Autophagy and oxidative stress in cardiovascular diseases

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Abstract

Autophagy is a highly conserved degradation process by which intracellular components, including soluble macromolecules (e.g. nucleic acids, proteins, carbohydrates, and lipids) and dysfunctional organelles (e.g. mitochondria, ribosomes, peroxisomes, and endoplasmic reticulum) are degraded by the lysosome. Autophagy is orchestrated by the autophagy related protein (Atg) composed protein complexes to form autophagosomes, which fuse with lysosomes to generate autolysosomes where the contents are degraded to provide energy for cell survival in response to environmental and cellular stress. Autophagy is an important player in cardiovascular disease development such as atherosclerosis, cardiac ischemia/reperfusion, cardiomyopathy, heart failure and hypertension. Autophagy in particular contributes to cardiac ischemia, hypertension and diabetes by interaction with reactive oxygen species generated in endoplasmic reticulum and mitochondria. This review highlights the dual role of autophagy in cardiovascular disease development. Full recognition of autophagy as an adaptive or maladaptive response would provide potential new strategies for cardiovascular disease prevention and management.

Keywords

autophagy; oxidative stress; atherosclerosis; cardiomyopathy; hypertension; heart failure

1. Introduction

Autophagy, first coined by Belgian biochemist Christian de Duve in 1966 [1], is characterized by the lysosome-dependent degradation of cytoplasm and damaged organelles such as mitochondria, endoplasmic reticulum and peroxisomes, as well as eliminating intracellular pathogens. In addition, autophagy serves as a dynamic recycling system to provide energy and building material for new protein and membrane production to promote survival under conditions of starvation [2]. Three types of autophagy have been defined: macroautophagy, microautophagy, and chaperone-mediated autophagy, each of which

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promotes proteolytic degradation of intracellular cargo at the lysosome. Autophagy related protein (Atg) composed protein complexes coordinate the formation of autophagosomes which fuse with lysosomes to generate autolysosomes. The contents are then degraded, and the breakdown products are released into the cytosol for synthetic and metabolic pathways[2]. Autophagy has been linked to cardiovascular diseases, as it is triggered by inflammation, hypoxia, oxidized lipoprotein, endoplasmic reticulum (ER) stress and reactive oxygen species (ROS), which are involved in atherogenesis [3-5]. Autophagy also promotes atherosclerotic plaque cell survival by degrading damaged intracellular components and protecting cells from apoptosis [6]. However, excessive autophagy may cause cell death. The observation that autophagy is activated during ischemia/reperfusion injury leads to the hypothesis that autophagy is a critical regulator of ischemia/reperfusion injury [7]. Indeed, autophagy promotes cell survival by eliminating dysfunctional mitochondria, which may otherwise release ROS leading to cell death [8]. Moreover, autophagy has diverse effects on cardiomyopathy - whereas augmented autophagy ameliorates dilated cardiomyopathy; autophagy activation promotes diabetic cardiomyopathy [9-11]. Furthermore, autophagy may antagonize ventricular hypertrophy by increasing protein degradation and decreasing tissue mass. Thus, autophagy may be an adaptive response in heart failure [12]. Autophagy also protects the heart by maintaining contractile function, as autophagosome dysfunction is associated with increased apoptosis, mitochondrial injury, intracellular Ca^{2+} dysregulation and cardiac dysfunction [13]. Lastly, autophagy mediates oxidative stress-elicited myocardial injury in response to ischemia/reperfusion [14]. This review will address the above points in detail.

1.1. Definition of autophagy

Autophagy, literally "self-eating" in Greek, is the major intracellular degradation system by which cellular components, including organelles and protein aggregates, are delivered to and degraded in the lysosome [2, 15]. The purpose of autophagy is not limited to elimination of cell cargo waste, but instead, autophagy serves as a dynamic recycling system to provide energy and building material for new protein and membrane production. Thus, autophagy helps maintain the health of cells and tissues by replacing impaired cellular components with fresh ones. In addition, it supplies endogenously-derived nutrients for energy generation to promote survival under conditions of starvation [2].

1.2. Induction of autophagy

In eukaryotic cells, autophagy is induced by starvation, hypoxia, hormones, ER stress, redox stress, mitochondrial damage, pathogen-associated molecular patterns or danger-associated molecular patterns to degrade protein aggregates, oxidized lipids, damaged organelles, and even intracellular pathogens [16-18]. In addition, autophagy promotes cellular senescence [19], cell surface antigen presentation [20, 21], lipid metabolism [22], protects against genome instability [23-25] and prevents necrosis [26]. Hence, autophagy plays a key role in preventing disease. Dysregulation of autophagy is linked to cancer[27], neurodegeneration [28], metabolic syndrome [29], liver disease [30], autoimmune diseases and infections [31].

1.3. Substrates of autophagy

Autophagy can be classified as nonselective or selective. As an adaptive response to nutrient starvation, nonselective autophagy promotes bulk degradation of cytoplasm and organelles to provides amino acids and lipid substrates for intermediary metabolism [2]. Deficiencies in autophagy result in maladaptation to starvation and poor survival due to insufficient amino acid production and protein synthesis. Autophagy also occurs at low levels to mediate global turnover of cytoplasmic materials under nutrient-rich conditions [15]. Selective autophagy involves degradation of organelles and protein aggregates. Selective autophagy of protein aggregates and peroxisomes [32] involves ubiquitination of target proteins, which are recognized by autophagy receptors, such as p62 or Nbr1, which bind both ubiquitin and microtubule-associated protein 1 light chain 3 (LC3) to deliver cargo to autophagosomes [2, 33, 34]. Under specific conditions, selective autophagy of organelles such as mitochondria (mitophagy) [35], ribosomes (ribophagy) [36], endoplasmic reticulum (reticulophagy) [37], peroxisomes (pexophagy) [38], and lipids (lipophagy) [39] occurs. For instance, mitophagy is triggered by mitochondrial permeability transition pore opening and loss of mitochondrial membrane potential [40].

