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The Role of Autophagy in the Heart

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Abstract

Autophagy is an evolutionarily conserved mechanism by which cytoplasmic elements are degraded intracellularly. Autophagy has also emerged as a major regulator of cardiac homeostasis and function. Autophagy preserves cardiac structure and function under baseline conditions and is activated during stress, limiting damage under most conditions. It reduces injury and preserves cardiac function during ischemia. It also reduces chronic ischemic remodeling and mediates the cardiac adaptation to pressure overload by restricting misfolded protein accumulation, mitochondrial dysfunction, and oxidative stress. Impairment of autophagy is involved in the development of diabetes and aging-induced cardiac abnormalities. Autophagy defects contribute to the development of cardiac proteinopathy and doxorubicin-induced cardiomyopathy. However, massive activation of autophagy may be detrimental for the heart in certain stress conditions, such as reperfusion injury. In this review, we discuss recent evidence supporting the important role of autophagy and mitophagy in the regulation of cardiac homeostasis and adaptation to stress.

1. INTRODUCTION

Proteins and organelles are continuously synthesized and degraded in cells, so that obsolete and dysfunctional elements are replaced with new ones. Proper turnover of proteins and organelles is critical for homeostasis, survival, and adaptation to stress (1, 2). Autophagy is an evolutionarily conserved mechanism of degradation of cytoplasmic elements and plays a pivotal role in the quality control of proteins and organelles (3–5). Autophagic digestion of cargo provides amino and fatty acids for the synthesis of adenosine 5′-triphosphate (ATP), proteins, and organelles. Autophagy also regulates secretion and intracellular trafficking (3–5). Although autophagy was originally identified as a nonspecific mechanism of degradation, cargo-specific forms of autophagy have also been discovered. Among these, mitophagy is a specialized form of autophagy devoted to the removal and digestion of damaged mitochondria (6–8).

Autophagy is rapidly activated by stresses, such as oxidative, metabolic, and genotoxic stress, as well as stress in the endoplasmic reticulum (ER). In most cases, autophagy is adaptive and limits derangements and death. However, in some conditions, autophagy facilitates cell death, including apoptosis and necrosis (3–5). Autophagy can also induce cell death with distinctive morphological characteristics and mechanisms of regulation, known as autosis (9). During the past decade, autophagy has also emerged as a major regulator of cardiac homeostasis and function. Autophagy maintains cardiac structure and function at baseline by eliminating misfolded proteins and damaged organelles (10, 11) and is further activated during stress, thereby limiting cardiac damage in many pathological conditions. Autophagy preserves cardiac function during ischemia and starvation by supplying substrates for ATP regeneration and reduces myocardial injury (12–16). Autophagy alleviates progression of contractile dysfunction and remodeling during hemodynamic overload by inhibiting misfolded proteins, mitochondrial dysfunction, and oxidative stress (10, 17). Cardiac autophagy is suppressed below physiological levels in failing hearts, contributing to the progression of heart failure (HF), and during aging (18, 19). Autophagy is also inhibited in the presence of proteotoxic stress, such as in desmin-related or doxorubicin (DOX)-induced cardiomyopathy (20, 21). On the other hand, autophagy is activated excessively under some conditions, including ischemia/reperfusion (I/R) and the acute phase of pressure overload (PO), where autophagy may facilitate myocardial injury (13, 22). Here, we discuss the current evidence supporting the important role of autophagy in mediating cardiac homeostasis and adaptation to stress and myocardial injury.

2. THE BIOLOGY OF AUTOPHAGY

2.1. Autophagic Machinery

The term autophagy was coined in 1963 by Christian de Duve, a Nobel laureate who hypothesized the existence of cellular machinery to transport cytosolic constituents to lysosomes for digestion (23). It is now known that three types of autophagy exist (**Figure 1**). Macroautophagy involves the formation of double-membrane vesicles called autophagosomes, which sequester cellular proteins, glucides, lipids, and organelles and then deliver them to lysosomes for degradation. Microautophagy refers to a process through which cellular elements to be degraded are directly engulfed by lysosomes (3–5). Chaperone-mediated autophagy (CMA) is characterized by the binding of proteins containing a KFERQ sequence to the chaperone Hsc70, which transports target proteins to lysosomes in a lysosome-associated membrane protein 2A (Lamp2A)-dependent manner (3–5). Macroautophagy (hereafter called autophagy) is critical for the broad regulation of cellular quality control processes, organelle degradation, and adaptation to stress, whereas the other two forms are involved in more specialized cellular functions (3–5).

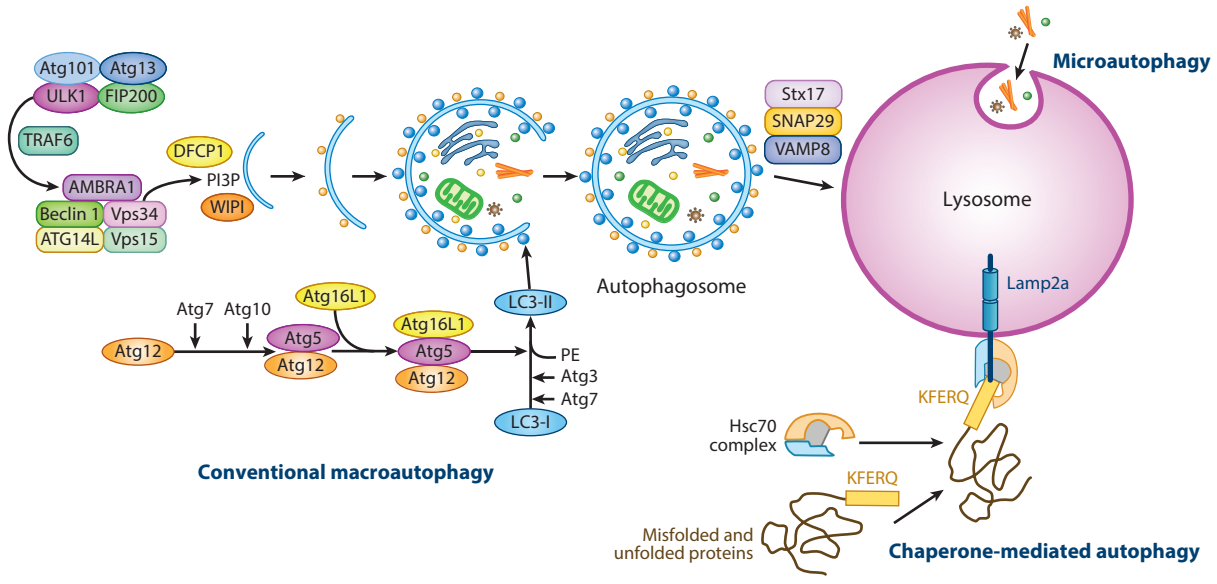


Figure 1

Schematic model of the major pathways in the regulation of the autophagic machinery. These pathway types include conventional macroautophagy, microautophagy, and chaperone-mediated autophagy. In macroautophagy, double-membrane vesicles called autophagosomes sequester cellular components and deliver them to lysosomes for degradation, whereas in microautophagy, lysosomes directly engulf cellular components. In chaperone-mediated autophagy, the chaperone Hsc70 transports target proteins to lysosomes for degradation. Abbreviations: AMBRA1, autophagy and Beclin 1 regulator 1; Atg, autophagy-related (gene); DFCP1, double FYVE-containing protein 1; FIP200, focal adhesion kinase family-interacting protein of 200 kD; Hsc70, heat-shock cognate 70; Lamp2a, lysosomal-associated membrane protein 2a; LC3, microtubule-associated protein 1 light chain 3; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol 3-phosphate; SNAP29, synaptosome-associated protein 29; Stx17, syntaxin 17; TRAF6, tumor necrosis factor receptor–associated factor 6; ULK1, unc-51–like autophagy-activating kinase 1; VAMP8, vesicle-associated membrane protein 8; Vps, vacuolar protein sorting; WIPI, WD (tryptophan-aspartic acid) repeat domain phosphoinositide-interacting protein.

Autophagy is regulated by specific autophagy-related (Atg) genes, encoding proteins that regulate the initiation and maturation of autophagosomes and fusion of autophagosomes to lysosomes to form autolysosomes (3–5). The existence of Atg genes was first described in yeast in several pioneering studies (3–5). To date, more than 35 Atg genes have been discovered, and most are conserved between yeast and mammals (24).

