current evidence. Nature Reviews Neurology. 2017;**13**(6):327-339

[77] Deschaintre Y, Richard F, Leys D, Pasquier F. Treatment of vascular risk factors is associated with slower decline in Alzheimer disease. Neurology. 2009;**73**(9):674-680

[78] Viticchi G, Falsetti L, Vernieri F, Altamura C, Bartolini M, Luzzi S, et al. Vascular predictors of cognitive decline in patients with mild cognitive impairment. Neurobiology of Aging. 2012;**33**(6):1127 e1-9

[79] Zhong W, Cruickshanks KJ, Huang GH, Klein BE, Klein R, Nieto FJ, et al. Carotid atherosclerosis and cognitive function in midlife: The beaver dam offspring study. Atherosclerosis. 2011;**219**(1):330-333

[80] de la Torre JC. The vascular hypothesis of Alzheimer's disease: Bench to bedside and beyond. Neuro-degenerative Diseases. 2010;**7**(1-3):116-121

[81] Nadar SK, Lip GY, Blann AD. Platelet morphology, soluble P selectin and platelet P-selectin in acute ischaemic stroke. The West Birmingham Stroke Project. Thrombosis and Haemostasis. 2004;**92**(6):1342-1348

[82] King SM, McNamee RA, Houng AK, Patel R, Brands M, Reed GL. Platelet dense-granule secretion plays a critical role in thrombosis and subsequent vascular remodeling in atherosclerotic mice. Circulation. 2009;**120**(9):785-791

[83] Jellinger KA, Attems J. Neuropathological evaluation of mixed dementia. Journal of the Neurological Sciences. 2007;**257**(1-2):80-87

[84] Murray IV, Proza JF, Sohrabji F, Lawler JM. Vascular and metabolic dysfunction in Alzheimer's disease: A review. Experimental Biology and Medicine. 2011;**236**(7):772-782

[85] Steinhubl SR, Badimon JJ, Bhatt DL, Herbert JM, Luscher TF. Clinical evidence for anti-inflammatory effects of antiplatelet therapy in patients with atherothrombotic disease. Vascular Medicine. 2007;**12**(2):113-122

[86] Coley N, Andrieu S, Gardette V, Gillette-Guyonnet S, Sanz C, Vellas B, et al. Dementia prevention: Methodological explanations for inconsistent results. Epidemiologic Reviews. 2008;**30**:35-66

[87] Easton JB, Houghton PJ. Therapeutic potential of target of rapamycin inhibitors. Expert Opinion on Therapeutic Targets. 2004;**8**(6):551-564

[88] Meijer AJ, Codogno P. Regulation and role of autophagy in mammalian cells. The International Journal of Biochemistry & Cell Biology. 2004;**36**(12):2445-2462

[89] Verheye S, Martinet W, Kockx MM, Knaapen MW, Salu K, Timmermans JP, et al. Selective clearance of macrophages in atherosclerotic plaques by autophagy. Journal of the American College of Cardiology. 2007;**49**(6):706-715

[90] Croons V, Martinet W, Herman AG, Timmermans JP, De Meyer GR. Selective clearance of macrophages in atherosclerotic plaques by the protein synthesis inhibitor cycloheximide. The Journal of Pharmacology and Experimental Therapeutics. 2007;**320**(3):986-993

[91] Martina JA, Diab HI, Lishu L, Jeong AL, Patange S, Raben N, et al. The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. Science Signaling. 2014;**7**(309):ra9

[92] Rahman K, Vengrenyuk Y, Ramsey SA, Vila NR, Girgis NM, Liu J, et al. Inflammatory Ly6Chi monocytes and their conversion to M2 macrophages drive atherosclerosis regression. The Journal of Clinical Investigation. 2017;**127**(8):2904-2915

[93] Spence JD, Solo K. Resistant atherosclerosis: The need for monitoring of plaque burden. Stroke. 2017;**48**(6):1624-1629

[94] Iadecola C, Gorelick PB. Converging pathogenic mechanisms in vascular and neurodegenerative dementia. Stroke. 2003;**34**(2):335-337