The Impact of Autophagy on Cardiovascular Senescence and Diseases

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Summary

The risk of cardiovascular disease increases with age, causing chronic disability, morbidity, and mortality in the elderly. Cardiovascular aging and disease are characterized by heart failure, cardiac ischemia-reperfusion injury, cardiomyopathy, hypertension, arterial stiffness, and atherosclerosis. As a cell ages, damaged organelles and abnormal proteins accumulate. A system for removing these cytoplasmic substrates is essential for maintaining homeostasis. Autophagy assists tissue homeostasis by forming a pathway by which these substances are degraded. Growing evidence suggests that autophagy plays a role in age-related and disease states of the cardiovascular system, and it may even be effective in preventing or treating cardiovascular disease. On the other hand, overexpression of autophagy in the heart and arteries can produce detrimental effects. We summarize the current understanding of the close relationship between autophagy and cardiovascular senescence.

Key words: Cardiovascular disease, Aging, Heart failure, Atherosclerosis

The human life span has significantly increased over recent decades and is expected to increase even further. Consequently, the prevalence of age-related cardiovascular disease is also increasing. Many risk factors for cardiovascular disease, such as hypertension, diabetes mellitus, dyslipidemia, and metabolic syndrome, emerge with age, and cardiovascular disease is the leading cause of chronic disability, morbidity, and mortality among the elderly.

The aging heart is associated with increases in left ventricular wall thickness, stiffness, and chamber size, as well as changes in the diastolic filling pattern, which can lead to cardiac dysfunction. Age-related loss of vascular elasticity and stiffness increases cardiac after-load in the elderly. As the body ages, damaged organelles and abnormal proteins accumulate, disrupting cellular homeostasis. A system for eliminating damaged organelles, proteins, and intracellular pathogens is critical for maintaining cellular homeostasis as the body ages. In recent years, it has become evident that autophagy plays a pivotal role in this regard. In particular, autophagy functions as part of a pathway that delivers cytoplasmic substrates to lysosomes for subsequent degradation and removal. Autophagy modulates homeostasis and the body’s response to stress, including energy deprivation in the heart, and altered autophagy is related to cardiovascular aging and disease. Because autophagy attenuates with age, interventions that increase autophagy may in turn reduce age-related cardiovascular disease, prolonging the lifespan. On the other hand, overexpression of autophagy in the heart can induce atrophy of cardiomyocytes, increasing the risk of ischemia-reperfusion injury. This review summarizes our current understanding of the close relationship between autophagy and cardiovascular senescence.

Three Types of Autophagy

Autophagy is classified into 3 subtypes: macroautophagy (commonly referred to as autophagy), microautophagy, and chaperone-mediated autophagy (CMA). In macroautophagy, a small vesicular sac, called the isolation membrane or phagophore, is formed. The phagophore elongates, engulfing long-lived proteins and organelles within the cytoplasm, eventually forming double-membranated structures called autophagosomes. The autophagosomes then fuse with lysosomes to degrade their cargo through the action of lysosomal enzymes.

Microautophagy is characterized by the direct delivery of cytosolic content into lysosomes by the invagination of its surface. Microautophagy is considered to play a roles in membrane homeostasis, maintenance of organelle size, and cell survival under nitrogen starvation. Microautophagy can be observed in either the non-selective or selective degradative process. Whereas the non-selective process of microautophagy is observed in both mammal-
ian and yeast cells, there are 3 types of selective microautophagy process, which is micropexophagy, piece-meal microautophagy of the nucleus, and micromitophagy, the latter of which is mainly observed in yeast cells.25) The mechanism and regulation of cargo selection of microautophagy in mammalian cells are poorly understood compared to in yeast.

CMA contains a KFERQ-like pentapeptide sequence that selectively degrades cytosolic proteins. During this process, the proteins bind to 70-kDa heat shock cognate 70 and translocate into the lysosomes through the lysosomal membrane receptor lysosomal-associated membrane protein 2A (LAMP-2A).26) CMA activates during oxidative stress and degrades oxidized proteins.27) The activities of CMA and macroautophagy decrease with age, resulting in an accumulation of oxidized proteins, a key characteristic of the aging process.28)

The role of microautophagy and CMA in cardiovascular disease is not fully understood. This review focuses on the role of macroautophagy, hereafter referred to as autophagy, on the cardiovascular system.