Autophagosome formation begins with generation of an isolation membrane (also called the phagophore), which most commonly originates from ER membranes, although it may also derive from Golgi, mitochondrial, or plasma membranes (24). Initiation of the isolation membrane is promoted by the unc-51–like autophagy-activating kinase (Ulk) macromolecular complex, comprising Atg13, either Ulk1 or Ulk2 (mammalian orthologs of Atg1), the focal adhesion kinase family interacting protein of 200 kD (FIP200), and Atg101 (24). The Ulk complex, in turn, activates another macromolecular protein assembly consisting of Beclin 1, Atg14L, Vps34, and Vps15 (24). Ulk1 phosphorylates Beclin 1 at serine 14, thereby activating the Vps34 complex (25). This complex is critical for expansion of the nascent autophagosome through formation of phosphatidyl inositol 3-phosphate (PI3P). PI3P promotes translocation of multiple autophagy proteins, including Atg18, Atg20, Atg21, and Atg24 to the phagophore assembly site, leading to growth of the phagophore (24). During the expansion, the phagophore sequesters cytoplasmic cargos,

maturates, and closes. The final maturation and closure of the autophagosome is mediated by the Atg conjugation system, similar to the ubiquitin conjugation system (24). First, conjugation of Atg12, a ubiquitin-like molecule, to Atg5 is mediated by Atg7, an E1-like protein, and by Atg10 and Atg3, E2-like proteins. The Atg12–Atg5 complex then promotes elongation of the maturating phagophore through interaction with Atg16, which acts as an E3-like enzyme to promote lipidation of microtubule-associated protein 1 light chain 3 (LC3), a ubiquitin-like molecule, through its conjugation to phosphatidylethanolamine. The conjugated LC3, called LC3-II, is incorporated into the phagophore, mediating its final maturation to autophagosome. The conjugation and subsequent deconjugation of LC3 to/from phosphatidylethanolamine are regulated by the cysteine protease Atg4 (24). Interestingly, maturation of syntaxin (Stx)17-positive autophagosomes can still be observed in cells lacking the Atg conjugation system, although successful completion of the process is significantly impaired (26). These data confirm that the conjugation reactions are important for autophagosome maturation, but they also suggest that unknown mechanisms can compensate for the final steps of autophagosome formation in the absence of functional conjugation machinery.

After maturation, autophagosomes fuse with lysosomes. This process is mediated by the soluble *N*-ethylmaleimide–sensitive fusion protein attachment protein receptor (SNARE) proteins STX17, SNAP29, and VAMP8 through an Atg14-dependent mechanism (27, 28). The Atg conjugation system also participates in the formation of autolysosomes by mediating fission of the autophagosomal membranes, resulting in degradation of the inner membrane. In fact, inner membrane degradation and autolysosome formation are delayed in the absence of the Atg conjugation system (26). Acid hydrolases in the lysosomes degrade the cargos, whereas LC3 at the outer autophagosomal membrane is recycled to the cytosol for generation of new autophagosomes.

2.2. Functions of Autophagy

Autophagy regulates multiple physiological processes critical for preservation of cellular function and viability under both stressed and unstressed conditions (3–5, 24). Removal of protein aggregates is particularly important in terminally differentiated cells such as adult cardiomyocytes that have nearly completely lost replicative abilities. Autophagosomes' large capacity allows them to sequester and deliver misfolded proteins and protein aggregates to lysosomes for degradation. Autophagy and the ubiquitin–proteasome system, the two major mechanisms of degradation, cross talk with each other and compensate for one another to maintain protein quality control (2). Ubiquitination marks proteins and organelles for degradation by autophagosomes (2). p62, which is degraded by autophagy and possesses both a ubiquitin-binding domain and an LC3-binding motif, recognizes cargos marked with ubiquitination and presents them to autophagosomes (2). This mechanism is well characterized in mitophagy but may also be involved in autophagic degradation of specific proteins. In mice with genetic disruption of autophagy, p62 and misfolded ubiquitinated proteins accumulate, leading to organ dysfunction, such as in liver and brain (29–31). Autophagy inhibition contributes to neurodegenerative disorders characterized by protein aggregate accumulation, collectively called proteinopathy, including Alzheimer's, Parkinson's, and Huntington's diseases (3).

Autophagy also plays a crucial role in maintaining ATP levels during starvation (3–5, 24). Mammalian cells with impaired autophagy develop abnormalities and die when subjected to nutrient deprivation (30, 32). Mice with systemic *atg5* deletion cannot activate autophagy and rapidly die after birth, exhibiting severe amino acid insufficiency, hypoglycemia, and myocardial damage, which suggests that autophagy activation is an important survival mechanism during neonatal starvation (33). Interestingly, neuronal-specific Atg5 overexpression is sufficient to rescue

atg5$^{-/-}$ mice, indicating that neuronal abnormalities are responsible for the early mortality observed in these mice, likely by causing suckling defects (34). Adult mice with either cardiac-specific *forkhead box O1* (*FoxO1*) gene deletion or systemic heterozygous deletion of *beclin 1* also develop cardiac dysfunction after 48 h of starvation, whereas wild-type mice display a preserved ejection fraction (15). Autophagy limits cell death in response to energy stress through several mechanisms. The first involves provision of new energy substrates, such as amino and fatty acids, derived from the digestion of proteins and lipids. Recycled amino acids are used to produce ATP through the tricarboxylic acid cycle and for gluconeogenesis in the liver. Second, autophagy is important for providing new amino acids for protein synthesis. Finally, autophagy activation limits mitochondrial dysfunction by eliminating damaged mitochondria, allowing for their replacement through coupled mitochondrial biogenesis. Mice with genetic disruption of autophagy or mitophagy display mitochondrial dysfunction and increased reactive oxygen species (ROS) (3–5, 24).

Autophagy is also involved in secretion of leaderless cytosolic proteins from cells. For example, autophagy is required for endothelial cell secretion of von Willebrand factor (35). Autophagy also positively regulates insulin secretion, and suppression of autophagy during fasting reduces insulin secretion from pancreatic β-cells (36). Secretion of cargo through autophagy utilizes a cytosolic cargo receptor and a SNARE system distinct from those utilized in ordinary lysosomal degredation.

Autophagy activation is required for endothelial cell-dependent neoangiogenesis. AGGF1, an angiogenic factor, activates autophagy in the mouse heart and reduces chronic ischemic remodeling by inducing neoangiogenesis. Genetic inhibition of autophagy abrogates the antiremodeling and angiogenic effects of AGGF1 (38).

Autophagy can be cargo specific. The most studied cargo-specific autophagy is mitophagy, devoted to selective sequestration and degradation of mitochondria. Other forms of cargo-specific autophagy include lipophagy and ER-phagy. In lipophagy, autophagy selectively degrades lipid droplets (3–5). Store-operated calcium ion (Ca^{2+}) entry-dependent lipophagy is a fundamental process for the degradation of lipid deposits in cardiomyocytes and for mitochondrial fatty acid oxidation (39). Lipophagy is activated in response to lipid overload to reduce accumulation of lipid droplets, and genetic disruption of autophagy causes significant lipid accumulation in the liver (40). ER-phagy is selective for ER, mediating the removal of overloaded ER and alleviating ER stress. FAM134, an ER protein, mediates ER recognition and removal by autophagosomes. Downregulation of FAM134 abrogates ER-phagy, leading to ER expansion, ER stress, and cell death in human neural cells (37).

Autophagy selectively degrades proteins as well. For example, autophagy selectively degrades Keap1 in a p62-dependent manner, leading to activation of nuclear factor erythroid 2-related factor 2 (Nrf2) (41). Autophagy also degrades Yes-activated protein 1 (YAP1), a transcription cofactor. In cells isolated from tuberous sclerosis complex (TSC)1–TSC2 double knockout mice, in which activation of mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) suppresses autophagy, YAP1 degradation is decreased, contributing to cancer progression in these mice (42).

However, excessive activation of autophagy causes cell death. Autophagy activation leads to selective degradation of catalase in response to caspase inhibition, enhancing ROS accumulation and cell death in mouse fibroblasts (43). Excessive elimination of mitochondria by autophagy can cause severe energy depletion (1). Furthermore, Levine's group described autosis, a novel form of cell death induced by autophagy (9). Autosis is characterized by unique morphological features: accumulation of autophagosomes and autolysosomes, disappearance of intracellular organelles, nuclear convolution, and characteristic perinuclear space at late stages. It is prevented by inhibitors of autophagy but not of apoptosis and necrosis. Autosis is observed in cells treated with

TAT-Beclin 1, an autophagy activator, in starved cells, and in hippocampal neurons from neonatal rats subjected to cerebral hypoxia/ischemia (9). Autosis is inhibited by knockdown of the Na^+, K^+-ATPase α1 subunit or inhibition of Na^+, K^+-ATPase by cardiac glycosides (9). How Na^+, K^+-ATPase mediates autosis is not well understood.

