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Autophagy in Ischemic Heart Disease

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Abstract

Autophagy is a major catabolic pathway by which mammalian cells degrade and recycle macromolecules and organelles. It plays a critical role in removing protein aggregates as well as damaged or excess organelles in order to maintain intracellular homeostasis and to keep the cell healthy. In the heart, autophagy occurs at low levels under normal conditions, and defects in this process cause cardiac dysfunction and heart failure. However, this pathway is rapidly upregulated under environmental stress conditions, including ATP depletion, reactive oxygen species, and mitochondrial permeability transition pore opening. Although autophagy is enhanced in various pathophysiological conditions such as during ischemia and reperfusion, the functional role of increased autophagy is not clear and is currently under intense investigation. In this review, we discuss the evidence for autophagy in the heart in response to ischemia and reperfusion, identify factors that regulate autophagy, and analyze the potential roles autophagy might play in cardiac cells.

Keywords

Apoptosis; Autophagy; Mitochondria; Myocardial ischemia

Introduction

The study of autophagy in mammalian systems and in disease states is advancing rapidly, and many investigators are entering this new and exciting field. Autophagy is a physiologic process that is involved in degradation of long-lived proteins and removing excess or damaged organelles¹. In this process, a double-membrane structure called the autophagosome sequesters cytoplasmic components such as ubiquitinated protein aggregates or organelles including mitochondria, peroxisomes, and endoplasmic reticulum (ER). Autophagy occurs constitutively in the normal myocardium, and disruption of this pathway results in development of left ventricular dilation and severe contractile dysfunction in the absence of any stress². Moreover, Danon disease is due to a defect in the autophagy-lysosomal pathway that is characterized by the development of cardiomyopathy, confirming that this pathway is essential for cellular homeostasis in the heart and that disruption of this process has adverse effects^{3, 4}. Autophagy is also induced when there is a change in the cellular environment, and is known to be an important survival mechanism that is rapidly activated in response to starvation when recycling of fatty acids and amino acids from proteins and lipids are required for survival⁵. Autophagy has been shown to be substantially increased in mouse hearts after fasting⁶. In addition, there is evidence that autophagy is enhanced in various pathological conditions including cardiac hypertrophy, cardiomyopathy, and heart failure^{4, 7–14}. Many studies have reported that autophagy is upregulated during myocardial ischemia and reperfusion^{15–21 18, 22 16, 20, 23}.

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However, the functional role of this enhancement is not clear. In this review, we discuss the evidence of enhanced autophagy in response to myocardial ischemia and reperfusion, identify signals that activate autophagy, and analyze the potential roles that autophagy might play in cells.

The Process of Autophagy

There are three main types of autophagy: macroautophagy, microautophagy and chaperone-mediated autophagy. Macroautophagy (hereafter referred to as autophagy) is the most common form of autophagy and is characterized by formation of a cup-shaped pre-autophagosomal double-membrane structure, which surrounds cytoplasmic material and closes to form the autophagosome. The outer membrane of the autophagosome then fuses with the lysosome to create a single membrane autophagolysosome, where the inner membrane of the autophagosome and its contents are digested⁵. This process is regulated by the autophagy (Atg) proteins. Activation of the class III PI3K/Vps34 and Beclin1 (Atg6) complex causes induction of autophagy which involves formation of an isolation membrane to which the Atg proteins are recruited^{24, 25}. Two ubiquitin-like protein conjugation pathways, Atg12-Atg5 and the microtubule associated protein light chain 3-phosphatidylethanolamine (LC3-PE) are involved in the expansion of the isolation membrane^{5, 26} (Figure 1). Atg12 and Atg5 are covalently conjugated when Atg12 is activated by the ubiquitin-activating E1 enzyme, Atg7 and then transferred to the E2 ubiquitin-conjugating enzyme Atg10. Atg10 is released when Atg12 is covalently conjugated to a lysine residue on Atg5²⁷. Atg12-Atg5 then binds to Atg16 and this trimeric complex then localizes to the outer membrane of the isolation membrane which is essential for recruitment of LC3 and elongation of the membrane^{28, 29}.

LC3 is synthesized as a larger precursor protein and is converted to LC3-I by the cysteine protease Atg4 which exposes a glycine residue at the C-terminal³⁰. Similar to Atg12, LC3-I is activated by Atg7 and then transferred to Atg3, an E2-like enzyme, which catalyzes the covalent conjugation with PE^{31, 32}. LC3-PE translocates to the autophagosome membrane in an Atg5-dependent manner²⁹. LC3 fused to a fluorescent protein such as GFP or mCherry is commonly used as a marker to monitor autophagy *in vivo*^{33–35}. The GFP-LC3 is diffusely distributed in the cytosol under normal conditions, but upon proteolysis of the C-terminus and lipidation, it is recruited into autophagosomes, which are evident as microscopic specks, or puncta (Figure 2). The extent to which GFP-LC3 is recruited into punctate structures correlates very well with the extent of autophagy and is now regarded as a reliable indicator of autophagic activity, with certain caveats^{36, 37}. Atg5 is rate-limiting for autophagy and overexpression increases autophagy^{38, 39}. Mutation of lysine 130, the site for the ubiquitin-like conjugation of Atg12, causes Atg5 to function as a dominant negative (Figure 2); Atg5^{K130R} is a useful tool to selectively block autophagy^{36, 40, 41}.