1.4. Types of autophagy

Three types of autophagy have been defined: macroautophagy, microautophagy, and chaperone-mediated autophagy, each of which promotes proteolytic degradation of intracellular cargo at the lysosome. Macroautophagy transfers soluble cytoplasmic materials and organelles to the lysosomes by utilizing the intermediate organelle called the autophagosome, which is a product of an isolation membrane (termed phagophore) enclosed within a small portion of the cytoplasm. The autophagosome then fuses with the lysosome to form an autolysosome and degrade the materials contained within [15] (Fig.1). In microautophagy, lysosomes directly engulf small pieces of the cytoplasm by inward invagination of the lysosomal membrane [15]. In chaperone-mediated autophagy, substrate proteins containing a KFERQ-like pentapeptide sequence are first bound with cytosolic Hsc70 then translocate into the lysosomal lumen, where they are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A, resulting in their unfolding and degradation [41]. Both macroautophagy and microautophagy are able to consume large structures through selective or non-selective mechanisms. The degradation products of all three types of autophagy can be used for new protein synthesis, energy production or gluconeogenesis. Among these, macroautophagy is the predominant form and the most extensively studied [42]. This review will focus on the role of macroautophagy (autophagy hereafter) in cardiovascular diseases.

1.5. Process of autophagy

The first Atg gene was identified in 1997 [43]. Genetic deletion of Atg genes revealed the critical roles of autophagy in physiological adaptation to stress and also a connection between defective autophagy and various diseases. Atg proteins are comprised of four major functional groups: (1) the Atg1/unc-51-like kinase (ULK) complex (Atg1/ULK1–Atg13–Atg17) that responds to upstream signals; (2) the Beclin 1/class III phosphatidylinositol 3-kinase (PI3K) complex (Atg6/Beclin1, Atg14, Vps34/PI3KC3, and Vps15) that mediates

vesicle nucleation [44]; (3) two ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems that mediate vesicle expansion; (4) Atg9 and its cycling system which provide lipids to the isolation membrane. These Atg-composed protein complexes coordinate the formation of autophagosomes. The Atg1/ULK1 complex (Atg1 in yeast and ULK1 in mammals) is an initial regulator of autophagosome formation [34]. Under nutrient-rich conditions, ULK1 is bound by mammalian target of rapamycin (mTOR) complex 1(mTORC1), which phosphorylates ULK1 and inhibits autophagy initiation. Whereas in starvation, mTORC1 dissociates from the ULK1 complex, releasing it to trigger autophagosome nucleation and elongation. In addition, ULK1 inhibits the kinase activity of mTORC1 through binding with raptor to induce its phosphorylation [45]. Adenosine monophosphate–activated protein kinase (AMPK) mediates the upstream signaling of mTORC1 which controls the cellular energy–sensing pathway [34]. In response to high concentrations of AMP and its effects on energy depletion, AMPK is activated, which, in turn, inhibits mTORC1 and promotes autophagy [2]. Autophagy induction promotes ULK1 complex activation, which phosphorylates Beclin-1 on Ser14, thereby enhancing the activity of Beclin1-Atg14-Vps34-Vps15 class III PI3K core complexes to promote autophagosome nucleation [46]. Additionally, under nutrient-rich conditions, Bcl-2 binds with the BH3 domain of Beclin 1 and inhibits autophagy. The phosphorylation of Bcl-2 and Beclin 1 disrupts this interaction and releases Beclin 1 [47]. When nutrients are abundant, Atg9 shuttles between the Golgi and endosomes. In starvation, Atg9 distributes to endosomal compartments to supply a membrane source for forming autophagosomes. Recent findings suggest Atg9 plays an essential role in regulating oxidative stress-induced JNK activation and autophagy through interaction with tumor necrosis factor receptor-associated factor 6 [48-50]. Two ubiquitin-like molecules, Atg12 and Atg8 (called LC3 in mammals) are involved in expansion of autophagosome membranes. Atg12 is first activated by Atg7 which acts like an E1 ubiquitin activating enzyme in an ATP-dependent manner. Atg12 is then covalently linked to Atg5 by Atg10, an E2-like ubiquitin carrier protein. Conjugated Atg5–Atg12 complexes bind Atg16L1 to form pre-autophagosomal structures that induce a curvature into the growing phagophore through asymmetric recruitment of processed LC3B-II. LC3B is proteolytically cleaved by protease Atg4 togenerate LC3B-I. The carboxy terminal glycine exposed by Atg4-dependent cleavage is activated by the E1-like Atg7 and then transferred to Atg3. As a result, phosphatidylethanolamine is conjugated to the carboxyl glycine to generate LC3B-II. Both Atg5–Atg12 and LC3B-II contribute recruitment and integration of LC3B-II into the growing phagophore. The synthesis and processing of LC3 are commonly used to monitor the progress of autophagy because LC3-II remains on mature autophagosomes until after fusion with lysosomes to generate autolysosomes. The contents are then degraded by proteases, lipases, nucleases, and glycosidases, and the breakdown products such as amino acids, lipids, nucleosides, and carbohydrates are released into the cytosol for synthetic and metabolic pathways [2] (Fig.1).