2.3. Signaling Mechanisms of Autophagy

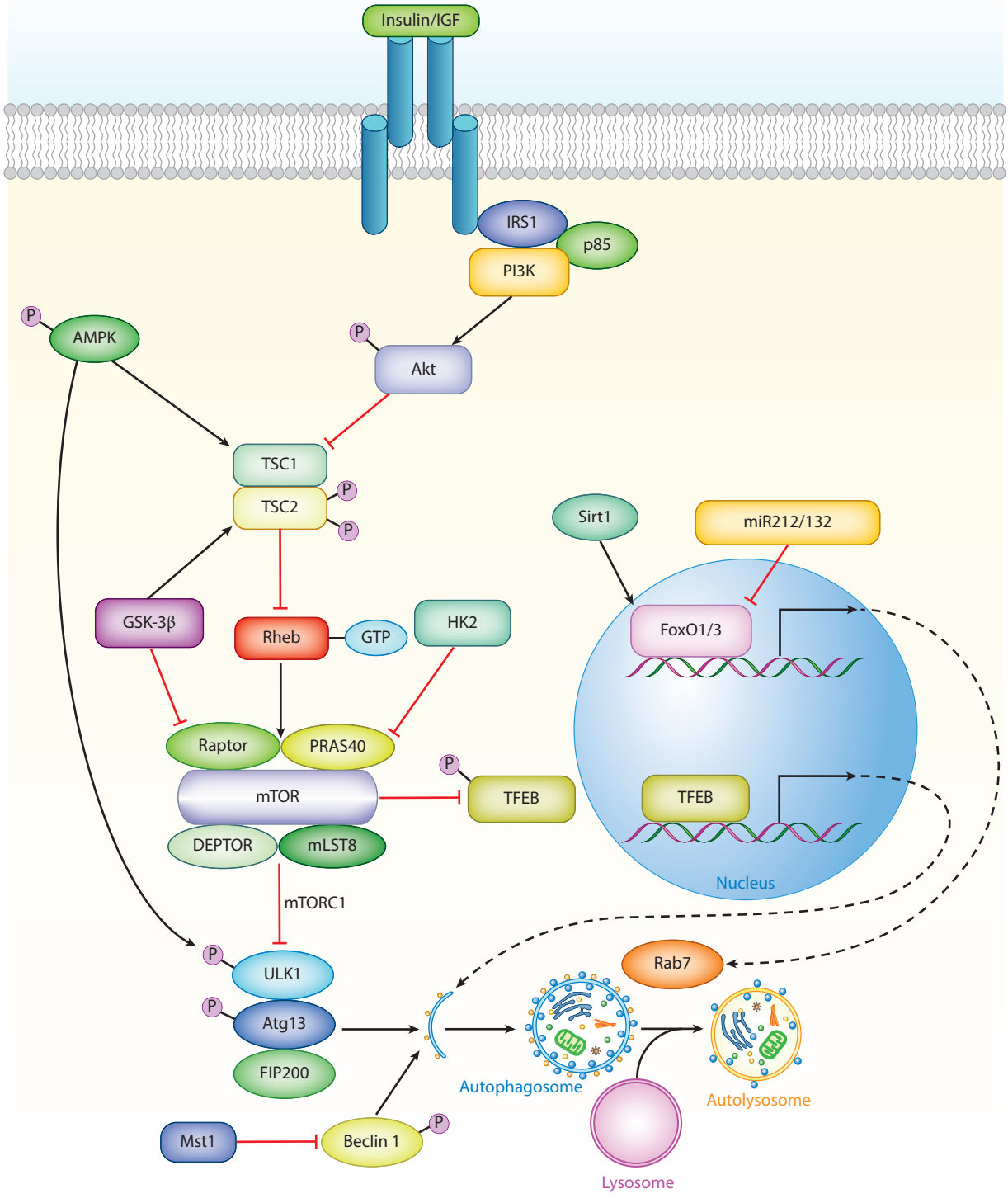
Several key signaling mechanisms directly regulate autophagy in cardiomyocytes at baseline and in response to stress (**Figure 2**).

2.3.1. mTOR. mTOR is a serine/threonine kinase that acts through two multiprotein complexes (44). mTORC1 is a master regulator of protein synthesis, cellular growth, metabolism, mitochondrial biogenesis, and autophagy. It is activated by nutrients and growth factors and is inhibited during starvation. mTORC2 regulates cell survival, insulin sensitivity, and cell polarity (45). mTORC1 negatively regulates autophagy through posttranslational and transcriptional mechanisms. mTORC1 phosphorylates Ulk1 at serine 757, thereby inhibiting autophagosome formation (46). In addition, mTORC1 represses transcription of Atg genes by inhibiting the nuclear transport of transcription factor EB (TFEB), a regulator of autophagy and lysosomal biogenesis (47). mTORC1 activity is reduced in nutrient-deprived cells, leading to autophagy activation (46). Forced mTORC1 activation in the heart during myocardial ischemia blunts autophagy activation and dramatically increases ischemic injury (12). Insulin signaling prevents excessive activation of autophagy during postnatal heart development through activation of the mTOR pathway, and mice with disrupted insulin signaling due to genetic deletion of IRS1 and IRS2 develop cardiomyopathy through unrestrained activation of autophagy (48). mTORC2 also regulates autophagy indirectly through protein kinase B (Akt) and mammalian sterile 20–like kinase 1 (Mst1) (44).

2.3.2. AMPK and GSK-3β. Autophagy is regulated by 5′-AMP-activated kinase (AMPK) and glycogen synthase kinase-3 beta (GSK-3β), serine/threonine kinases whose activity is finely controlled by the energy status. AMPK is activated by ATP depletion and stimulates autophagy

Figure 2

Schematic model of the intracellular signaling mechanisms regulating autophagy. The autophagic machinery is modulated by various posttranslational modifications. mTORC1 negatively regulates autophagy through both posttranslational and transcriptional mechanisms. AMPK activates both TSC1/2 and ULK1, thereby facilitating initiation of the autophagic machinery. Similarly, GSK-3β phosphorylates and activates TSC1/2, thereby suppressing mTORC1 activity, which in turn activates autophagy. Pro-apoptotic Mst1 attenuates autophagic activity by enhancing molecular interaction between Beclin 1 and Bcl-2 family proteins through Beclin 1 phosphorylation. TFEB promotes both lysosome biogenesis and autophagy activation. Activation of FoxO1 stimulates autophagy and enhances autophagic flux through upregulation of Rab7. miR-212/132 downregulates FoxO3a, thereby inhibiting autophagy. Solid arrows denote stimulation; dashed arrows indicate that transcriptional targets of FoxO1/3a and TFEB stimulate autophagosome formation and autophagosome–lysosome fusion, respectively; T-shaped indicators show inhibition; and small circles containing P symbolize protein phosphorylation. Abbreviations: Akt, protein kinase B; AMPK, 5′-AMP-activated kinase; Atg13, autophagy-related gene 13; DEPTOR, DEP domain–containing mTOR-interacting protein; FIP200, focal adhesion kinase family-interacting protein of 200 kD; FoxO, forkhead box O; GSK-3β, glycogen synthase kinase-3 beta; GTP, guanosine triphosphate; HK2, hexokinase 2; IGF, insulin-like growth factor; IRS1, insulin receptor substrate 1; miR-212/132, miRNA-212/132; mLST8, mammalian lethal with SEC13 protein 8; Mst1, mammalian sterile 20-like kinase 1; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; PI3K, phosphoinositide 3-kinase; PRAS40, proline-rich Akt substrate of 40 kDa; Rab7, Ras-related in brain 7; Raptor, regulatory-associated protein of mTOR complex 1; Rheb, Ras homolog enriched in brain; Sirt1, sirtuin 1; TFEB, transcription factor EB; TSC, tuberous sclerosis complex; ULK1, unc-51–like autophagy-activating kinase 1.


Insulin/IGF
IRS1
p85
PI3K
AMPK
Akt
TSC1
TSC2
GSK-3β
Rheb
GTP
HK2
Sirt1
miR212/132
FoxO1/3
Raptor
PRAS40
mTOR
TFEB
DEPTOR
mLST8
TFEB
Nucleus
mTORC1
ULK1
Atg13
FIP200
Rab7
Autophagosome
Autolysosome
Mst1
Beclin 1
Lysosome

through inhibition of mTORC1. AMPK activates TSC1/2, which inhibit Rheb, an mTORC1 activator (49). AMPK also directly activates autophagy by phosphorylating Ulk1 at serines 313 and 777, thus dissociating Ulk1 from mTORC1 (46). AMPK is required for autophagy activation during ischemia in cardiomyocytes (13). Similar to AMPK, GSK-3β is stimulated during energy stress and phosphorylates and activates TSC1/2, leading to mTORC1 inhibition and stimulation of autophagy (49). GSK-3β is activated and promotes autophagy through inhibition of mTOR during myocardial ischemia (14), whereas GSK-3β is inhibited during reperfusion, allowing mTORC1 activation and restraining autophagy (14).

