ANTIOXIDANTS & REDOX SIGNALING
Volume 9, Number 9, 2007

DOI: 10.1089/ars.2007.1689

Forum Review

The Role of Autophagy in Mediating Cell Survival and Death During Ischemia and Reperfusion in the Heart

HIROMITSU TAKAGI, YUTAKA MATSUI, and JUNICHI SADOSHIMA

ABSTRACT

Autophagy is a major mechanism for degrading long-lived cytosolic proteins and the only known pathway for degrading organelles. Autophagy is activated by many forms of stress, including nutrient and energy starvation, oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and infections. Although autophagy recycles amino acids and fatty acids to produce energy and removes damaged organelles, thereby playing an essential role in cell survival, inappropriate activation of autophagy leads to cell death. In the heart, activation of autophagy can be observed in response to nutrient starvation, ischemia/reperfusion, and heart failure. In this review, the signaling mechanism and the functional significance of autophagy during myocardial ischemia and reperfusion are discussed. *Antioxid. Redox Signal.* 9, 1373–1381.

INTRODUCTION

Ischemic heart disease is one of the most prevalent health problems in the western world. In the setting of myocardial ischemia, an important therapeutic goal is to restore the coronary blood flow rapidly and to minimize the size of myocardial infarction. However, the process of reperfusion itself could increase oxidative stress and subsequent cell death of heart cells, collectively termed ischemia/reperfusion (I/R) injury, which necessitates the development of novel cardioprotective strategies.

Autophagy is a major mechanism for degrading long-lived cytosolic proteins and the only known pathway for degrading organelles (48). There are at least three types of autophagy: (a) macroautophagy, (b) chaperon-mediated autophagy, and (c) microautophagy. In macroautophagy, either organelles or cytoplasmic proteins are sequestrated in double membrane-bound vesicles, termed autophagosomes, subsequently degraded upon fusion with lysosomes. Since macroautophagy is the primary means of the cytoplasm-to-lysosome delivery of protein/organelle to be degraded, it is commonly referred to simply as autophagy (48). In chaperon-mediated autophagy, chaperons, including Hsc73, recognize substrates (58, 63) and deliver them to lysosomes without a need of vesicular trafficking. Although protein degradation by macroautophagy is generally nonselective, that by chaperon-mediated autophagy is more selective. Microautophagy is an uptake of cytosolic proteins by direct invagination of the vacuolar/lysosomal membrane. Microautophagy has been postulated to occur based on morphological features of lysosomes (48, 61), but the molecular mechanism mediating mircoautophagy or its functional significance remains to be elucidated.

One of the most well-established functions of autophagy is an adaptation to nutrient starvation, where autophagy not only degrades unnecessary proteins but also extracts amino acids and fatty acids as energy sources for cell survival. Autophagy is rapidly induced in response to nutrient starvation and is essential for survival against starvation in a wide variety of cell types. For example, although autophagy is low during embryogenesis in mice, autophagy is upregulated immediately after birth in various tissues, including the heart (53) to compensate for the lack of energy until the newborn receives sufficient levels of nutrients through breast feedings.

Autophagy also occurs constitutively at low levels even under normal (unstressed) conditions in cells. Hepatocytes in

Cardiovascular Research Institute, Department of Cell Biology and Molecular Medicine, University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark, New Jersey.

liver-specific *atg7* KO mice develop ubiquitin-positive aggregates (50), indicating that baseline autophagy is essential for clearance of degraded proteins. Protein turnover is slowed, and old proteins accumulate in aged cells. Interestingly, the activity of autophagy decreases with aging (19). Moreover, *bec-1*, an autophagy gene, is required for normal Dauer morphogenesis and the life span extension observed in a worm mutant lacking the insulin signaling gene *daf-2* (66). Thus, autophagy might be required for life span extension as well.

Importantly, however, inappropriate activation of autophagy could facilitate cell death, termed autophagic cell death or type II programmed cell death (6, 27). Increasing lines of evidence suggest that autophagy is activated by many kinds of stress, such as ischemia and inflammation in mammals (1, 9, 10, 65, 100). Whether autophagy is protective or detrimental against stress in mammalian cells is not fully understood. In this review, we will discuss the signaling mechanism and the functional significance of autophagy in the heart and cardiac myocytes therein, with an emphasis on the role of autophagy in mediating cell survival and cell death in response to I/R.