1.6. Autophagic flux measurement

Because the accumulation of autophagosomes may indicate autophagy induction or impairment of autophagolysosomal maturation, autophagic flux assays are necessary to distinguish between autophagy induction and a block in downstream steps [51]. Several of these assays are discussed here. (1) LC3 turnover assay: based on the observation that LC3-

II is degraded in autolysosomes, LC3 turnover is one principal method currently used to measure autophagic flux. Inhibition of autophagosome-lysosome fusion and thereafter protein degradation by lysosomotropic reagents or lysosomal proteases inhibitors will result in the accumulation of LC3-II, and the elevated levels of LC3-II indicate that autophagic flux is increased [52]. (2) Degradation of LC3 and its substrates: LC3-II increases transiently upon autophagy induction, and deceases during prolonged autophagy activation. Thus the amount of total cellular LC3 and its substrate p62, quantified by immunoblot analysis or flow cytometry, inversely correlates with autophagic flux [53]. (3) Delivery of mRFP-GFP-LC3 to the lysosome: based on the concept that the low pH inside the lysosome quenches the fluorescent signal of GFP, but not RFP. Therefore, an mRFP-GFP-LC3 tandem construct labels autophagosomes with yellow and autolysosomes with red. The increase of both yellow and red punctae indicates increased autophagic flux. Notably, each of these three methods has its own limitations. For example, autophagic flux can be detected even under basal conditions, LC3 and p62 can be transcriptionally regulated during autophagy, and sometimes autolysosomes are observed as yellow due to different acidification and degradation capacities of the lysosome. Given the limitations of the individual assays discussed above, a combination of these methods would provide the most precise prediction of autophagic flux in different cell types and experimental contexts [51].

2. Autophagy and cardiovascular diseases

Autophagy plays dual roles in cardiovascular diseases through adaptive or maladaptive regulation. Physiological autophagy serves as a protective mechanism to maintain normal cardiovascular function. However, impaired autophagy contributes to disease development. A better understanding of the function of autophagy in the cardiovascular system could provide new therapeutic avenues for disease prevention or control. As a consequence, the role of autophagy in the cardiovascular system is currently under intense investigation.

2.1. Autophagy in atherosclerosis and cardiac ischemia

2.1.1. Autophagy in atherosclerosis—The discovery of autophagy-like ultrastructural features such as vacuolization and formation of myelin figures by transmission electron microscopy and expression of the autophagy marker LC3-II by western blot analysis of human carotid plaques reveals that autophagy is one major component in the process of atherosclerosis [54]. It is well established that inflammation, hypoxia, oxidized lipoprotein, ER stress and ROS are all involved in atherogenesis. *In vitro* studies have demonstrated that these factors present in atherosclerotic plaques could also serve as the triggers of autophagy [3-5]. Notably, autophagy in atherosclerotic plaques could be either beneficial or detrimental. On one hand, basal autophagy promotes plaque cell survival by successful degradation of damaged intracellular components, and thus protects cells against oxidative stress [6]. In addition, the engulfment of defective mitochondria by autophagosomes limits the release of cytochrome C into the cytosol and protects cells from apoptosis [55]. Autophagy is induced in endothelial cells (ECs) or smooth muscle cells (SMCs) in response to oxidized lipoprotein or lipid peroxidation products to promote cell survival [5]. Autophagy also may mediate some anti-inflammatory effects of resveratrol in EC [56]. Another study shows upregulation of autophagy attenuates 7-ketocholesterol-(a major

component of oxidized lipoproteins) induced cell death in SMCs [57]. Together these findings indicate that autophagy might be protective in atherogenesis. Indeed, recent studies suggest autophagy favors plaque stabilization by regulating lipid metabolism. Autophagy delivers lipid droplets to lysosomes, where they are hydrolyzed by lysosomal acid lipase to generate free cholesterol for efflux. Therefore, macrophage foam cell cholesterol efflux is mediated by autophagy [58]. Wild-type p53-induced phosphatase 1(Wip1) is considered to control autophagy-dependent macrophage cholesterol efflux [59]. Autophagy in macrophages is also protective in that disruption of autophagy in macrophages in advanced plaques increases apoptosis and oxidative stress, thus worsening efferocytosis and promoting plaque necrosis [60]. Loss of macrophage autophagy increases plaque formation in part through inflammasome hyperactivation and increased cholesterol crystal formation, suggesting that intact autophagy suppresses the inflammasome, which is essential in atheroprotection [61]. As a consequence, successful autophagy could stabilize the atherosclerotic plaques and reduce adverse vascular events [3, 62].

On the other hand, excessive autophagy in SMCs or ECs may cause cell death. SMC death destabilizes plaques due to the decreased synthesis of collagen which results in a thinner fibrous cap. Autophagy mediated EC death is also the result of sustained ER stress in the atherosclerotic lesion [4]. EC death may be detrimental by promoting thrombosis and clinical events. However, macrophage death is considered a promising approach for plaque stabilization [63]. Recent evidence suggests that phagocytosis of macrophages dying through autophagy results in inflammasome activation and inflammatory factor release [64]. The above findings suggest that appropriate manipulation of autophagy would foster its beneficial effects in stabilizing the plaque by promoting cell survival and reducing cell death.

2.1.2. Autophagy in cardiac ischemia/reperfusion—The direct effect of atherosclerosis is cardiac ischemia, and autophagy activation in response to myocardial ischemia was documented 30 years ago [7]. Hypoxia triggers significant formation of autophagosomes adjacent to swollen and fragmented mitochondria [7], suggesting there may be interaction between autophagy and mitochondria during cardiac ischemia. The findings that autophagy inhibitors promoted cardiac myocyte death under glucose starvation suggested that autophagy could be protective by replenishing depleted energy stores and removing the toxic dysfunctional organelles. Going further, this study indicates that AMPK may participate in ischemia-induced autophagy that promotes cell survival by eliminating dysfunctional mitochondria, which would otherwise become a source of ROS and pro-apoptotic mediators [8].