2.3.3. Mst1. Mst1 is a serine/threonine kinase that is activated by proapoptotic stimuli, including oxidative stress, and inhibits autophagy through phosphorylation of Beclin 1 at threonine 108. Beclin 1 is negatively regulated through interaction with Bcl-2 family antiapoptotic molecules. Phosphorylation of Beclin 1 by Mst1 enhances its interaction with Bcl-2, and this Beclin 1–Bcl-2 interaction not only inhibits autophagy but also dissociates Bcl-2 from Bax, thereby activating Bax and stimulating apoptosis. Stress-induced activation of Mst1 promotes cardiomyocyte death through both suppression of autophagy and stimulation of apoptosis. Conversely, suppression of Mst1 protects cardiomyocytes against cardiac stress by stimulating autophagy and inhibiting apoptosis (17).

2.3.4. ROS signaling. ROS contribute to autophagy activation during starvation by oxidizing Atg4 at cysteine 81 (50). Atg4 oxidation inhibits its protease activity, thereby increasing the amount of lipidated LC3 and autophagosome formation. Physiological levels of ROS are required for autophagy activation during nutrient deprivation and ischemia in cardiomyocytes (51). NADPH oxidase 4 (Nox4), a ROS-producing enzyme, is rapidly activated in the ER in response to glucose deprivation in cardiomyocytes. Nox4-derived ROS promote autophagy by activating the PERK/ATF4 pathway, whereas downregulation of Nox4 inhibits autophagy and exacerbates myocardial injury in response to myocardial ischemia (51). It should be noted that excessive oxidative stress impairs cardiac autophagy. Saturated fatty acids impair autophagic flux through activation of Nox2-derived superoxide, and inhibition of superoxide rescues autophagic flux in cardiomyocytes subjected to lipid overload (52).

2.3.5. Transcriptional mechanisms. TFEB, whose nuclear localization and activity are regulated by phosphorylation by ERK2 and mTOR, promotes autophagosome formation, autophagosome-lysosome fusion, and cargo degradation during starvation by upregulating Atg genes and lysosomal enzymes (53). TFEB-dependent autophagy mediates the protective effect of intermittent fasting against I/R injury in the heart (54). FoxO1 is deacetylated and activated by sirtuin 1 (Sirt1) in energy-deprived cardiomyocytes. FoxO1 activation promotes autophagy and stimulates autophagic flux through upregulation of Rab7 and Atg genes (15). Krüppel-like factor 4 is a master regulator of cardiac mitochondrial homeostasis that governs mitochondrial dynamics, clearance, biogenesis, and metabolism (55). Finally, miRNAs have emerged as important regulators of autophagy. miRNA-212/132 promotes cardiomyocyte hypertrophy though downregulation of FoxO3a and inhibition of autophagy (56). miRNA-22 is progressively upregulated in the heart during aging, contributing to the aging-induced decline in autophagy through peroxisome proliferator-activated receptor alpha inhibition. miRNA-22 inhibition improves cardiac adaption to chronic myocardial infarction (MI) in old mice by stimulating autophagy (57).

2.3.6. Metabolites. Recent studies have identified several key intermediates in the metabolic pathway as being essential for activation/inactivation of autophagy. Cytosolic acetyl-coenzyme

A (acetyl-CoA), a product of the enzymatic reaction of pyruvate dehydrogenase or β-oxidation, negatively regulates autophagy through activation of p300. Acetyl-CoA is depleted in starved cells, which causes autophagy activation. Conversely, pharmacological induction of acetyl-CoA inhibits autophagy and reduces maladaptive hypertrophy in response to PO (58). Hexokinase 2 (HK2), a glycolytic enzyme, activates autophagy in cardiomyocytes. HK2 interacts with mTORC1 during glucose deprivation, inactivating mTORC1 and upregulating autophagy (59). Nicotinamide adenine dinucleotide (NAD^+), a pyridine nucleotide and electron carrier, also positively regulates autophagy. Nicotinamide phosphoribosyltransferase promotes NAD^+ accumulation and activates autophagy in cardiomyocytes through activation of Sirt1 (60). Finally, saturated fatty acids were shown to promote autophagy in a mouse model of diabetic cardiomyopathy through de novo synthesis of C14-ceramide by ceramide synthase-5 (61).

3. MITOCHONDRIAL QUALITY CONTROL

Maintaining mitochondrial quality is particularly important in cardiomyocytes, as the mitochondria of the heart produce 6 kg/day of ATP to continuously support its pumping function (8). Several key cellular mechanisms contribute to mitochondrial quality control at multiple levels, including fission and fusion, degradation of senescent and dysfunctional mitochondria, and biogenesis, collectively termed mitochondrial quality-control mechanisms (1). Here, we overview how damaged mitochondria are degraded and how mitochondrial quality is maintained in the heart.

3.1. Mitochondrial Dynamics

Mitochondrial dynamics, including fusion and fission, represent the first response to mitochondrial damage, such as mitochondrial membrane potential depolarization, to maintain mitochondrial quality (1). In fusion, single mitochondria join to generate tubular and elongated organelles. Fusion between healthy and reversibly damaged mitochondria allows functional repair of the damaged portions. For example, fusion reduces mitochondrial DNA damage during aging (1). Also, fusion generally increases mitochondrial oxidative capacity and efficiency. Mitochondrial fusion occurs frequently in freshly isolated adult cardiomyocytes, with both rapid and slow kinetics, despite adult myocytes having more fragmented and densely packed mitochondria in the interfibrillar space (8). Fission is characterized by fragmentation of mitochondria into short and rounded organelles. In theory, fission eliminates damaged portions of mitochondria, which are then removed by mitophagy, thus preserving the healthy portions of the mitochondria and maintaining their function. In addition, fission increases mitochondrial resistance to stress (8).

Several proteins regulate mitochondrial dynamics. Mitofusin (Mfn) 1 and 2 and optic atrophy 1 (Opa1) promote mitochondrial fusion. Mfn1/2 promote fusion of the outer mitochondrial membrane (OMM), whereas Opa1 regulates fusion of the inner mitochondrial membrane (IMM). Mitochondrial peptidases YME1L and OMA1 regulate processing of Opa1, which in turn affects fission and fusion of mitochondria. Mfn1/2 have partially overlapping functions in mitochondrial fusion, and mice with deletion of both Mfn1/2 in cardiomyocytes accumulate aberrant and dysfunctional mitochondria and develop cardiomyopathy (62, 63). However, mice with cardiac deletion of Mfn2 develop spontaneous cardiac hypertrophy and mildly reduced left ventricular function, whereas mice with Mfn1 deletion have normal cardiac function (64, 65). Genetic mutations in *Mfn2* also cause Charcot-Marie-Tooth disease type 2A, a neurodegenerative disease. These differences may be explained by the fact that Mfn2 also functions as a Parkin receptor. Notably, cardiac-specific deletion of either Mfn1 or 2 confers protection against oxidative stress,

suggesting that Mfns preserve cardiac and mitochondrial function at baseline but may contribute to mitochondrial damage during stress (64). Supporting this notion, cardiomyocyte overexpression of Mfn2 was found to induce apoptosis in response to oxidative stress (66). Cardiac deletion of Opa1 was also found to induce cardiac hypertrophy and dilation in response to mechanical stress (67), and accelerated proteolysis of Opa1 in the heart caused by an imbalance between YME1L and OMA1 induces HF (68). However, controlled systemic overexpression of Opa1 stabilizes mitochondrial cristae and protects against myocardial ischemia (69).

The guanosine triphosphatase (GTPase) dynamin-related protein 1 (Drp1) promotes mitochondrial fission by binding to Mff, Fis1, and MiD49/51 at the OMM (8). Genetic disruption of mitochondrial fission is also detrimental in the heart. Mice with cardiac-specific Drp1 deletion develop dilated cardiomyopathy (DCM) under baseline conditions, which is associated with early mortality (11, 70). In addition, in mice with inducible *Drp1* gene disruption, defective mitophagy causes increased I/R injury and HF in response to PO (11). Python mice having a *C425F* mutation in *Drp1* develop mitochondrial dysfunction and DCM, and both mitochondrial fission and mitophagy are attenuated (71). *Mff* knockout mice also develop DCM and die prematurely. Surprisingly, concomitant *Mfn1* gene deletion rescues *Mff* knockout mice, improving cardiac function and mitochondrial oxidative capacity and extending life span (72). These results suggest that a balance between fission and fusion is critical for the preservation of cardiac structure and function, and a molecular intervention to alter mitochondrial dynamics by manipulating Mfn configuration was recently proposed (73). It should be noted that Drp1 also possesses noncanonical functions (74). Thus, results of loss-of-function studies of Drp1 and other fission/fusion proteins may not necessarily represent the functions of fission/fusion alone.