Autophagy in myocardial ischemia and reperfusion injury

Many studies have reported that the number of autophagosomes increases in the heart during both ischemia and reperfusion^{15–21 18, 22 16, 20, 23}. Autophagy was initially reported to be enhanced in fetal mouse hearts in organ cultures after being subjected to hypoxia combined with glucose deprivation and subsequent reperfusion¹⁹. Later, another study found that brief ischemia (20 min) did not induce autophagy until reperfusion in Langendorff perfused rabbit hearts¹⁵. However, longer ischemia (40 min) caused an increase in the number of autophagosomes which was further increased during reperfusion, suggesting that both ischemia and reperfusion can induce autophagy. Gurusamy et al. found that 30 min of ischemia and two hours of reperfusion caused upregulation of autophagy in a Langendorff model as assessed by an increase in LC3-II and Beclin 1⁴². Another study found enhanced autophagy and lysosomal activity in pig hearts subjected to six episodes of ischemia and reperfusion²¹. Enhanced

autophagy has also been observed in isolated cardiac cells where exposure to hypoxia/reoxygenation or simulated I/R (sI/R) caused an increase in the number of autophagic vesicles^{17, 20, 42}. We found that autophagic activity was inhibited during ischemia, but was re-activated during reperfusion in HL-1 myocytes¹⁶.

Signals that Induce Autophagy in Ischemia/Reperfusion

ATP levels and AMPK

Multiple signals are likely to activate autophagy during I/R. For instance, autophagy has been reported to be upregulated in response to reduced cellular content of ATP⁴³. In cultured cardiac myocytes, glucose deprivation caused significant reduction in the levels of ATP which coincided with upregulation of autophagy¹⁸. Moreover, myocardial ischemia causes a decrease in ATP levels and an increase in the AMP/ATP ratio resulting in activation of the AMP-activated protein kinase (AMPK)⁴⁴. Activated AMPK enhances uptake and oxidative metabolism of fatty acids as well as increases glucose transport and glycolysis⁴⁵. Matsui et al. reported that glucose deprivation induced autophagy via activation of AMPK and inhibition of mTOR in isolated cardiac myocytes¹⁸. mTOR is a negative regulator of autophagy and functions as a sensor for cellular energy and amino acid levels and is negatively regulated by AMPK via a pathway involving the tuberous sclerosis complex (TSC1/2) and its substrate Rheb, a Ras-related small GTPase⁴⁶. Moreover, induction of autophagy in response to myocardial ischemia was reduced in transgenic mice overexpressing a dominant negative AMPK¹⁸.

Hypoxia and Bnip3

The BH3-only protein Bnip3 is an important contributor to I/R injury¹⁷ and is upregulated by hypoxia^{47, 48}. Many studies have reported that Bnip3 induces upregulation of autophagy in different cells, including cardiac cells. We found that overexpression of Bnip3 resulted in significant induction of autophagy in HL-1 myocytes¹⁷ and adult cardiac myocytes (unpubl. observation). Also, overexpression of a Bnip3 dominant negative protein reduced autophagy induced by simulated I/R in HL-1 myocytes, suggesting that Bnip3 contributes to activation of autophagy during I/R¹⁷. Autophagy is also upregulated during hypoxia. For instance, Zhang et al. found that upregulation of autophagy during hypoxia required hypoxia-dependent factor-1-expression of Bnip3⁴⁹, and Azad et al. reported that expression of Bnip3 siRNA or a dominant-negative form of Bnip3 reduced hypoxia-induced autophagy⁵⁰. In contrast, the study by Papandreou et al. reported that hypoxia-induced activation of autophagy in cancer cell lines was not dependent on nutrient deprivation, or expression of Bnip3, but involved activation of AMPK⁵¹. Clearly, more studies are needed to define the role of Bnip3 in hypoxia-mediated autophagy, but a reasonable interpretation is that Bnip3 causes mitochondrial damage with secondary upregulation of autophagy to remove damaged organelles.