A second wave of autophagy activation is triggered by reoxygenation following ischemia. More autophagosomes are detected after ischemia/reperfusion as compared to hypoxia alone [7]. Autophagy could be either adaptive or maladaptive in the context of ischemia/reperfusion injury. Some studies suggest autophagic flux is impaired during ischemia/reperfusion at the level of autophagosome formation and lysosome degradation. In addition, impaired autophagosome clearance is associated with increased ROS and mitochondrial permeabilization, resulting in cell death. This process is accompanied by ROS-mediated Beclin-1 upregulation and a decline in lysosome-associated membrane protein-2 and

impaired autophagosome processing [65]. In contrast, other studies show that overexpression of Beclin1 enhances autophagic flux and protects cardiac cells from ischemia/reperfusion injury [66]. Consistent with this result, Beclin $1^{+/-}$ mice manifested attenuated autophagy, with less infarction and suppressed apoptosis [8]. Additionally, new signaling involving BCL-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) is a recently identified player in ischemia/reperfusion injury. As a downstream target of hypoxia-inducible factor 1α, BNIP3 permeabilizes cardiac mitochondria and promotes mitochondrial fission, causing mitochondrial dysfunction and cardiomyocyte death during ischemia/reperfusion injury. This process is related to autophagosome formation. Forced expression of BNIP3 stimulates autophagy in cardiac myocytes [67]. Autophagy serves as a protective mechanism in the setting of BNIP3 expression in cardiac myocytes, as inhibition of autophagy increases BNIP3-induced cardiomyocyte death by preventing removal of damaged mitochondria [67]. Conversely, BNIP3 expression appears to trigger autophagy and leads to a decline in lysosome abundance in cardiac myocytes. The resultant autophagosome accumulation prevents its pro-survival role and triggers cardiomyocyte death [37].

Another important signaling protein in ischemia/reperfusion is glycogen synthase kinase-3β (GSK-3β), which regulates autophagy during prolonged ischemia in a time-dependent manner. In the initial phase of ischemia, activation of GSK-3β stimulates autophagy, whereas during subsequent reperfusion, inactivation of GSK-3β inhibits autophagy. Such time-dependent regulation of endogenous GSK-3β adapts the cardiac myocyte to ischemia and protects against reperfusion injury [68]. In another pathway, ischemia-induced autophagy activation is dependent on AMPK activation. In contrast, reperfusion-augmented autophagy is dependent on Beclin1 instead of AMPK. Collectively, these results indicate the distinct roles of autophagy during ischemia and reperfusion. Autophagy may play an adaptive role during ischemia and a maladaptive role during reperfusion [8]. Thus, precise control of GSK-3β or AMPK at different stages of ischemia/reperfusion would provide a novel strategy to enhance survival under ischemic conditions and protect against cardiac myocyte death from reperfusion injury.

2.2. Autophagy in cardiomyopathy

Cardiomyopathy is classified as dilated, hypertrophic or restrictive cardiomyopathy. Lamin A/C gene (LMNA) mutation-related dilated cardiomyopathy is characterized by left ventricular enlargement and decreased systolic function accompanied by arrhythmias and other systemic diseases. Defective autophagy is one major feature in LMNA cardiomyopathy, which could be caused by overexpression of dual specificity phosphatase 4 in the heart and upregulated AKT-mTOR signaling. Pharmacological interventions improve cardiac function, which is correlated with enhanced autophagy [9-11]. A recent finding is that mammalian target of rapamycin complex 1 mediates LMNA deficiency-induced dilated cardiomyopathy. Elevated mTORC1 signaling impairs autophagy, and mTORC1 inhibition with rapalogs extends survival in the LMNA deficient mice through restoration of autophagic flux and improved cardiac function [69]. The desmin-related cardiomyopathy (DRC) characterized by accumulation of misfolded proteins is triggered by a missense mutation in the alphaB-crystallin (CryAB) gene. Autophagy is considered an adaptive

response in this proteo-toxic form of cardiomyopathy, which is evidenced by accelerated heart failure and early mortality in DRC mice with genetic ablation of beclin1, a gene required for autophagy [70]. In addition, transgenic overexpression of the mutant desmin or CryAB (R120G) in mice *in vivo as well as in vitro* upregulates p62 mRNA and protein levels which protects cardiomyocytes from misfolded protein induced cell injury and death by maintaining responsive autophagosome formation and autophagy [71]. Atg7 induces basal autophagy. Sustained Atg7 expression rescues impaired autophagy in the CryAB (R120G) hearts with decreased cardiac hypertrophy and prolonged survival, suggesting autophagy activation would be a viable therapeutic strategy for ameliorating desmin-related cardiomyopathy [12, 72]. Dilated cardiomyopathy is linked with suppressed mitophagy, which is the result of deficient mitofusin-2. Ablation of mitofusin-2 prevented the translocation of Parkin to the mitochondria and Parkin-mediated ubiquitination [73]. The proapoptotic kinase Mst1 phosphorylates Beclin1 at Thr-108, which enhances its interaction with Bcl-2 and/or Bcl-xL, and inhibits the phosphatidylinositide 3-kinase activity of the Atg14L-Beclin1-Vps34 complex, thus suppressing autophagy and providing a mechanism for the development of dilated cardiomyopathy in man [74]. Very recently, the cardioprotective effects of macrophage migration inhibitory factor (MIF) against doxorubicin-induced cardiomyopathy has been recognized to function through augmented autophagy [75]. Knock down of myeloid cell leukemia-1 (MCL-1), an anti-apoptotic BCL-2 protein, in the adult heart led to rapid development of cardiomyopathy and death, which was associated with impaired induction of autophagy in the heart [76]. The above studies imply that defective autophagy contributes to cardiac dysfunction and cardiomyopathy development; therefore, pharmacologic interventions to augment autophagy might improve cardiac function and ameliorate cardiomyopathy.