3.2. Mitophagy

Mitophagy is cargo-specific autophagy specifically devoted to degradation of mitochondria. It is the major mechanism of mitochondrial degradation, although mitochondria can also be degraded by other mechanisms, such as mitochondrial protease–dependent degradation, the ubiquitin–proteasome system, the endosomal system, and microautophagy (1). Mitophagy is crucial for mitochondrial quality control and cell survival (6–8). Inspection of Mito-Keima mice indicated that basal mitophagic activity is greater in the heart than in other organs, such as thymus (75), possibly reflecting high levels of oxidative phosphorylation, ROS, and mitochondrial damage in cardiomyocytes (6–8). Mitophagy is activated during stress, allowing elimination of damaged mitochondria. Mitophagy is also tightly coupled to mitochondrial biogenesis (6–8, 76). Thus, eliminated mitochondria are continuously replaced with fresh ones. Mitophagic mechanisms can be divided into Parkin-dependent and Parkin-independent mechanisms (**Figure 3**).

3.2.1. Parkin-dependent mitophagy. Phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1)/Parkin is one of the best characterized pathways mediating mitophagy. PINK1 is a serine/threonine kinase that is degraded by matrix metalloproteinases and presenilin-associated rhomboid-like proteases in healthy mitochondria (77). When mitochondria are damaged, PINK1 accumulates in the OMM, where it promotes recruitment of Parkin, an E3-ubiquitin ligase that ubiquitinates proteins on the OMM of damaged mitochondria (78). Two main mechanisms have been proposed to mediate PINK1-mediated recruitment of Parkin to the OMM of damaged mitochondria. One is through phosphorylation of Mfn2 at threonine 11 and serine 442 (79). Alternatively, PINK1 recruits Parkin through phosphorylation of ubiquitin at serine 65, which allows ubiquitin to interact with and activate Parkin (80). PINK1 also phosphorylates Parkin at serine 65, thereby activating Parkin (81). Thus, PINK1-induced phosphorylation of ubiquitin and Parkin

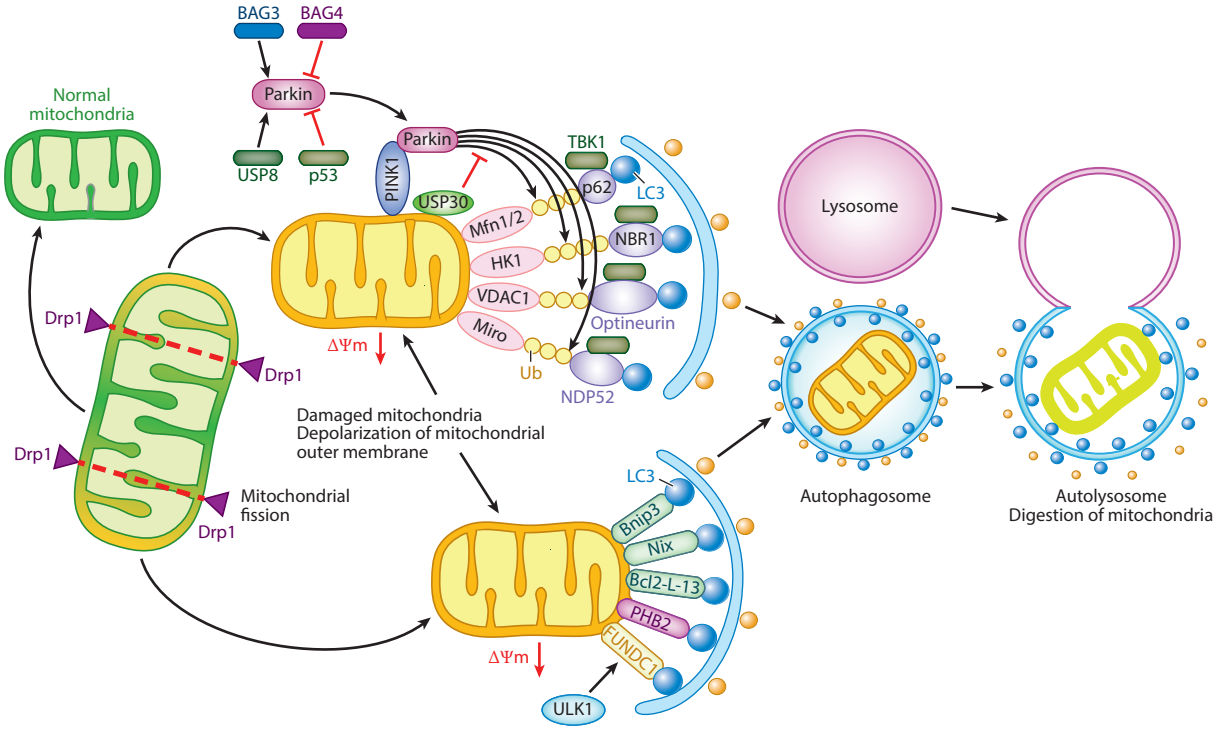


Figure 3

Schematic model of the molecular mechanisms regulating mitophagy. There are two signaling pathways for selective activation of mitophagy: the PINK1-Parkin–mediated pathway and the mitophagic receptor–mediated pathway. In PINK1-Parkin–mediated mitophagy, PINK1 recruits Parkin to the outer membrane of damaged mitochondria and activates it. Activated Parkin ubiquitinates target proteins, which in turn recruit mitophagy receptors/adaptors that interact with LC3, thereby promoting autophagosome recruitment. Several different molecules either positively or negatively regulate this process. In the mitophagic receptor–mediated pathway, mitophagic receptors Parkin-independently bind to LC3, thereby recruiting autophagosomes to damaged mitochondria. Arrows denote stimulation, and T-shaped indicators denote inhibition. Abbreviations: BAG, Bcl2-associated athanogene; Bcl2-L-13, Bcl2-like protein 13; Bnip3, Bcl2/adenovirus E1B 19-kDa protein-interacting protein 3; Drp1, dynamin-related protein 1; FUNDC1, FUN14 domain–containing protein 1; HK1, hexokinase 1; LC3, microtubule-associated protein 1 light chain 3; Mfn1/2, mitofusin 1/2; Miro, mitochondrial Rho; NBR1, neighbor of BRCA1; NDP52, nuclear dot protein of 52 kDa; Nix, Nip3-like protein 13; p53, tumor protein 53; PHB2, prohibitin 2; PINK1, phosphatase and tensin homolog–induced kinase 1; TBK1, TANK-binding kinase 1; Ub, polyubiquitin chain; ULK1, unc-51–like autophagy-activating kinase 1; USP, ubiquitin-specific peptidase; VDAC1, voltage-dependent anion-selective channel 1.

amplifies both the mitochondrial recruitment and activation of Parkin. Other molecules also play a role in regulating Parkin mitochondrial translocation. HSPA1L, a member of the HSP70 family, and BAG4 have mutually opposing roles in the regulation of Parkin translocation to the OMM (82). Cytosolic p53 also prevents Parkin translocation through direct interaction with Parkin (83). On the other hand, ubiquitin-specific peptidase 8, a deubiquitinating enzyme, removes K6-linked ubiquitin chains from Parkin, thereby promoting recruitment of Parkin to OMM proteins (84).

Once recruited to the OMM of damaged mitochondria, Parkin ubiquitinates several targets, facilitating the interaction between ubiquitinated proteins and mitophagy receptors/adaptors, including p62/sequestosome 1, neighbor of BRCA1 (NBR1) and histone deacetylase 6 (HDAC6), a ubiquitin-binding domain-containing deacetylase (85). p62/sequestosome 1 and NBR1 contain ubiquitin- and LC3-binding domains. Interaction with LC3 promotes sequestration of damaged

mitochondria into autophagosomes, whereas HDAC6 may be involved in autophagosome-lysosome fusion (85). Moreover, PINK1 is involved in recruitment of other LC3 receptors, such as optineurin, NDP52, and Tax1-binding protein 1, which amplify LC3 recruitment, thus facilitating mitophagy (86, 87). In particular, TANK-binding kinase 1 (TBK1)-dependent phosphorylation of optineurin strongly amplifies mitophagy, in agreement with the observation that deletion of optineurin is associated with decreased autophagic engulfment of depolarized mitochondria (88). Damaged mitochondria are also recognized by LC3 through prohibitin 2 (PHB2), an IMM receptor that contains an LC3-binding domain, when the OMM is ruptured by a proteasomal-dependent mechanism. PHB2 is required for clearance of paternal mitochondria after embryonic fertilization in *Caenorhabditis elegans*, indicating that this mechanism is evolutionarily conserved (89). Parkin also regulates mitochondrial dynamics. In particular, Parkin was found to promote ubiquitination of Mfn1/2 and their consequent degradation in a proteasome- and p97- (an AAA^+ ATPase) dependent manner (90). It should be noted that Parkin also has mitophagy-independent functions (1). Thus, the phenotypes observed in loss-of-function studies of Parkin may not be fully attributable to Parkin-dependent mitophagy, and caution should be exercised in interpreting them.