Calcium

A consequence of ischemia is elevated levels of intracellular Ca²⁺ due to the plasma membrane sodium calcium exchanger (NCX) operating in reverse, loading the cell with Ca²⁺⁵². It was recently demonstrated that increased cytosolic calcium is a potent inducer of autophagy. Ca²⁺ mobilizing agents such as vitamin D compounds, ionomycin, ATP, and thapsigargin inhibited the autophagy regulator mTOR, which resulted in massive accumulation of autophagosomes in a Beclin1- and Atg7-dependent manner⁵³. Thus, it is very likely that elevated intracellular Ca²⁺ is one of the many factors that induces autophagy during ischemia.

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS)

ROS are generated in the myocardium during both ischemia and reperfusion^{54, 55}, which can activate autophagy in cardiac myocytes. For instance, LPS treatment increased ROS and induced autophagy in neonatal cardiac myocytes^{56, 57}. Mitochondria are major sources of ROS which can activate apoptosis, but it has recently been reported that mitochondria also induce autophagy through ROS. For instance, Chen et al. found that treatment with rotenone or TTFA to inhibit mitochondrial electron transport chain (mETC) complexes I and II, respectively, induced significant autophagy in U87 and HeLa cells⁵⁸. Moreover, rotenone and TTFA treatment induced substantial ROS production in cells and the presence of a ROS scavenger decreased autophagy, suggesting that ROS generated from the mETC activates autophagy. Moreover, evadiamine, a quinoxaline alkaloid, was reported to induce autophagy via increased ROS production in HeLa cells⁵⁹. At least one critical step in the autophagy pathway has been reported to be regulated by ROS. Atg4 is a cysteine protease that cleaves LC3 to expose a glycine residue allowing for the covalent bonding of LC3 to PE. Atg4 is also responsible for recycling of LC3 by cleaving PE from PE-conjugated LC3. Hydrogen peroxide generated during starvation was found to regulate autophagosome formation by inactivating Atg4 by oxidation of an essential cysteine residue, which led to accumulation of LC3-PE on the phagophore membrane and formation of autophagosomes⁶⁰.

Nitric oxide (NO) has been reported to play an important role in protecting the heart against ischemia/reperfusion injury⁶¹, whereas excess levels of NO contribute to heart failure⁶². There is evidence that NO activates autophagy in cells. Treatment with the NO donor S-nitrosocysteine induced autophagy, and electron microscopy showed autophagosomes engulfing damaged mitochondria in neurons⁶³. Moreover, Yang et al. found that nitric oxide induced autophagy in HeLa cells⁵⁹. In contrast, Rabkin and Klassen evaluated changes in gene expression in neonatal cardiac myocytes treated with the NO donor SIN-1 and found that gene expression of the autophagy genes *beclin 1*, *Atg5*, and *Atg12* did not change in the cells, suggesting that NO does not cause upregulation of autophagy in cardiac myocytes⁶⁴. However, this study only measured changes in gene expression and did not assess whether NO induced formation of autophagosomes in cells, and as the study by Matsui et al. demonstrated, autophagy can be induced without upregulation of autophagy genes such as *beclin1*¹⁸. Thus, further studies examining the relationship between NO and autophagy in cardiac myocytes is necessary.

mPTP opening

The mitochondrial permeability transition pore (mPTP) opening has been reported to induce autophagy in mammalian cells^{65–67}. For instance, Arrington et al. found that overexpression of the Ca²⁺-activated cysteine protease Calpain 10 resulted in mitochondrial swelling and increased autophagy which was blocked by the mPTP inhibitor cyclosporine A⁶⁶. We found that inhibition of the mPTP with cyclosporine A decreased the upregulation of autophagy after sI/R in HL-1 myocytes (unpubl. observation). Since reperfusion triggers opening of the mPTP⁶⁸, it is possible that the mPTP serves as an upstream signal for autophagy in I/R.

ER stress and the Unfolded Protein Response (UPR)

The endoplasmic reticulum (ER) is important in protein synthesis and folding, as well as Ca²⁺ homeostasis. Perturbation of the ER environment induces ER stress and lead to the accumulation of unfolded or misfolded proteins which activates the UPR. The UPR is a protective and compensatory mechanism which activates multiple functions to avoid damage to the cell. It rapidly decreases protein synthesis, induces expression of ER chaperone proteins, and increases the degradation of misfolded proteins⁶⁹. The UPR is activated in mouse hearts subjected to ischemia/reperfusion^{70, 71}, as well as in surviving cardiac myocytes in the border zone of the infarct in a mouse model of *in vivo* myocardial infarction^{70, 72}. Moreover, transgenic