In contrast, autophagy may promote cardiomyopathy. For instance, histone deacetylases (HDACs) regulate cardiac plasticity. HDAC activity is required for stress-induced cardiomyocyte autophagy which is linked to load-induced cardiac hypertrophy. HDAC inhibitors have antihypertrophic effects due to the unique action of inhibiting augmented autophagic flux [77]. Diabetic cardiomyopathy, first introduced by Rubler in 1972 [78], has been defined as ventricular dysfunction that occurs independently of coronary artery disease and hypertension. Changes in myocardial structure, Ca^{2+} signaling and metabolism have been implicated in the pathogenesis of diabetic cardiomyopathy. Recently, the essential role of autophagy in diabetic cardiomyopathy has been investigated intensively. Diabetes induces cardiomyocyte apoptosis and suppresses cardiac autophagy, which is linked with diabetic cardiomyopathy. Activation of AMPK by metformin restores cardiac autophagy and prevents cardiomyopathy presumably through disruption of Beclin1-Bcl-2 complex and protects against cardiac apoptosis [79, 80]. Interestingly, deficiency in autophagy protects cardiac function in type 1 diabetes through upregulation of Rab9 regulated alternative autophagy and mitophagy [81]. Another study shows that cardiac hypertrophy and dysfunction in type-2 diabetes is dependent on saturated fatty acid and sphingolipid synthesis. In particular, ceramide synthase-5 is involved in lipid-induced autophagy and lipotoxic cardiomyopathy [82]. Due to the important effects of autophagy in metabolic disease-induced cardiomyopathy, autophagy could serve as a biomarker for cardiac dysfunction and cardiomyopathy in diabetes.

2.3. Autophagy in heart failure

To date, heart failure remains one of the leading causes of death in the United States. An estimated 5 million Americans have heart failure with a mortality rate of approximately 50% in 5 years [83]. Heart failure is a progressive disease characterized by adverse ventricular remodeling which involves changes in the balance between cardiomyocyte protein synthesis and degradation. It is recognized that the autophagy–lysosome pathway is a house-keeper in cardiomyocytes under physiological conditions. However, the role of autophagy in heart failure is controversial. For instance, autophagy may antagonize ventricular hypertrophy by increasing protein degradation and decreasing tissue mass. As a result, autophagy may be an adaptive response to heart failure. In the mouse heart, autophagy induced by sustained expression of Atg7 ameliorates ventricular dysfunction, decreases cardiac hypertrophy, and prolongs survival. These findings suggest that activation of autophagy may be a viable therapeutic strategy for improving cardiac performance under proteotoxic conditions [12]. However, the efficiency of protective autophagy declines with age, leading to abnormal intracellular protein aggregates, which result in enhanced oxidative stress, decreased ATP production, and cell death. Oxidative stress sensitizes the heart to the renin-angiotensin-aldosterone system, inducing autophagic type-II programmed cell death and increasing the propensity for adverse cardiac remodeling, diastolic dysfunction and heart failure [84]. Angiotensin II (AngII) increases mitochondrial ROS in cardiomyocytes, concomitant with increased autophagy in hearts of angiotensin II-treated mice [47]. AngII type I (AT1) receptor mediates autophagosome formation in response to AngII stimulation. This response, however, is blocked by co-expression of the AngII type 2 (AT2) receptor in neonatal cardiomyocytes [85]. A robust autophagic response in cardiomyocytes is elicited by pressure overload stress, this response is maladaptive, as excessive autophagy in load-induced heart failure leads to autophagic cell death, loss of cardiomyocytes, and may contribute to the worsening of heart failure. Mechanical unloading with a left ventricular assist device attenuated autophagy, reduced energy demand and improved function of the failing human heart [86].

Autophagy in cardiomyocytes is affected by a variety of stimuli, such as lipid and glucose. High fat diet (HFD) disrupts autophagosome maturation at the step of autophagosomes fusion with lysosomes. This impaired autophagic flux is associated with increased apoptosis, mitochondrial injury, intracellular Ca^{2+} dysregulation and cardiac dysfunction [13]. Akt2 was considered to play a predominant role in HFD-induced cardiac hypertrophy and contractile dysfunction. Akt2 ablation has cardioprotective effect in that it rescues HFD-induced disruption of the autophagosome maturation process and facilitated the transition from autophagosomes to autophagolysosomes[13]. Conversely, compared with normal glucose (5.5 mM), high glucose (17 or 30 mM) decreases autophagic flux in cardiomyocytes. In addition, high glucose-induced cardiomyocyte death is attenuated by suppression of autophagy by 3-methyladenine or silencing of the Becn1 or Atg7 gene. In contrast, augmentation of autophagy with rapamycin or overexpression of Becn1 or Atg7 predisposes cardiomyocytes to high glucose toxicity. These results indicate that reduced autophagic flux is an adaptive response that serves to limit high glucose-induced cardiac toxicity [87]. Autophagy also protects the heart from intermittent hypoxia-induced systolic dysfunction by maintaining contractile function [88]. miR-212 and miR-132, two important

miRNA involved in cardiac hypertrophy, are upregulated in hypertrophic cardiomyocytes. Overexpression of miR-212 and miR-132 leads to impaired autophagic response to starvation, hypertrophy and heart failure. MiR-212/132 deletion protects mice from pressure-overload-induced heart failure [89]. The cytokine MIF elicits cardioprotective effects through AMPK activation. As an illustration of its importance, MIF deficiency exacerbates left-ventricular dysfunction following starvation. This process is mediated by interrupted starvation-induced autophagic vacuole formation and exacerbated starvation-induced cell death. These results indicate that MIF preserves cardiac contractile function under starvation by regulating autophagy [90]. A recent study showed that mitochondrial homeostasis and autophagy are dependent on MCL-1 which is based on the observation that cardiomyocyte-specific MCL-1 knockout mice developed rapid cardiomyopathy and heart failure [76]. Finally, the regulatory associated protein of mTOR (Raptor) is an mTOR binding partner that mediates mTOR signaling to downstream targets. Ablation of raptor reduces myocardial mTORC1 activity, leading to heart failure, dilated cardiomyopathy and high mortality, which is associated with apoptosis and augmented autophagy in cardiomyocytes [91]. In summary, autophagy, controlled by a variety of factors, may antagonize ventricular hypertrophy and alleviate heart failure by increasing protein degradation and decreasing tissue mass. On the other hand, excessive autophagy under certain conditions such as pressure overload leads to cell death, and may contribute to the worsening of heart failure.