Molecular Mechanisms of Autophagy Formation

Since the details of autophagic processes have been presented in several recent reports; only the basic concept of autophagy and the essential components for understanding autophagy formation are described in this review (Figure 1).29) Autophagy consists of multiple, sequential steps. These include initiation and nucleation, elongation of phagophores, and maturation of autophagosomes.12,13,30) In this process, autophagy related gene (Atg) proteins, evolutionarily conserved and identified for the first time in yeasts, are required.31) More than 30 Atg proteins that were first characterized in Saccharomyces cerevisiae regulate autophagy.32-35)

The initiation process requires activation of the class III phosphatidylinositol-3-kinase (PI3K) and Beclin1 to recruit autophagy-specific proteins and form phagophores. This step of autophagy also involves Unc-51-like kinase 1 (Ulk1) responding to upstream signals and interacting with Atg5, Atg12, Atg13, and Atg16. Subsequently, 2 ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems mediate the elongation of membranes that form a double membrane-structured autophagosome. In particular, LC3-II, generated by the proteolytic cleavage of LC3, is associated with the formation of autophagosomes. Levels of LC3-II correlate with the amount of autophagosomes and is considered a marker of autophagosome formation. This process depends on the E1-like enzyme Atg7. In the final step of autophagy, the outer membrane of the autophagosome fuses with a lysosome to form a single membrane-structured autolysosome. This process is called autophagic flux and is mediated by soluble N-ethylmaleimide-sensitive factor attachment protein receptors, an endosomal sorting complex required for transport; GTPase Rab7 proteins; and the lysosomal-associated membrane proteins LAMP-1 and LAMP-2. Lysosomal contents are then degraded by lipases, nucleases, protease, and glycosidases to recycle degraded products for synthetic and metabolic pathways.36)

As for monitoring autophagy, it is important to measure not only the numbers of autophagosomes but also autophagic flux.37) Measurement of the number of double membrane-structured autophagosomes by electron microscopic analysis, the amount of LC3-II protein expression by immunoblot, and GFP-LC3 dots using adenovirus GFP-LC3-transduced cells by microscopy are useful to examine autophagosome formation. In contrast, observation of single membrane-structured autolysosomes by electron microscopy and measurement of both LC3-II and p62 protein expression by immunoblot are required to evaluate autophagic flux. Since p62 is a protein degraded by autolysosomes, proceeding autophagic flux contributes to increased LC3-II and decreased p62 protein expression. Chloroquine is a specific inhibitor to fuse autophagosomes with lysosomes resulting in impeding autophagic flux. Thus, the measurement of LC3-II and p62 with administration of chloroquine is also useful to evaluate autophagic flux.

As described above, autophagy requires Atg5 and Atg7. However, a recent study reported on a different...
form of autophagy that does not require Atg5 and Atg7, called “alternative autophagy” (Figure 2). In contrast to Atg5/Atg7-independent alternative autophagy, Atg5/Atg7-dependent autophagy is called “conventional autophagy”. Unlike conventional autophagy, alternative autophagy does not require LC3 but requires Ulk1, Beclin1, and membrane trafficking protein Rab9. Atg5-knockout mice embryos develop normally until the perinatal period, whereas Beclin1-deficient mice have embryonic lethality, suggesting that Atg5-independent alternative autophagic pathways exist during embryonic development.

These 2 distinct autophagic pathways in mammalian cells are remarkably adaptable to cellular stress responses. Previous reports have indicated that the source of the conventional autophagic pathway is the endoplasmic reticulum and the alternative autophagic pathway stems from the Golgi apparatus. However, there is an incomplete understanding of the mechanisms of alternative autophagy, the physiological and pathological assessments of both pathways, and the functioning of the 2 distinct pathways in vivo.

**Effect of Autophagy on the Heart**

**Autophagy and cardiac aging:** Autophagy is a crucial component of the aging process and is involved in the modulation of cardiac homeostasis and cardiac stress response. Once stimulated, autophagy upregulates in response to nutrient deprivation, ischemia, and hypoxia to degrade organelles and damaged proteins. This process recycles amino acids for maintaining cellular functions, energy production, and the synthesis of new enzymes.

Whereas there is accumulating evidence suggesting that autophagy may be sufficient to extend life span, autophagy in the heart decreases with age. It has been reported that cardiac-specific ablation of Atg5 protein in mice contributes to the development of dilated cardiomyopathy with age, suggesting that autophagy has a crucial role in maintaining cardiac homeostasis and that cardiac aging results when autophagy is inhibited. Caloric restriction and inhibition of the mammalian target of rapamycin (mTOR) have been shown to enhance longevity and promote autophagy. Caloric restriction and mTOR can reverse age-related cardiac hypertrophy and diastolic dysfunction. Inhibition of autophagy by Atg5 knockdown in Drosophila abolishes the protective effects of rapamycin on age-related changes.