mice overexpressing the monocyte chemoattractant protein-1 (MCP-1) in the heart develop ischemic heart disease and increase the expression of many ER stress response genes⁷³. Several studies have also shown that simulating ischemia or ischemia/reperfusion in cultured neonatal rat or adult mouse ventricular myocytes activates the UPR^{70, 71, 74, 75}. Recently, ER stress has been linked to induction of autophagy^{76–78}. For instance, Tannous et al. reported that pressure overload promoted upregulation of the UPR regulator and ER chaperon BiP, which correlated with upregulation of autophagy in mouse hearts⁷⁹. Ogata et al. found that treatment with ER stressors tunicamycin and thapsigargin caused upregulation of autophagy in neuroblastoma cells, and IRE1, a major sensor of ER stress, was required for induction of autophagy where thapsigargin or tunicamycin failed to induce autophagy in IRE1 deficient mouse embryonic fibroblasts (MEFs)⁷⁸. We showed that release of ER calcium is required for induction of autophagy⁸⁰. Li et al. reported that GRP78/BiP was required for stress-induced autophagy where knockdown of GRP78 expression by siRNA in cells prevented induction of autophagy by ER stress as well as by nutrition deprivation⁸¹. These studies suggest that the UPR signaling pathways are required for induction of autophagy in response to ER stress.

Functional Role of Autophagy in Myocardial Ischemia and Reperfusion

The functional role of autophagy in the heart is currently under intense investigation and studies have characterized this process both *in vitro* and *in vivo*. Interestingly, upregulation of autophagy has been reported to both contribute as well as be the cause of cell death in the heart. Many studies have reported that enhanced autophagy contributes to cell death during I/R. For instance, Aki et al. found that glucose deprivation caused a significant increase in autophagy and cell death, and that inhibiting autophagy with 3-methyladenine (3-MA) or LY294002 reduced cell death, suggesting that autophagy contributes to cell death⁸². Another study reported that inhibiting autophagy by downregulation of Beclin 1 or treatment with 3-MA reduced cell death in isolated cardiac myocytes after simulated to sI/R²⁰. Interestingly, Matsui et al. found that autophagy was protective during ischemia, but that it switched to a detrimental role during reperfusion. They reported that hearts from *beclin 1* (*beclin 1*^{+/-}) heterozygous mice exhibited reduced levels of autophagy during reperfusion compared to wild type litter mates which correlated with decreased apoptosis and reduced infarct size¹⁸. Increased levels of autophagy have also been reported to contribute to cell death in pressure overload-induced heart failure¹⁴. Zhu et al. found that mice with *Beclin 1*^{+/-} had reduced levels of autophagy which coincided with diminished cardiac remodeling induced by pressure overload compared to wild type, whereas transgenic mice with cardiac specific overexpression of *Beclin 1* had increased autophagy and enhanced pathological remodeling in response to stress.

In contrast, many studies have reported that autophagy is a beneficial response to I/R. One of the first studies of autophagy in the heart reported that increased levels of autophagy correlated with functional recovery and salvage of the myocardium after I/R, but impairment of the autophagosome-lysosome pathway during extended ischemia, likely due to depletion of ATP, was associated with irreversible injury and contractile dysfunction¹⁵. Similarly, Yan et al. reported that cardiac myocytes with enhanced autophagy were negative for apoptosis, whereas apoptotic cells were negative for autophagy in a porcine model of chronic myocardial ischemia and hibernating myocardium, suggesting that autophagy served to protect cells against apoptosis in this model²¹. Autophagy has also been shown to protect in cell culture models that mimic I/R injury. For instance, Matsui et al. reported that inhibition of autophagy enhanced glucose deprivation-mediated cell death¹⁸. We found that enhancing autophagy protected against sI/R mediated cell death in HL-1 myocytes, whereas inhibition of autophagy enhanced cell death¹⁶. These studies suggest that upregulation of autophagy promotes survival during ischemia/reperfusion.

Ischemic preconditioning is the well-recognized phenomenon in which a brief episode of ischemia followed by reperfusion confers protection against a subsequent more severe ischemic insult. Recently, Gurusamy et al. reported that preconditioning enhanced autophagy and that inhibition of autophagy abolished the cardioprotective effects of preconditioning⁴². Many agents that have been shown to be cardioprotective also induce autophagy. For instance, rapamycin, a powerful inducer of autophagy, was shown to reduce infarct size in a Langendorff model⁸³. Statins, which have been shown to be beneficial in the post-MI setting regardless of effects on cholesterol levels, are powerful inducers of autophagy; however, pravastatin, which showed less benefit in the PROVE IT-TIMI 22 trial, failed to induce autophagy^{84, 85}. Exercise is another intervention that has been shown to confer cardioprotection against I/R injury^{86, 87} as well as to induce autophagy⁸⁸.