Mitophagy can be activated in the absence of mitochondrial depolarization. One such mechanism is the mitochondrial unfolded protein response (UPR^{mt}). Accumulation of misfolded proteins in mitochondria activates UPR^{mt}, which in turn activates mitophagy as an adaptive response. UPR^{mt} induces accumulation of PINK1 on the OMM, thereby activating Parkin-mediated mitophagy even in healthy mitochondria (91). Prolonged activation of UPR^{mt} is potentially harmful, however, because it can also propagate defective mtDNA and facilitate mitochondrial heteroplasmy (92).

Recent evidence suggests that Parkin is also involved in mitochondrial degradation mediated by mechanisms distinct from macroautophagy. For example, mitochondrial-derived vesicles directly transfer part of mitochondria to lysosomes, a mechanism similar to microautophagy (93). A Rab5-dependent endosomal pathway also contributes to the clearance of damaged mitochondria. Interestingly, this mechanism is activated before the onset of autophagy but requires Parkin. Damaged mitochondria are internalized in Rab5-positive endosomes through the endosomal sorting complexes required for transport (ESCRT) machinery and then delivered to lysosomes (94).

3.2.2. Parkin-independent mitophagy. Although Parkin-mediated mitophagy is the best characterized form of mitophagy, several Parkin-independent mechanisms exist. Bcl2-like protein 13 (Bcl2-L-13) is a mammalian homolog of yeast Atg32, which serves as a mitophagy receptor and plays a pivotal role in the induction of mitophagy in yeasts. Bcl2-L-13 contains the WXXI motif, an LC3-binding domain, and can promote mitochondrial fragmentation and mitophagy in Parkin-deficient cells (95). Under hypoxic conditions, Bcl2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3) is upregulated by hypoxia-inducible factor 1, promoting mitophagy and protecting cells from ROS accumulation (96). Bnip3 interacts with Nip3-like protein 13 (Nix), and both proteins have LC3-binding motifs and translocate to the OMM, thereby recruiting autophagosomes to damaged mitochondria (97). The OMM protein FUN14 domain-containing protein 1 (FUNDC1) also induces mitophagy in a Parkin-independent manner. Under basal conditions, FUNDC1 cannot interact with LC3 due to phosphorylation at serine 13 and tyrosine 18 by casein kinase 2 and Src, respectively. Under stress conditions, Ulk1 phosphorylates FUNDC1 at serine 17, and PGAM5 dephosphorylates it at serine 13, thereby promoting mitophagy (98). Whether these various LC3 receptors/adaptors act in parallel or in a stimulus-specific manner is currently unknown.

Other mechanisms of mitochondrial degradation that do not utilize the conventional autophagic machinery also appear to contribute to mitochondrial quality control in some cell types. For example, autophagy can take place even in the absence of LC3 or Atg5/Atg7, and autophagosomes can be generated from trans-Golgi membrane through Rab9A/B-dependent mechanisms (99). Similar findings were reported in starved HeLa cells, where mitogen-activated protein kinase 1 (MAPK1) and MAPK14 are required to degrade damaged mitochondria (100). The functional significance of mitophagy mediated through unconventional mechanisms remains to be elucidated.

4. THE ROLE OF AUTOPHAGY IN THE HEART

The activity and functional significance of autophagy in the heart vary substantially, depending on the nature of stress and timing of assessment. In this section, we discuss the role of autophagy in various pathophysiological conditions in the heart.

4.1. Autophagy During Aging

Autophagy is required for maintenance of cardiac structure and function under unstressed conditions. Mice with inducible cardiac-specific *atg5* deletion develop cardiac dilation and dysfunction and die early. Cardiac dysfunction in these mice is associated with misfolded protein accumulation, sarcomere disarray, and mitochondrial derangements (10). Cardiac aging is associated with impairment of protein quality control, accumulation of dysfunctional organelles, and imbalance between pro-oxidants and antioxidants, all of which contribute to increased susceptibility to stress (18). Autophagy is a major regulator of aging and life span in many organisms (18) and is progressively decreased in the heart during aging, suggesting that downregulation of autophagy underlies the progression of cardiac aging (18, 19). Key regulators of autophagy, including FoxOs, TFEB, and GSK-3α, are downregulated during aging in the heart. ROS-induced DNA damage contributes to NAD^+ depletion, which in turn inhibits the Sirt1/FoxO pathway, resulting in downregulation of *Atg* genes (101, 102). LAMP2 is also downregulated during aging, suggesting impairment of autophagic flux (103). Moreover, cardiac aging is accompanied by accumulation of lipofuscin in lysosomes, which suppresses lysosomal activity (18). Several interventions to stimulate autophagy not only extend life span but also alleviate cardiac aging and increase stress resistance. For example, systemic Atg5 overexpression activates autophagy and extends life span in mice (104), whereas the beneficial effects of spermidine upon aging are lost in *atg5* knockout mice (19).

Mitophagy is fundamental for preservation of cardiac function at baseline. Baseline cardiac mitophagy is downregulated during aging, accompanied by a decline in mitochondrial function (19). Mice with cardiac-specific *parkin* deletion rapidly develop a lethal cardiomyopathy due to impaired mitophagy-dependent elimination of fetal mitochondria, which must be replaced by adult mitochondria soon after birth (105). Similarly, disruption of Mfn2-dependent mitochondrial recruitment of Parkin during weaning induces lethal cardiomyopathy due to accumulation of fetal mitochondria (105). Parkin translocation to mitochondria and activation of mitophagy are inhibited by cytosolic p53. p53 knockout preserves mitochondrial function in mice during aging (83), and Parkin overexpression ameliorates aging-induced cardiac dysfunction and senescence, suggesting that p53 and Parkin may control cardiac aging through regulation of mitophagy (83). UPR^{mt}, caused by either matrix protein misfolding or an imbalance between mitochondrial and nuclear proteins, induces mitophagy and other cell protective mechanisms, including secretion of mitokines, circulating factors that transmit the effect of UPR^{mt} to other organs (106).

Elucidation of the endogenous mechanisms connecting UPR^{mt} to mitophagy may provide hints toward stimulating mitophagy in aging hearts.

4.2. Autophagy During Myocardial Ischemia and Reperfusion

Activation of autophagy represents a prosurvival mechanism during myocardial ischemia. Autophagy activation ensures the availability of energy substrates, removes damaged mitochondria, and reduces oxidative stress that would otherwise exacerbate myocardial cell death (3–5). Autophagy activation during ischemia is triggered by activation of the AMPK pathway and inhibition of the Rheb/mTORC1 pathway. Disruption of these mechanisms impairs autophagy activation and exacerbates myocardial injury (12, 13). Nox4 also contributes to autophagy activation and cardioprotection during ischemia via activation of ATF4 (51). Nox4 activation and autophagy during energy deprivation appear to be negatively regulated by the tyrosine kinase Fyn (107). In the pig heart, autophagy may counteract apoptosis in the ischemic area during chronic ischemic hibernation (108). Thus, interventions to stimulate autophagy may be protective during myocardial ischemia.

The signaling mechanisms that mediate reperfusion injury are distinct from those that mediate ischemic injury. During reperfusion, nutrients and oxygen are restored to the ischemic tissue, but ROS markedly accumulate, leading to progressive myocardial injury. Autophagy is massively activated during reperfusion, independently of AMPK. In this situation, Beclin 1 is strongly upregulated in the heart during reperfusion in a ROS-dependent manner (13). Mice with systemic heterozygous *beclin 1* deletion display significantly reduced autophagy and ischemic injury, indicating that Beclin 1-dependent upregulation of autophagy during reperfusion is maladaptive (13). One possible explanation for the detrimental effect of autophagy during I/R is that it induces autosis in cardiomyocytes (9). Because autosis is not necessarily constrained by inhibition of lysosomal degradation, excessive autophagosome formation at the expense of intracellular membrane sources, rather than lysosomal destruction, may be responsible for cellular dysfunction and death. Currently, however, it remains unclear whether I/R induces autosis in the heart and, if so, when it is activated and whether it contributes to overall death during I/R. Careful comparison of the time courses of autophagy and cell death would help shed light on the precise role of autophagy and the nature of cardiomyocyte death during I/R.

It should be noted that whether upregulation of Beclin 1 during I/R induces myocardial injury by stimulating autophagy remains the subject of debate. One report suggests that Beclin 1 interferes with autophagosome-lysosomal fusion through unknown mechanisms (109). In this scenario, Beclin 1 both positively and negatively regulates autophagy. Thus, autophagy functions efficiently only when Beclin 1 is within the optimum concentration range. Further investigation is required to clarify how Beclin 1 function is regulated during reperfusion and how it affects overall cardiomyocyte function.