2.4. Autophagy in hypertension

Hypertension is classified as primary hypertension and secondary hypertension. Primary hypertension accounts for 90–95% of cases without obvious medical cause. The remaining 5–10% of cases are secondary effects of diseases in kidneys, arteries, heart or endocrine system. The heart exhibits robust hypertrophic growth in response to hypertension, which is regulated by autophagy. Essential cellular elements eliminated by excessive autophagy may provoke cell death and contribute to hypertension-related heart disease [92]. Excessive proliferation and resistance to apoptosis of pulmonary artery smooth muscle cells (PASMCs) are features of pulmonary arterial hypertension. PASMC proliferation facilitates vascular remodeling, leads to narrowed vascular lumen and increased pulmonary vascular resistance, and eventually increased pulmonary arterial pressure. Recently, chloroquine, a widely used antimalarial and antirheumatoid drug, was shown to prevent the development of monocrotaline-induced pulmonary hypertension by inhibition of autophagy. Monocrotaline-induced pulmonary hypertension is characterized by increased expression of LC3B-II and reduced expression of p62 in muscularized small pulmonary arteries, which is accompanied by increased medial thickness and proliferation of PASMCs. Chloroquine inhibited autophagy and restored p62 levels in the media of small pulmonary arteries *in vivo*, and was associated with inhibition of proliferation and induction of apoptosis in PASMCs in small pulmonary arteries. The underlying mechanism was attributed to chloroquine preventing acidification of the lysosome and subsequent processing of the autophagosome, thus preventing degradation of bone morphogenetic protein type II receptor (BMPR-II). The conserved intact BMPR-II signaling along with impaired autophagy contributes to a pro-apoptotic, anti-proliferative phenotype in PASMCs, indicating that autophagy is involved in pulmonary hypertension [93]. In another model, sympathetic premotor neurons that maintain

vasomotor tone in the rostral ventrolateral medulla (RVLM) play a pivotal role in neurogenic hypertension. Drugs inhibiting autophagy in RVLM decreased hypertension in spontaneously hypertensive rats, suggesting autophagy could be a therapeutic target in the control of neurogenic hypertension [94]. Taken together, these studies suggest that autophagy promotes pulmonary hypertension and neurogenic hypertension. Inhibition of autophagy could provide a new therapeutic strategy in the management of hypertension.

2.5. Autophagy and oxidative stress in cardiovascular disease

Emerging evidence indicates oxidative stress plays an important role in cardiovascular disease development. Dysregulation of autophagy renders cardiomyocytes more prone to ischemia-induced injury and cardiac remodeling [95]. H_2O_2 significantly increased both autophagosomes and autolysosomes and, thus, autophagic flux in cardiac myocytes. N-2-mercaptopropionyl glycine (MTG), an antioxidant, attenuates autophagy in the presence of H_2O_2 *in vitro*, as well as autophagic flux in ischemia/reperfusion-induced oxidative stress *in vivo*. The impaired autophagy *in vivo* is accompanied by a decrease in the size of myocardial infarction. Moreover, Beclin1+/- mice have reduced myocardial infarction after ischemia/reperfusion, whereas MTG treatment results in no additional reduction of infarct size. These results imply that autophagy mediates myocardial injury by increasing the oxidative stress elicited by ischemia/reperfusion [14]. In cardiac myocytes, ROS are also stimulated by glucose deprivation. Inhibition of ROS disrupted autophagy induced by glucose deprivation, emphasizing the important effects of oxidative stress on the activation of autophagy in cardiac myocytes induced by energy stress [96]. In the context of hypertension and diabetes, oxidative stress sensitizes the heart to the renin-angiotensin-aldosterone system, induces autophagic type-II programmed cell death, and leads to accelerated cardiac remodeling and cardiac dysfunction. Adiponectin, an adipokine known to mediate cardioprotective effects, demonstrates antioxidant potential to attenuate autophagy induced by excessive ROS in cardiomyocytes by inhibiting H_2O_2-induced AMPK/mTOR signaling [84]. Mitophagy plays an important role in mitochondrial quality control and cellular homeostasis through selective degradation of dysfunctional mitochondria. Dysfunctional mitochondria produce excess ROS which triggers oxidative stress. In the diabetic heart, mitophagy was decreased as evidenced by decreased Pink and Parkin, accompanied by decreased Lamp1 levels. As expected, impaired mitophagy in the diabetic heart increased ROS generation and oxidative protein damage, and the antioxidant enzyme MnSOD was also decreased. Notably, Beclin-1 deficiency restored mitophagy, and the MnSOD level was partially restored, and thereafter, ROS generation and oxidative protein damage was attenuated. These results indicate that inhibition of autophagy improves mitophagy and protects the heart from diabetes-induced oxidative injury [81]. Furthermore, autophagy also mediates inflammatory responses in cardiac myocytes. For instance, monocyte chemotactic protein-1 (MCP-1) promotes the development of heart failure by inducing oxidative stress. MCP-1-induced protein (MCPIP) mediates the downstream effects of oxidative stress, including ER stress, autophagy and cell death, and these processes are inhibited by inhibitors of oxidative stress. In addition, inhibitors of ER stress inhibit autophagy and cell death. These findings indicate that the elevated MCP-1 levels associated with chronic inflammation may contribute to the development of heart failure through oxidative stress-induced ER stress and autophagy [97].