Many other studies have also found that autophagy protects against various cellular stressors. For instance, LPS treatment of neonatal myocytes induced production of ROS and autophagy, but did not induce cell death⁵⁷. However, when autophagy was inhibited with 3-MA in LPS-treated cells, there was significant cell death. This suggests that increased oxidative stress by LPS leads to upregulation of autophagy by the cells to protect itself from damage by these reactive oxygen species. Chen et al. found that autophagy provided protection against cell death in response to mild oxidative stress, but excessive oxidative stress inhibited the autophagic process, resulting in irreversible damage to the cell and activation of apoptosis⁵⁸.

Thus, these studies suggest that autophagy has a dual role in the heart depending on the stimulus. It has been speculated that too much autophagy can cause cell death by excessive degradation of essential proteins and organelles. Clearly, further work will be necessary to establish the role of autophagy in cardioprotection and cell death.

Potential Mechanisms of Cardioprotection by Autophagy in I/R

ATP Maintenance

The primary function of autophagy is catabolic. Within the autophagolysosome, proteins and membranes are degraded into amino acids which can feed into the tricarboxylic acid (TCA) cycle to generate ATP. Fatty acids generated from breakdown of organelle membrane lipids which can be used as fuel by mitochondria in the context of nutrient limitations. Matsui et al. found that glucose deprivation significantly reduced the cellular ATP content which correlated with upregulation of autophagy in cardiac myocytes¹⁸. Moreover, inhibition of autophagy with 3-MA further reduced the levels of ATP and increased cell death in response to glucose deprivation¹⁸. This suggests that activation of autophagy during ischemia promotes survival by maintaining ATP production.

Mitophagy

Mitochondrial integrity is essential to cellular survival. Damaged mitochondria may generate excessive reactive oxygen species (ROS), and if uncoupled, could consume ATP. Therefore their elimination by autophagy is an efficient cytoprotective response. Furthermore, damaged or unstable mitochondria may release cytochrome *c*, AIF, and other apoptosis-promoting factors which would promote damage to neighboring mitochondria. Autophagy is the only process by which the cell can degrade organelles, and transmission electron microscopy studies have shown that many autophagosomes formed during I/R contain mitochondria^{17, 42}. Moreover, ultrastructural analysis of hearts lacking Atg5 showed aggregation of mitochondria and disorganized sarcomeres. Mitochondria also play a major role in inducing autophagy in the cell; autophagy is upregulated when mitochondria fail to maintain ATP levels⁴³, or when mitochondria are damaged⁸⁹. HL-1 cells subjected to simulated ischemia/reperfusion (sI/R) exhibit widespread mitochondrial fragmentation that begins during ischemia and precedes the

development of autophagy¹⁶. A number of investigators have suggested that mitochondrial fragmentation is a prerequisite for efficient mitochondrial autophagy (mitophagy) (reviewed in^{90, 91}). Mitochondrial fission is known to precede autophagy, and some have suggested that fission is a prerequisite for autophagic removal of mitochondria. Indeed, it has been suggested that in aging, mitochondrial fission is impaired, resulting in the accumulation of large, senescent mitochondria⁹¹. As we have recently published⁹², mitochondrial fission occurs during ischemia, well before a detectable increase in autophagy. Furthermore, inhibition of mitochondrial fission in response to simulated ischemia prevents upregulation of autophagy.

Protein Clearance

The autophagy-lysosomal pathway is important in degradation of long-lived proteins, whereas the ubiquitin-proteasome system (UPS) regulates turnover of short-lived proteins⁹³. It has been reported that the function of the proteasome is inhibited in by ischemia/reperfusion injury which is likely due to the substantial increase in the number of substrates that the proteasome degrades as well as decreased activity of the 20S and/or 26S proteasome^{94, 95}. There is evidence that these two degradation systems are functionally coupled. During certain conditions, accumulation of ubiquitinated proteins might exceed proteasomal degradation resulting in a buildup of ubiquitinated proteins and formation of protein aggregates. These large protein complexes are poor substrates for the proteasome⁹⁶, but can be degraded by the autophagosome-lysosome pathway. Deletion of *atg5* or *atg7* in the brain disrupts autophagy and results in the accumulation of polyubiquitinated proteins in neurons^{97, 98}, and deletion of *atg5* in the heart increases the levels polyubiquitinated proteins as well as the activity of the proteasome². Peripheral myelin protein 22 (PMP22) is a short-lived molecule that forms aggregates in response to mutations or inhibition of the proteasome, and removal of the PMP22 aggregates is mediated by autophagy. The accumulation of the aggregates was suppressed by induction of autophagy, and enhancement of autophagy during proteasome inhibition prevented protein aggregate formation and correlated with a reduction in accumulated proteasome substrates. Conversely, simultaneous inhibition of autophagy and the proteasome increased the formation of aggregates⁹⁹. Moreover, it has been reported that inhibition of the proteasome triggered activation of autophagy and that suppression of autophagy caused accumulation of ubiquitinated proteins in isolated cardiac myocytes, suggesting that autophagy is an important mechanism in clearing protein aggregates in cardiac myocytes⁷⁹. Activation of autophagy with rapamycin has been shown to increase clearance of aggregate-prone proteins and decreased accumulation of protein aggregates *in vitro* and *in vivo* 100–102. Recently, Tannous et al. reported that accumulation of ubiquitinated proteins in the heart in response to pressure overload which was not due to diminished proteasome activity, suggesting that the accumulation of ubiquitinated proteins exceeded the capacity of the proteasome⁷⁹. This suggests that both proteasomal and autophagic clearance pathways are activated in response to pressure-overload stress. It is not clear exactly how autophagy attenuates ER stress, but it is likely that the protective effect of autophagy is at the level of protein clearance, which would reduce ER stress caused by these proteins.