The function of autophagy during I/R may also depend upon the identity of its cargo. For example, unlike general autophagy, mitophagy appears to protect the heart during I/R. Mitophagy inhibition in cardiac-specific heterozygous *Drp1* knockout mice causes accumulation of damaged and dysfunctional mitochondria, which in turn promotes cardiomyocyte death during reperfusion (11). Disruption of PGAM5 also promotes necroptosis in the heart in response to I/R, through inhibition of mitophagy (110), and mitophagy inhibition by bicarbonate increases ischemic injury (111). Conversely, activation of mitophagy through ischemic preconditioning and simvastatin treatment reduces the size of I/R–induced infarcts (112). Mitophagy is activated in the human right atrial appendage during cardiac surgery, together with increases in mitochondrial biogenesis, which promote turnover of cardiac mitochondria in response to myocardial I/R injury (113). Thus,

small molecules selectively activating mitophagy may be beneficial for the prevention of I/R injury.

4.3. Autophagy During Heart Failure

The role of autophagy during cardiac hypertrophy, remodeling, and HF is also context dependent. Complete disruption of autophagy appears to be detrimental during PO. Mice with cardiac-specific *atg5* deletion develop cardiac dysfunction when subjected to transverse aortic constriction, with significant accumulation of misfolded proteins, altered mitochondria, and damaged sarcomeres (10). However, in mice subjected to severe PO, Beclin 1 overexpression in cardiomyocytes exacerbates pathologic remodeling, whereas heterozygous *beclin 1* disruption reduces cardiac hypertrophy and dysfunction (22). Trichostatin A, an HDAC inhibitor, and dimethyl α-ketoglutarate, a precursor of cytosolic acetyl-CoA, reduce cardiac hypertrophy and reverse cardiac dysfunction in mice subjected to PO by inhibiting autophagy (114). The level of autophagy activation in response to PO may depend upon the severity of PO and the timing of measurement. Thus, dissecting the specific conditions under which autophagy becomes detrimental during PO is of interest.

Autophagy activation appears to be beneficial during cardiac remodeling after MI, which causes volume overload. Pharmacological inhibition of autophagy exacerbates cardiac dysfunction and dilation during the chronic phase of MI. Although autophagy is modestly activated both at the border zone and in the remote area of MI, its activity appears insufficient to control the quality of proteins and organelles, as misfolded proteins accumulate and mitochondrial dysfunction develops in post-MI hearts. We believe that signaling mechanisms negatively regulating autophagy, including Mst1, are activated and suppress autophagy below the required level. Indeed, *Mst1* gene deletion alleviates cardiac remodeling and dysfunction in the post-MI heart through activation of autophagy. Mst1 inhibition also reduces infarct size, suggesting that autophagy may contribute to the healing process of the heart, possibly through activation of reparative mechanisms, angiogenesis, or even facilitation of regeneration. These beneficial effects are abrogated by monoallelic *beclin 1* deletion, suggesting that enhanced autophagy plays a significant role in mediating the protective effect of Mst1 inhibition in post-MI hearts (17).

Mitophagy is also necessary for cardiac adaptation to PO. Although mitophagy is activated in response to PO, it is activated only transiently, in the acute phase of PO, and inactivated thereafter. Mitochondrial and cardiac dysfunction develop after mitophagy is inactivated. TAT-Beclin 1, an inducer of autophagy, ameliorates cardiac function by reactivating mitophagy (115). These results suggest that mitophagy is adaptive, preserving mitochondrial function during pathological hypertrophy. How mitophagy is inactivated after transient activation during PO is unknown. Mitophagy is also involved in cardiac remodeling and HF after MI. Parkin is upregulated and mitophagy is observed in the peri-infarct zone in post-MI hearts, whereas Parkin-deficient mice exhibit larger infarcts and increased cardiac remodeling (16).

Interestingly, the time course of mitophagy activation in response to PO is distinct from that of Parkin translocation to mitochondria (115), and Parkin recruitment is independent of PINK1 after MI (116), suggesting that mitophagy activation in the heart in response to hemodynamic stress may be regulated by unique signaling mechanisms rather than the well-established PINK1–Parkin mitophagy. Mitochondrial autophagy is important for proper degradation of mitochondrial DNA by mitochondrial DNase II. When coordination between mitophagy and DNase II is disrupted, improperly degraded mitochondrial DNA induces sterile inflammation and contributes to the development of HF during PO (117).

4.4. Autophagy and Genetic Cardiomyopathy

Genetic defects in autophagy cause cardiomyopathy. An X-linked deletion of *lamp2* causes Danon's disease, a lysosomal storage disease associated with hypertrophic cardiomyopathy (HCM), skeletal muscle alterations, and neurological disorders. Deletion of the *lamp2b* isoform disrupts fusion between autophagosomes and lysosomes, impairing degradation of autophagic cargos (118). Mice with muscle-specific deletion of *ragA/B*, components of the lysosomal Ragulator–Rag complex, display lysosome dysfunction due to the loss of lysosomal v-ATPase function and reduced autophagic flux, and they develop a progressive HCM (119). The molecular mechanism connecting the lack of autophagy with cardiac hypertrophy is currently unknown.

In a model of desmin-related cardiomyopathy caused by an αB-crystallin mutation, partial deletion of *beclin 1* dramatically increases cardiac intracellular aggregates called aggresomes, thereby exacerbating HF and mortality (120). Thus, autophagy is an adaptive mechanism that alleviates accumulation of aggresomes. Autophagy is impaired in cardiomyocytes with overexpression of mutated αB-crystallin (20). Thus, aggresomes may directly inhibit autophagy by physically obstructing autophagic flux, exhausting autophagic machinery, or activating negative regulators of autophagy. Reactivation of autophagy through Atg7 overexpression or exercise training reduces protein aggregation, cardiac dysfunction, and mortality in mice with mutated αB-crystallin (121). Autophagy activation through HDAC6 inhibition or expression of small ubiquitin-like modifier E2 enzyme UBC9 also ameliorates cardiac structure and function in these mice (122, 123). Molecular mechanisms through which these interventions can reactivate autophagy remain to be elucidated. Autophagic flux is impaired in rat cardiomyocytes treated with amyloidogenic immunoglobulin light chain isolated from patients with amyloid cardiomyopathy due to marked lysosomal dysfunction and downregulation of genes involved in lysosome biogenesis, including TFEB. Reactivation of autophagic flux through treatment with rapamycin reduces contractile dysfunction and cardiomyocyte death induced by amyloidogenic immunoglobulin light chain deposition in an in vivo zebrafish model (124).

Autophagic flux is impaired in a mouse model of DCM and left ventricular noncompaction caused by a mutation in the *PLEKHM2* gene (125). Likewise, activation of autophagy by temsirolimus improves cardiac function in a mouse model of DCM caused by *lamin A/C* gene mutation (126). Myocardial autophagosome abundance, as assessed by electron microscopy, is directly correlated with outcome in patients with DCM, confirming that autophagy is beneficial in limiting the progression of human DCM (127). Expression of Vps34 is reduced in the hearts of patients with HCM, and *vps34* knockout mice exhibit cardiac derangements resembling HCM. Autophagic flux and ESCRT-mediated protein degradation are impaired, and accumulation of K63-polyubiquitinated αB-crystallin is observed in the hearts of *vsp34* knockout mice (128).

In summary, in many forms of cardiomyopathy, autophagy is either primarily or secondarily impaired, which in turn deteriorates protein and organelle quality control mechanisms. Elucidation of the specific molecular mechanisms by which autophagy is impaired or through which autophagy is reactivated should lead to the development of effective interventions to alleviate cardiac dysfunction in these patients.

4.5. Autophagy and Diabetic Cardiomyopathy

Autophagy is generally altered in diabetic conditions (129). In many mouse models of type II diabetes, cardiac autophagy is impaired at the level of either autophagosome formation or autophagosome-lysosome fusion, depending on the cause of diabetes, disease duration, severity of metabolic derangements, and associated cardiac alterations (129). In mice fed a high-fat

diet (HFD), myocardial autophagy is impaired at baseline and in response to myocardial ischemia through activation of the Rheb/mTORC1 pathway, in association with inhibition of AMPK. Reduced autophagy was observed in the hearts of Ossabaw pigs fed an atherogenic diet during the transition from obesity to metabolic syndrome (130). Autophagic flux was also severely impaired in the hearts of mice fed a Western diet (45% of calories from fat), through activation of Nox2 and protein kinase C (52). In addition, lipid overload also affects CMA by interfering with Lamp2a (131).

Autophagy defects contribute to HFD-induced loss of cardioprotection during ischemia. Rapamycin administration abrogates the increased ischemic injury observed in HFD-fed mice subjected to acute prolonged ischemia, whereas the beneficial effects of rapamycin are lost with heterozygous *beclin 1* deletion, indicating that these effects are mediated by autophagy reactivation (12). Physical exercise reactivates myocardial autophagy in mice with HFD-induced type II diabetes by reducing interaction of Beclin 1 with Bcl-2, which in turn improves glucose tolerance (132). HFD-induced suppression of cardiac autophagy is aggravated by adiponectin gene deletion, whereas reactivation of autophagy by rapamycin reduces cardiac abnormalities, suggesting that autophagy defects contribute to the development of diabetic cardiomyopathy (133).