**Autophagy in heart failure:** Heart failure is a progressive disease, characterized by cardiac remodeling. The initial response of the heart to pressure overload is hypertrophic growth. Persistent pressure overload forces changes in dilatation, leading to systolic dysfunction and heart failure. Although cardiac hypertrophy is induced in response to cardiac stress and is beneficial in the short term, over time it certainly contributes to heart failure.

Pressure overload induces an autophagic response in cardiac myocytes by overexpression of Beclin1. This increases autophagic flux, pathological remodeling response (such as hypertrophy), and fibrotic changes that often lead to systolic dysfunction. In contrast, heart failure is partially rescued by inhibiting autophagy with Beclin1 hetero-knockout. These data indicate that autophagy can manifest as a maladaptive response to pressure overload in cardiovascular disease.

In relation to the renin-angiotensin-aldosterone system, angiotensin II stimulation activates autophagy via the angiotensin II type 1 (AT1) but not angiotensin II type 2 (AT2) receptors in neonatal rat cardiomyocytes. Angiotensin II stimulation of AT1 receptors alone activates autophagy, whereas stimulation of AT2 receptors strongly decreases autophagy in a rat model of heart hypertrophy. Thus, an AT1 receptor antagonist may treat heart failure.
by reducing cell death caused by excessive autophagy. However, another study suggested that low autophagic flux in cardiac hypertrophy is required to elicit an adaptive response, and complete obstruction of this process generally has extremely negative results.\textsuperscript{(55)} For example, cardiac-specific abrogation of Atg5 leads to cardiomyopathy.\textsuperscript{(66)} An optimal zone of cardiomyocyte autophagy is required to adapt to pressure overload, and excessive autophagy may lead to unnecessary protein degradation of critical cellular components.

Recent studies suggest that cytosolic acetyl-coenzyme inhibits deacetylation of autophagy proteins and is therefore a negative metabolic regulator of autophagy. Therefore, maintenance levels of the protein acetylation are important for inhibiting the adverse autophagy induced by cardiac pressure overload.\textsuperscript{(56)} Another study demonstrated that maladaptive autophagy depends on histone deacetylases. Cardiac remodeling results from pressure overload and can be prevented by inhibiting histone deacetylation and suppressing autophagy.\textsuperscript{(57)}

**Autophagy in ischemic heart disease:** Ischemic heart disease is a major contributor to global morbidity and mortality. Autophagy in cardiomyocytes plays a paradoxical role in the body’s response to ischemia-reperfusion injury.\textsuperscript{(58-60)} Ischemia reduces supplies of nutrients and oxygen that are delivered to the myocardium. Autophagy is activated through AMP-activated protein kinase (AMPK)-mediated inhibition of mTOR in an adaptive manner in order to replenish metabolic substrates and eliminate damaged mitochondria.\textsuperscript{(59,60)} AMPK is a necessary molecule for initiating autophagy during periods of cardiac ischemia, and pharmacological inhibition of autophagy increases cardiomyocyte death, again suggesting that autophagy functions as an adaptive mechanism.\textsuperscript{(61)}

Once nutrients and oxygen are restored to the previously ischemic tissue during reperfusion, cardiac autophagy upregulates dramatically in rat, rabbit, and swine and primary neonatal cardiomyocytes.\textsuperscript{(62-65)} A previous study suggests that cardiac autophagy, upregulated by reperfusion, can be adaptive or detrimental. Reperfusion induces excessive autophagy by Beclin1 upregulation, which is an essential molecule for inducing autophagy and adapting to cardiac stress.\textsuperscript{(66)} During reperfusion, persistent activation of Beclin1 can be detrimental, causing excessive levels of autophagy, leading to cardiomyocyte damage and increasing cardiac injury.\textsuperscript{(67)}