Autophagy and Bcl-2 Family Proteins

The Bcl-2 family proteins are important regulators of apoptosis in the cardiovascular system. Transgenic mice with cardiac specific overexpression of anti-apoptotic Bcl-2 are protected against I/R injury^{103–105}, whereas mice deficient for pro-apoptotic Bax have reduced I/R injury¹⁰⁶. Recently, the Bcl-2 family proteins have been linked to autophagy. Structural and functional analysis showed that Beclin1 is a novel BH3-only protein and that anti-apoptotic Bcl-2 and Bcl-X_L can bind to the BH3 domain and inhibit Beclin1^{107, 108}. A BH3 mutant of Beclin1 which has reduced affinity for Bcl-X_L/Bcl-2 was a more potent inducer of autophagy than wild type Beclin1, and mutants of Bcl-2 and Bcl-X_L that were unable to interact with

Beclin1 failed to inhibit autophagy^{6, 108}. Pattingre et al. found that overexpression of Bcl-2 in the heart reduced starvation-induced autophagy compared to wild type⁶. Based on the literature, it is possible that cardiac specific overexpression of Bcl-2 protects against I/R injury by blocking apoptosis and by preventing activation of autophagy by inhibiting Beclin1. This is supported by the fact that Beclin1^{+/-} hearts have reduced autophagy and infarct size after I/R¹⁸. In cells where autophagy serves as a mechanism of cell death, activation of both apoptosis and autophagy may be a way for the cell to fully ensure cell death. Also, Rashmi et al. found that the BH3-only protein Bik induced cell death via the autophagic pathway in MEFs lacking Bcl-2 but not in wild type MEFs¹⁰⁹. If the role of Bcl-2 is to maintain Beclin1 inactive, then these cells may be more sensitive to activation of autophagy leading which may lead to excessive induction of autophagy and cell death. Several of the pro-apoptotic BH3-only members have been reported to activate autophagy. For example, the BH3-only protein Bad or the BH3-mimetic ABT737 directly activated autophagy by disrupting the interaction between Bcl-2/Bcl-X_L and Beclin1^{108, 110}. As discussed above, Bnip3 causes rapid upregulation of autophagy in various cells^{17, 49, 50, 111, 112}, possibly by competing with Beclin1 for binding to Bcl-2⁴⁹, and by inhibiting mTOR by directly interacting with Rheb¹¹³. To date, Bnip3 is the only BH3-only protein that has been reported to induce autophagy in cardiac cells, but based on data in other cell types, it is likely that other BH3-only proteins will induce autophagy in the heart.

A newly identified BH3-only protein, ApoLipoprotein L1 (ApoL1), was also found to induce cell death via autophagy. Overexpression of ApoL1 in cells led to cell death which was reduced by inhibiting autophagy with 3-MA or wortmannin, and autophagy deficient cells were resistant to ApoL1-mediated cell death¹¹⁴. In contrast, upregulation of autophagy in response to Bnip3 in HL-1 cardiac myocytes was shown to serve as a protective response against Bnip3-mediated injury^{17, 22}. The Bnip3 homolog Nix (Bnip3L), which has been implicated in cardiac hypertrophy and development of cardiomyopathy¹¹⁵, was recently reported to play an essential role in mitophagy in erythrocytes. Nix-dependent loss of mitochondrial membrane potential was important for targeting the mitochondria into autophagosomes for clearance during erythroid maturation, and interference with this function impaired erythroid maturation¹¹⁶. It is clear that different BH3-only proteins have different effects on cells depending on the stimulus and the cell type.