Conversely, autophagy serves to protect against disease development by mediating protein quality control. AntimycinA (AMA) induces mitochondrial stress and increases mitochondrial superoxide generation, as well as augments nuclear DNA oxidation and cell death in cardiomyocytes. Upregulated autophagy by rapamycin promotes mitochondrial clearance and protects cardiomyocytes from the AMA-induced cell injury. In addition, the accumulation of ubiquitinylated proteins induced by AMA is suppressed by autophagy. Hence, autophagy induction could become a potential therapeutic strategy against oxidative stress-mediated injury in cardiomyocytes [98]. Autophagy also plays a protective role in AngII induced oxidative stress. Atg5+/- mice have impaired autophagy and increased production of ROS, which activates nuclear factor κB (NF-κB) in macrophages and increases cardiac inflammation. As a consequence, Atg5+/- mice are associated with increased cardiac fibrosis. Thus, intact autophagy signaling protects the heart from hypertension-induced inflammation and cardiac injury [99]. In addition to mitochondria, ROS production in cardiomyocytes during glucose deprivation is dependent on NADPH oxidase-4 expressed in the ER. ROS produced in the ER induce autophagy in the cardiomyocytes and promote survival in response to energy stress. These effects are mediated by the protein kinase RNA-activated-like endoplasmic reticulum kinase/eukaryotic initiation factor-2α/activating transcription factor-4 pathway [100]. To summarize, depending on the pathologic environment, autophagy may mediate oxidative stress-induced cell injury and cell death through autophagic type-II programmed cell death or protect against cell injury from ROS produced by mitochondria or the ER.

3. Manipulation of cardiac autophagy

The transition of autophagy from an adaptive phase to a maladaptive phase plays a vital role in driving the development of cardiovascular disease. As described above, autophagy can have either beneficial or detrimental effects in the cardiovascular system. Adaptive autophagy promotes survival in response to hypoxia and oxidative stress by removing damaged organelles as well as recycling macromolecules to maintain energy levels and support protein synthesis. In contrast, prolonged hypoxia and subsequent reperfusion result in maladaptive autophagy which causes cell death through excessive self-digestion of essential organelles and proteins or inducing apoptosis. Thus, manipulation of autophagy may represent a potential therapeutic target to treat or prevent development of heart disease. Lysosomal-associated membrane protein 2 (Lamp-2) is a ubiquitous lysosomal membrane protein required for the proper fusion of lysosomes with autophagosomes [101]. Lamp-2 depletion results in the inhibition of cytoprotective autophagy signaling secondary to the failure of fusion between lysosomes and autophagosomes which is linked to Danon disease, characterized by severe cardiomyopathy [102]}. The failure of autolysosome fusion is a hallmark of the maladaptive autophagy which results in the accumulation of autophagic vacuoles and the autophagosome-associated proteins LC3 and p62, coupled with increased apoptosis [103]. Autophagic flux assays may be used to determine the autophagic status in pathological conditions; however, the lack of suitable assays for measuring the ongoing autophagic flux in humans limits the feasibility of identifying the optimal window of autophagic activation to exploit the cardioprotective effects of macroautophagy without disrupting cardiac homeostatic mechanisms. A combination of measurement of circulating

LC3 levels in the blood and *in vivo* imaging of autophagy markers in the heart would provide better information about disease progression and generate corresponding therapeutic approaches. Indeed, animal studies have yielded promising results. Upregulated autophagy has beneficial effects on cardiovascular disease management. *In vivo* work indicates sustained expression of autophagy-related 7 in the CryAB (R120G) hearts leads to decreased cardiac hypertrophy, ameliorated ventricular dysfunction, and prolonged survival [12]. In addition, Inhibition of mTOR by everolimus limits infarct size and attenuates adverse left ventricular remodeling after myocardial infarction in mice [8]. Moreover, chronic AMPK activation by metformin prevents cardiomyopathy by upregulating autophagy in diabetic mice [104]. Recently, Xu *et al.* reported that rapamycin restored autophagy activity in the hearts of cardiomyocyte-phosphatase and tensin homolog knockout mice, reversing hypertrophic cardiomyopathy [105]. In contrast, downregulation of autophagy may also protect cardiac function. For instance, histone deacetylase inhibitors attenuate cardiac hypertrophy and improve cardiac function by suppressing autophagy [77]. In addition to testing autophagy inhibitors or activators such as mTOR inhibitors and AMPK activators, it is worthwhile to explore the effects of current medications already used clinically such as β-blockers, Ca^{2+} channel blockers, vasodilators and statins on autophagy, as well as safe dose ranges to ensure their positive effects in cardiac autophagy and protect cardiac function.

4. Conclusions

This review summarizes the most current findings on how autophagy is executed and regulated, and how the integration or disruption of autophagy pathways contributes to cardiovascular physiology and disease. Defining the mechanisms of autophagy pathways in different cell types in the cardiovascular system and the importance of autophagosomal degradation of organelles such as endoplasmic reticulum and mitochondria during oxidative stress has the potential to yield novel therapeutic approaches for the treatment of cardiovascular diseases.