Cardiac autophagy is inhibited in many mouse models of type I diabetes as well. AMPK activation reactivates autophagy in diabetic hearts by promoting dissociation of Bcl-2 from Beclin 1 (134). Reactivation of autophagy through overexpression of Beclin 1 aggravates diabetes-induced cardiac abnormalities (135). Conversely, partial deletion of *beclin 1* or ablation of *atg16* reduces cardiac autophagy in diabetic mice, but, surprisingly, it ameliorates cardiac structure and function. These results suggest that autophagy inhibition is an adaptive mechanism in type I diabetic mouse hearts. Interestingly, inhibition of autophagy through genetic disruption of *beclin 1* or *atg16* was associated with reactivation of mitophagy, suggesting that stimulation of mitophagy may be one mechanism by which suppression of general autophagy protects diabetic hearts (135). The molecular mechanism by which suppression of autophagy paradoxically promotes mitophagy remains to be elucidated.

4.6. Autophagy During Doxorubicin-Induced Cardiomyopathy

Cardiomyopathy is a serious side effect of anticancer drugs, including DOX. DOX dose-dependently increases cardiomyocyte death through downregulation of GATA4 and activation of autophagy (136). GATA4 overexpression inhibits autophagy and blunts DOX-induced toxic effects in cardiomyocytes. Similarly, 2-deoxyglucose antagonizes DOX-induced cardiomyocyte death by inhibiting autophagy (137). Molecular mechanisms through which autophagy potentiates DOX toxicity in these models remain to be elucidated. In contrast, another report showed that DOX inhibits autophagic flux in cultured cardiomyocytes and the heart independently of mTOR activation (21). Autophagic flux can be inhibited due to impaired lysosomal acidification and function caused by suppression of V-ATPase activity, which in turn leads to an accumulation of undegraded autolysosomes. *Beclin 1*-deficient mice display a better cardiac phenotype and autophagic flux when treated with DOX, whereas mice with Beclin 1 overexpression showed more severe DOX-induced cardiac abnormalities (21). These results suggest that exaggerated autophagosome formation inhibits autophagic flux and contributes to DOX-induced cardiotoxicity (21). Another study demonstrated that DOX promotes cardiomyocyte death through inhibition of TFEB and impairment of autophagic flux. Restoration of TFEB levels reduced the detrimental cardiomyocyte effects of DOX (138). Together, these studies suggest that maintaining autophagic flux at an appropriate level by preserving lysosomal function may be important to prevent progression of DOX cardiotoxicity. Additionally, DOX blocks cardiac mitophagy through p53-dependent

inhibition of Parkin mitochondrial translocation, which in turn induces accumulation of dysfunctional mitochondria (83).

5. AUTOPHAGY-MODULATING CLINICAL INTERVENTION

Several drugs are emerging as potential candidates for modulating autophagy in heart disease patients. Among the most characterized inducers of autophagy are compounds that inhibit mTORC1 or activate AMPK. mTORC1 inhibitors protect against chronic cardiac remodeling, myocardial ischemia, cardiac hypertrophy, and cardiac aging (12). AMPK activators, such as metformin and 5-aminoimidazole-4-carboxamide ribonucleotide, stimulate autophagy and protect the heart against ischemia and diabetic cardiomyopathy (139, 140). Short-term caloric restriction may also represent a strategy to reduce cardiac stress via induction of autophagy. Caloric restriction reduces MI by activating autophagy during I/R (141). Intermittent fasting confers cardioprotection by stimulating autophagy and lysosomal function (54). Exercise activates autophagy through phosphorylation of Bcl-2, which in turn promotes endurance during acute exercise (132) and improves cardiac function in desmin-related cardiomyopathy (121). Spermidine protects the heart in hypertensive rats either directly or by reducing blood pressure, and diet-derived spermidine intake correlates with a reduction in cardiovascular diseases (19). Trehalose, a natural disaccharide, activates autophagy independently of mTOR and clears protein aggregates (142). TAT-Beclin 1, derived from an internal region of Beclin 1, interacts with Golgi-associated plant pathogenesis-related protein 1, a negative regulator of autophagy, thereby promoting autophagy by mobilizing endogenous Beclin 1 (143). TAT-Beclin 1 improves mitochondrial function and mitophagy, preventing PO-induced HF (115). Modulation of specific miRNAs may represent another possible approach to modulating cardiac autophagy. Inhibition of miRNA-22, a strong inhibitor of autophagy in cardiomyocytes, using locked nucleic acid–anti-miR-22, activates autophagy and attenuates cardiac remodeling in aged mice (57). The recent development of reliable indicators of autophagy and mitophagy, namely GFP-LC3-RFP-LC3ΔG (144) and Mito-Keima (75), respectively, may facilitate high-throughput screening of small molecules regulating autophagy and mitophagy.

6. PERSPECTIVES

Data obtained in animal models indicate that modulation of autophagy is a promising mode of treatment of heart disease in human patients. Here, we discuss outstanding issues and future directions of research regarding the role of autophagy in the heart.

Although there has been dramatic progress in autophagy research in the heart over the past decade, the ability to evaluate whether autophagy is activated or inhibited in a given condition and to clarify its functional significance still needs to be improved. One critical issue is the assessment of autophagy and autophagic flux in a given condition. This is not an easy task, and thus, extreme caution must be exercised when drawing conclusions regarding autophagic activity. Multiple methods should be used, ideally including indicator dyes such as tandem fluorescent LC3 (11), Mito-Keima (75), or GFP-LC3-RFP-LC3ΔG (144), following applicable guidelines (145). It is always essential to assess flux using lysosome inhibitors. The functional significance of autophagy modulation should be evaluated using multiple molecular interventions affecting autophagy.

An obvious outstanding issue is whether autophagy induces cell death in the heart. That autophagy can kill cardiomyocytes has been demonstrated in several mouse models in which autophagy is either excessively or undesirably activated (3–5). Whether autophagy-induced cardiomyocyte death occurs in the heart in pathophysiologically relevant conditions, such as I/R,

MI, PO, and DOX toxicity, should be further investigated. In particular, whether or not autosis, a specific form of cell death, is induced in these conditions is of great interest. Because cardiac glycoside effectively prevents autosis (9), addressing this issue is highly significant clinically.

The role of autophagy in nonmyocytes is poorly understood in the heart. In particular, the role of autophagy in cardiac stem cells is of great interest. Signaling mechanisms activated by the local environment positively control differentiation of cardiac and skeletal progenitor cells through autophagy and mitophagy (146, 147). The role of autophagy in stem cells might be lineage dependent, however, because autophagy maintains stemness of hematopoietic stem cells by suppressing metabolism through clearance of active mitochondria. The age-dependent decline in autophagy leads to decreases in the capacity for self-renewal and regeneration of hematopoietic stem cells (148). The role of autophagy in other cell types, including cardiac fibroblasts, endothelial cells, and inflammatory cells in the heart, is also of great interest. Autophagy is activated in cardiac fibroblasts in response to volume overload and induces degradation of procollagen I and fibronectin, thereby contributing to cardiac remodeling (149).

Most studies regarding cardiac autophagy have focused on macroautophagy mediated through the Atg conjugation system and through LC3. However, the existence of macroautophagy independent of the Atg conjugation system has been reported (99). Furthermore, the other forms of autophagy, namely CMA and microautophagy, have been far less well investigated in the heart. During PO, mitophagy is only observed in the heart after conventional autophagy has been downregulated (115). Similarly, in a mouse model of diabetic cardiomyopathy, mitophagy is stimulated when macroautophagy is inhibited (135). Whether autophagy and mitophagy are regulated by distinct molecular mechanisms in the heart and, if so, how their activities affect each other, should be clarified.

Increasing evidence suggests that autophagy can target specific cargos. Morphological analyses of cargos with electron microscopy and unbiased proteomic analyses of various subcellular fractions, including lysosomes, might identify novel cargos degraded through autophagy. Autophagy is often triggered by metabolic cues. Thus, it seems reasonable to speculate that autophagic degradation of cellular constituents generates feedback signals. For example, CMA degrades enzymes involved in fatty acid oxidation in the liver, so downregulation of CMA induces hepatic steatosis (150). It would be interesting to test whether macroautophagy affects metabolism and other cellular functions through degradation of specific substrates in cardiomyocytes.

Finally, new methods to evaluate autophagy in human subjects should be developed. These methods are fundamental for understanding the involvement of autophagy in heart disease patients. Indirect circulating biomarkers may represent a possibility, and specific probes tagging autophagosomes should be developed. Furthermore, it is important to perform biochemical or electron microscopy studies using myocardial biopsies. These should improve understanding of how autophagy affects prognosis in subjects affected by heart disease and how it should be modified.

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