Conclusions

Enhanced levels of autophagy in cardiac myocytes are observed in many cardiovascular diseases, but the functional role of autophagy in these settings is unclear. Increasing evidence from *in vitro* and *in vivo* studies suggest that basal levels of autophagy are important for maintaining cellular homeostasis and for protecting cells against excess or dysfunctional organelles. However, it appears that enhancing autophagy can also promote survival in response to milder stress, such as brief hypoxia and lower levels of oxidative stress, possibly by removing damaged and harmful organelles as well as recycling of macromolecules to maintain energy levels and protein synthesis. In contrast, it is possible that severe stress, such as prolonged hypoxia and subsequent reperfusion, results in excessive and/or long-term upregulation of autophagy which causes cell death by promoting cell death by excessive self digestion of essential organelles and proteins. Substantial loss of cardiac myocytes after I/R can lead to contractile dysfunction and heart failure. Thus, manipulation of autophagy may represent a potential future therapeutic target to treat or prevent development of heart disease. However, there are still many unanswered questions about its role(s) in the heart and it is possible that different pathways trigger death-associated and survival-associated autophagy. Therefore, it is important to elucidate the molecular mechanism of how and under what conditions autophagy contributes to survival or cell death in the heart.

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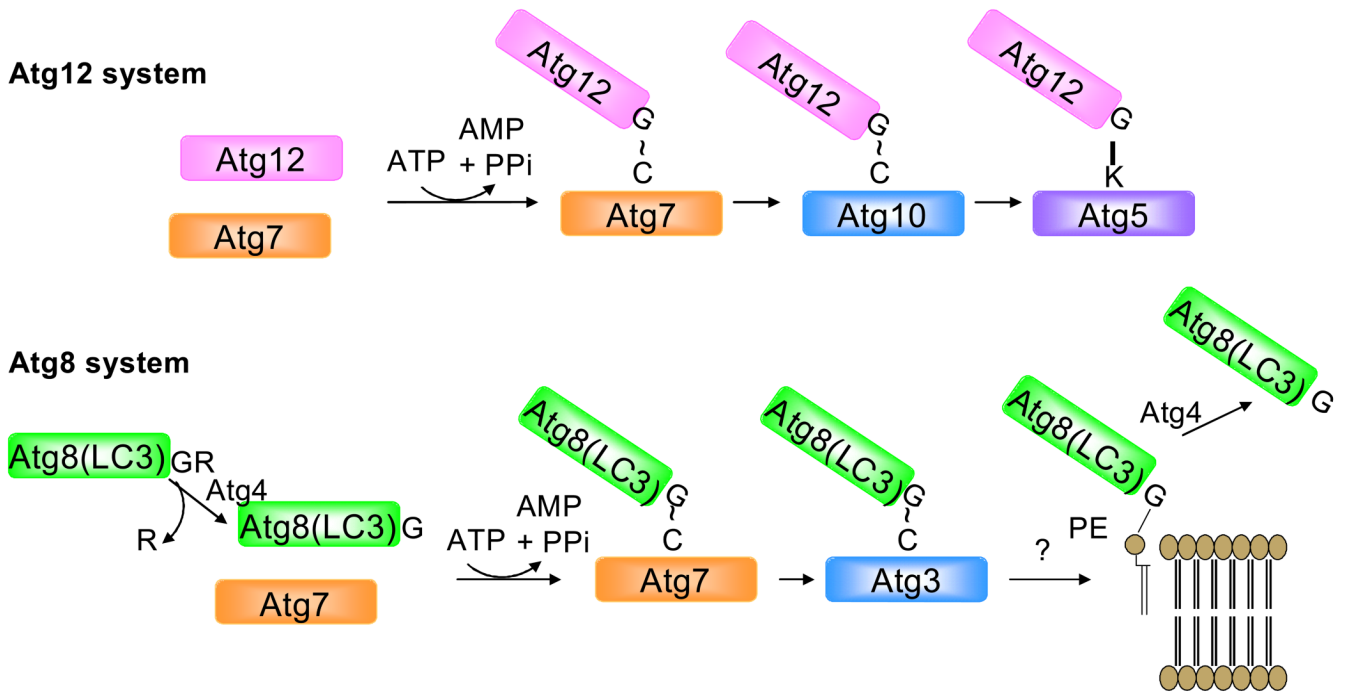


Figure 1.

Ubiquitin-like conjugation systems are required for autophagosome formation. In the first case, Atg7 and Atg10 are responsible for conjugating Atg12 onto the acceptor lysine of Atg5. This is a pre-requisite for the recruitment of Atg8, which is conjugated onto phosphatidylethanolamine in the phagophore membrane in a reaction dependent upon Atg7 and Atg3.