Acknowledgments

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Abbreviations

AMA	antimycin A
AMPK	adenosine monophosphate–activated protein kinase
AngII	angiotensin II
AT1	angII type I
AT2	angII type 2
Atg	autophagy related protein
BMPR-II	bone morphogenetic protein type II receptor
BNIP3	BCL-2/adenovirus E1B 19-kDa interacting protein 3
CryAB	alphaB-crystallin
DRC	desmin-related cardiomyopathy
ECs	endothelial cells
ER	endoplasmic reticulum
GSK-3β	glycogen synthase kinase-3β
HDACs	histone deacetylases
HFD	high fat diet
LC3	light chain 3
LMNA	lamin A/C gene
MCL-1	myeloid cell leukemia-1
MCP-1	monocyte chemotactic protein-1
MCPIP	MCP-1-induced protein
MIF	macrophage migration inhibitory factor
MTG	N-2-mercaptopropionyl glycine
mTOR	mammalian target of rapamycin

mTORC1	mammalian target of rapamycin complex 1
NF-κB	nuclear factor κB
PASMCs	pulmonary artery smooth muscle cells
PI3K	class III phosphatidylinositol 3-kinase
Redox	reduction-oxidation
ROS	reactive oxygen species
RVLM	rostral ventrolateral medulla
SMCs	smooth muscle cells
ULK	unc-51-like kinase

Highlights

This is a revision of review article that highlights:

- Introduced types and process of autophagy

- Summarized the correlation of autophagy with cardiovascular diseases

- Highlighted the interaction of autophagy and oxidative stress in the development of cardiovascular diseases
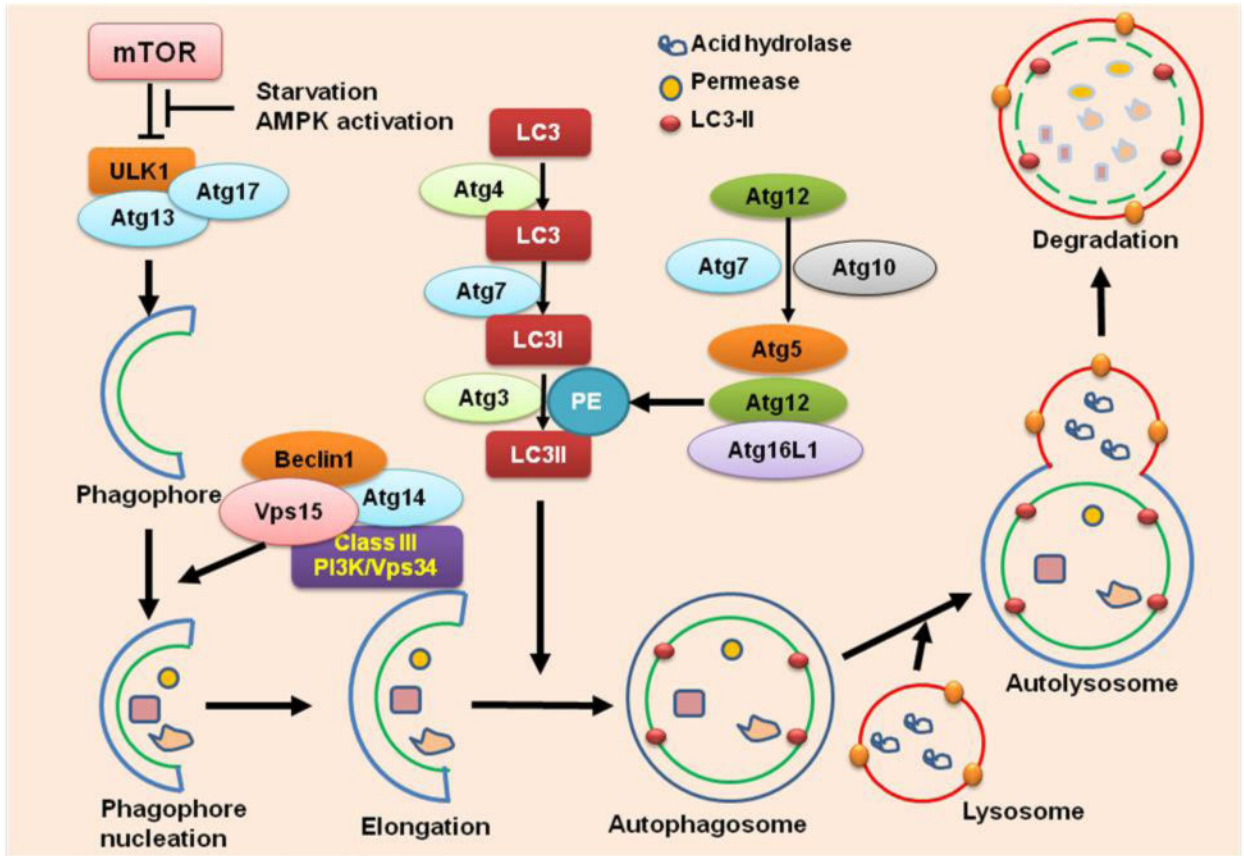
Figure 1. Intracellular pathways regulating macroautophagy
Macroautophagy transfers soluble cytoplasmic materials and organelles to the lysosomes by utilizing the intermediate organelle called the autophagosome, which is a product of an isolation membrane (termed phagophore) enclosed within a small portion of the cytoplasm. The autophagosome then fuses with the lysosome to form an autolysosome and degrades the materials contained within. Autophagy is orchestrated by the Atg-composed protein complexes. Starvation or AMPK activation inhibits mTOR, leading to the release and activation of the ULK1 complex (ULK1/Atg7/Atg13), which stimulates phagophore formation, and activates the Beclin1 complex (Beclin1/Class III PI3K/Vps34/Vps15/Atg14). The Beclin1 complex promotes phagophore nucleation and elongation. Two ubiquitin-like molecules, Atg12 and Atg8 (called LC3 in mammals) are involved in expansion of autophagosome membranes. Conjugated Atg5–Atg12 complexes bind Atg16L1 to form pre-autophagosomal structures which induce the recruitment of processed LC3-II. LC3-II remains on mature autophagosomes until after fusion with lysosomes to generate autolysosomes. The contents are then degraded by proteases, lipases, nucleases, and glycosidases, and the breakdown products such as amino acids, lipids, nucleosides, and carbohydrates are released into the cytosol for synthetic and metabolic pathways. PE: phosphatidylethanolamine.