**Autophagy in protein aggregation-related cardiomyopathy:** Autophagy dysfunction leads to disease progression, such as cardiomyopathy and skeletal myopathy.\textsuperscript{(67,68)} Glycogen accumulation causes hypertrophic cardiomyopathy.\textsuperscript{(69)} Danon disease is characterized by defects in autophagosome-lysosome fusion, where a mutation in LAMP2 disrupts intracellular catabolism, contributing to the accumulation of autophagic substrates in cardiomyocytes and skeletal muscle cells.\textsuperscript{(69,70)} Pompe disease is a lysosomal storage disease characterized by defective glycogen metabolism. Enzyme replacement therapy is an effective treatment for Pompe disease, but some resistance to treatment may result from accumulation of autophagic debris. The suppression of autophagosome formation, through the deletion of Atg7, contributes to the amelioration of the phenotype, facilitating successful enzyme replacement therapy.\textsuperscript{(71)}

**Diabetic cardiomyopathy:** Diabetic cardiomyopathy, initially described by Rubler in 1972, involves diabetes-associated changes in myocardial structure and function that occurs independent of coronary artery disease or hypertension.\textsuperscript{(72)} Recent studies have demonstrated that autophagic flux (the maturation process that turns autophagosomes into autolysosomes) is inhibited in diabetic cardiomyopathy.\textsuperscript{(73-75)} In diabetes, autophagy helps maintain normal cellular architecture and function. Activation of AMPK by metformin in diabetic mice retrieves cardiac autophagy, protecting cardiac apoptosis with Beclin1 activation by disrupting the Beclin1-Bcl2 complex, which is the inhibited form of Beclin1.\textsuperscript{(76,77)} Interestingly, it is reported that diabetic cardiomyopathy in type 1 diabetes is protected by up-regulation of Rab9, which modulates alternative autophagy and mitophagy instead of conventional autophagy.\textsuperscript{(78)}

**Effect of Autophagy on the Vasculature**

**Autophagy and vascular aging:** Aging is a risk factor for cardiovascular disease, and autophagy plays a crucial role in promoting longevity.\textsuperscript{(78)} Recently, several studies reported that age-related impairment of autophagy was related to vascular aging, potentially contributing to endothelial dysfunction, arterial stiffness, and vascular pathologies, such as atherosclerosis and calcification.\textsuperscript{(79,80)} Aging properties may be affected by increasing reactive oxygen species (ROS) levels in vasculature.\textsuperscript{(63)} Because autophagy clearly modulates oxidation-reduction deficiency, this process can cause worsening of cardiovascular disease, which is exacerbated by oxidative stress.\textsuperscript{(81)} Endothelial cells of old mice have lower Beclin1 levels and higher SQSTM1/p62 levels than those of young mice, suggesting that age-related changes in the vasculature reduce autophagic flux.\textsuperscript{(79)} Administration of trehalose or spermidine induces autophagy and reverses age-related arterial stiffening.\textsuperscript{(82-85)} Similarly, a recent study indicated that resveratrol, known to activate endothelial autophagy, reduces the development of arterial aging in rhesus monkeys.\textsuperscript{(83)}

**Autophagy in atherosclerosis:** Atherosclerosis is a chronic inflammatory disease of the arteries that progresses with age in accordance with risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, obesity, and cigarette smoking.\textsuperscript{(86,87)} Atherosclerosis involves the formation of arterial plaques. These plaques impinge on the structural components of vasculature, which is composed of vascular smooth muscle cells (VSMCs), an extracellular matrix, inflammatory cells (such as macrophages, T lymphocytes, dendritic cells, and mast cells), and lipids.\textsuperscript{(88,89)} Acute conditions, such as myocardial infarction, stroke, and sudden death, can result from the rupture of atherosclerotic plaques.

Autophagy in atherosclerotic plaques can play either a detrimental role. Recent studies demonstrate that autophagy plays a crucial role in the stability of atherosclerotic plaques and atherogenesis. This may eventually lead toward new therapeutic strategies for atherosclerosis, and it is therefore important to investigate the
mechanisms involved in plaque stability and plaque rupture to improve therapy strategies. VSMCs comprise the fibrous cap and may facilitate plaque rupture. Unstable plaques include a higher proportion of inflammatory lipid cells and a lower proportion of VSMC compared to stable lesions. Transmission electron microscopy reveals that autophagy occurs in the fibrous caps of experimental or human atherosclerotic plaques that contain VSMCs, and levels of LC3 are elevated in advanced human plaques on Western blot analysis. This suggests that autophagy is closely related to atherogenesis. Autophagy in atherosclerosis is caused by ROS, tumor necrosis factor-α, oxidized low-density lipoprotein (oxLDL), inflammatory mediators, osteopontin, and advanced glycation end products.