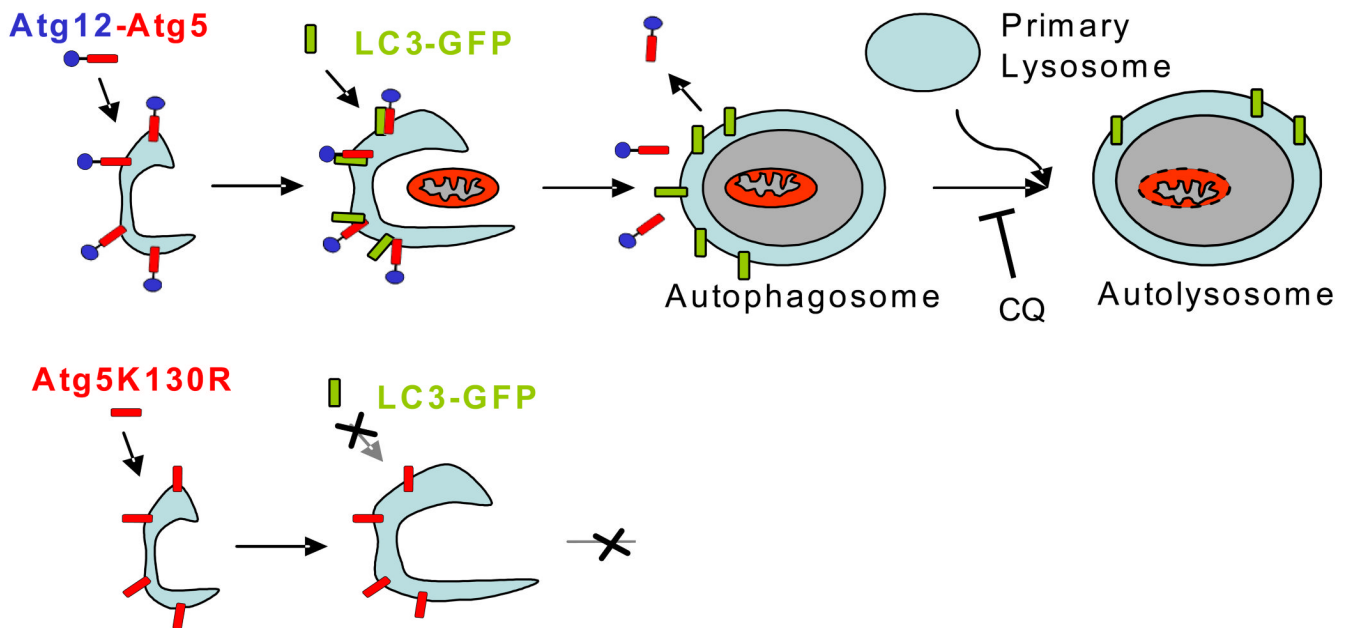
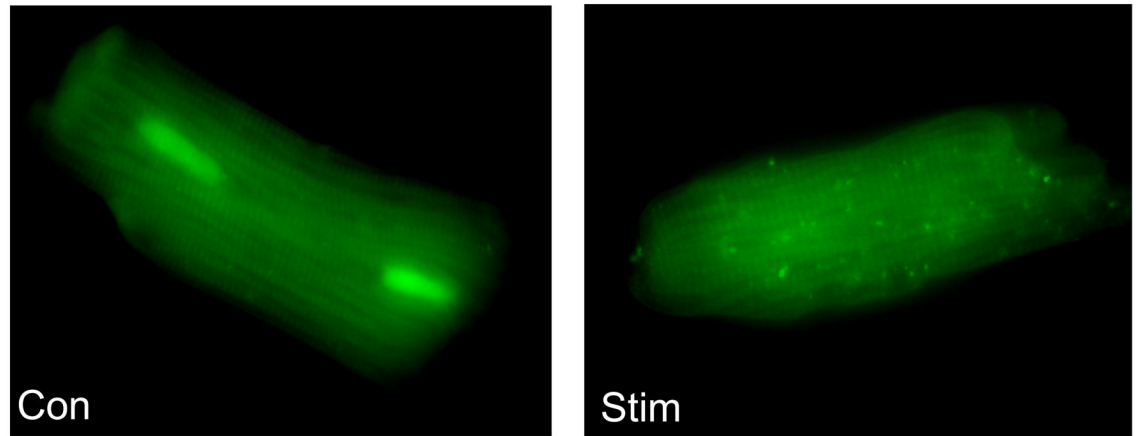


Figure 2.

GFP-LC3 is present diffusely in the cytoplasm until it is incorporated into the developing autophagosomal membrane. The accumulation of GFP-LC3 on these structures can be visualized by fluorescence microscopy as bright fluorescent puncta. Fusion with the lysosome can be prevented with chloroquine (CQ) or Bafilomycin A1. Selective disruption of autophagy can be accomplished by using a point mutant of Atg5 in which the acceptor lysine is mutated to arginine (Atg5K130R). This prevents the incorporation of Atg12 and stalls the process before incorporation of LC3, phagophore enlargement, or target engulfment.