Several lines of evidence support the benefits of autophagy in VSMCs. In VSMCs and endothelial cells (ECs), autophagy activates in response to lipid peroxidation products or oxidized lipoproteins. Autophagy may play a role in the anti-inflammatory effects, induced by resveratrol administration, in ECs. Furthermore, ECs treated with oxLDL induced autophagy, which protects endothelial function. Other reports suggest that 7-ketocholesterol (7-KC)-induced autophagy reduced the death of VSMCs attributable to low concentrations of statins. Recently, it was shown that activation of autophagy by 7-KC was mediated by Nox4-induced ROS production and played a protective role. These studies indicate that autophagy could be important for protecting cells from developing atherosclerosis. Conversely, excessive autophagy in VSMCs or ECs is associated with reducing collagen synthesis, which may destabilize plaque and provoke platelet aggregation and lesion thrombosis.

**Autophagy in hypertension:** Hypertension is a common risk factor for cardiovascular disease and affects approximately 30% of the global population. Persistent high blood pressure contributes to robust hypertrophic cardiac growth. The main mechanisms of blood pressure regulation relate to the actions of renin-angiotensin-aldosterone, as well as the sympathetic and parasympathetic nervous systems and the antidiuretic effects of hormones. Hormones and peptides within these systems regulate autophagy, and disorders affecting these systems may lead to the development of hypertension.

Sympathetic premotor neurons maintain vasomotor tone in the rostral ventrolateral medulla (RVLM) and play an important role in neurogenic hypertension. Administration of drugs to inhibit autophagy in RVLM spontaneously reduces blood pressure in hypertensive rats, suggesting that autophagy could be a therapeutic option for treatment of neurogenic hypertension.

**Conclusion and perspective:** This review summarizes existing evidence of the crucial role of autophagy in cardiovascular disease and senescence. Autophagy is related to age-associated cardiovascular diseases, such as cardiac hypertrophy, heart failure, ischemic heart disease, cardiomyopathy, hypertension, and atherosclerosis. Because autophagy is essential for maintaining cardiovascular homeostasis, age-related impairment of autophagy may contribute to the development of cardiovascular disease. Therefore, pharmacological modulation of autophagy may be an emerging treatment option for patients with cardiovascular disease. A recent report revealed that dietary supplementation of the natural polyamine spermidine normalized the age-dependent impairments of aortic pulse wave velocity and arterial endothelium-dependent dilation by induction of autophagy. Another report demonstrated that administration of spermidine enhanced cardiac autophagy, which augmented elimination of damaged mitochondria and maintained mitochondrial respiration, leading to reduction of cardiac hypertrophy and prevention of diastolic dysfunction in old mice. In addition, spermidine-induced augmentation of autophagy contributed to delay the progression to heart failure in Dahl rats that were fed a high-salt diet. As shown in the Table, the relationship between autophagy and cardiovascular physiology is complex and excessive autophagy leads to cell death, so it is important to note that close attention must be paid to dos-

### Table. Beneficial and Detrimental Effects of Autophagy

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<th><strong>Beneficial effects of autophagy</strong></th>
<th><strong>Detrimental effects of autophagy</strong></th>
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<td>Artery</td>
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<td>atherosclerotic plaque stability and modulating atherogenesis</td>
<td>provoke plaque destabilization</td>
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<td>anti-inflammatory effects in ECs</td>
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<td>protect endothelial cell function</td>
<td>reduce collagen synthesis</td>
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<td>reverse arterial stiffening</td>
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<td>reduce the development of arterial aging</td>
<td>amplify the pathological remodeling</td>
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<td>Heart</td>
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<td>adaptive response to pressure overload</td>
<td>increase cardiomyocyte death during I/R</td>
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<td>protect cardiac apoptosis and improve cardiac function in diabetes</td>
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**References**

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Conflicts of interest:

The Framingham study suggests that there is a gender difference in the pathogenesis and progression of cardiovascular disease. The molecular mechanisms that comprise these gender differences are complex and are not fully understood. Pre-menopausal women are less prone to cardiovascular disease than age-matched men; however, this difference disappears following menopause. Issues relating to autophagy and gender remain poorly understood. Future studies should investigate gender differences in cardiovascular disease and aging, with a focus on the relationship between sex hormones and autophagy. These inquiries may give rise to gender-specific medicines that take advantage of the beneficial effect of autophagy induction in cardiovascular disease.

Disclosures

Conflicts of interest: None.

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20: 183-90.