MOLECULAR MECHANISMS OF AUTOPHAGY

The process of autophagy can be divided into at least four steps, namely induction, formation of autophagosome, autophagosome fusion with the lysosome, and degradation (Fig. 1). Autophagy is tightly regulated by evolutionarily conserved mechanisms. In yeast, >20 *ATG* genes have been identified to mediate autophagy (47), and homologous genes also exist in mammals.

In mammalian cells, the first step, induction of autophagy, is regulated by class I and class III phosphoinositide 3 kinases (PI3Ks). Activation of the class I PI3K by growth factors, such as insulin, inhibits autophagy through activation of Akt/PKB (79) and mTOR (mammalian target of rapamycin) (11, 74). On the other hand, activation of the class III PI3K/Vps34 complex containing Beclin1 (a mammalian homolog of yeast Atg6) promotes autophagy (44, 106). In yeast, class III PI3K/Vps34 and Atg6 mediate vesicle trafficking, including endosomes, and the transport of lysosomal proteases (45, 75). The downstream target of mTOR regulating autophagy is not well understood. Although Atg1 kinase complex is shown to be regulated by TOR in yeast, the relationship between these two molecules remains to be clarified (42).

After induction, the second step, formation of autophagosomes, is initiated by the emergence of isolation membrane. The isolation membrane is elongated to sequester the cytosolic components and form the autophagosome, which has a double membrane structure. This step is primarily mediated by two ubiquitin-like conjugation systems, LC3 (mammalian homolog of yeast ATg8)-phosphatidylethanolamine (LC3-PE) and Atg12–Atg5 (77). These conjugation systems are widely conserved in eukaryotes and have an essential role in mediating autophagy (56). In the Atg12–Atg5 conjugation system, Atg7, which acts as an E1 ubiquitin-activating enzyme (46), and Atg10, which functions like an E2 ubiquitin-conjugating enzyme (83), are required, where Atg7 binds to Atg12, which is in turn activated by ATP hydrolysis, transferred to Atg10, and then conjugated with Atg5. The Atg12–Atg5 conjugate then binds to another protein, Atg16 (71). The Atg12–Atg5–Atg16 complex is localized in the outer membrane of the isolation membrane and considered to play an essential role in mediating elongation of the isolation membrane to form autophagosomes (70, 72).

The other conjugation system is LC3-PE. LC3 was originally characterized as a microtubule-associated protein (54). LC3, synthesized as a full length precursor protein, is converted to LC3-I through cleavage by Atg4, a cysteine protease. LC3-I is then activated by Atg7 in an ATP-dependent manner, transferred to Atg3, an E2 ubiquitin-conjugating enzyme-like molecule, and finally conjugated to the phospholipid phosphatidylethanolamine (PE) (89). LC3-PE (LC3 II) is then recruited to the autophagosomal membrane in an Atg5-dependent manner (72). LC3 II is localized in the outer and the inner membranes of the isolation membrane works as an important component of autophagosomes.

Following the fusion of the two sides of an autophagosome, the outer membrane of autophagosomes fuse with lysosomes to generate the autophagolysosome, the third important step of autophagy. Rab7, a Rab GTPase, is targeted to the autophagosome membrane and mediates fusion of the autophagosome with the lysosome, to allow maturation (30, 39). Lysosomal receptor proteins, namely Lamp-1 and Lamp-2, are involved in Rab7-mediated fusion of autophagosomes with lysosomes (39). Acidification of autophagosomes is also required for the fusion with the lysosome (98).

The final step in autophagy is fusion of the autophagosome with the lysosome. During this step, the contents of the autophagolysosome, as well as the lysosomal transport proteins, are degraded by hydrolytic enzymes, such as cathepsin D (38, 93). This allows degraded products, such as amino acids, to be recycled.

STIMULATION OF AUTOPHAGY DURING I/R

Induction of autophagosomes in the heart after I/R was first reported >30 years ago. Sybers *et al.* (85) showed that fetal mouse hearts in organ cultures exhibited autophagic vacuoles containing damaged organelles. Interestingly, autophagic vacuoles were increased by transient deprivation of oxygen and glucose followed by resupply of them, a procedure similar to I/R (85). Decker and Widenthal (18) reported that in the rabbit perfused heart autophagic vacuoles are induced by 20–40 min of hypoxia and that lysosomal autophagy is dramatically increased after reperfusion. In these two reports, induction of autophagic vacuoles was later accompanied by functional recovery of the heart. Decker and Widenthal showed, however, that large nonfunctional lysosomes were observed when hypoxia was extended to 1 h (18). We have shown that autophagy can be also observed in the porcine model of chronic myocardial ischemia (99, 100). Taken together, autophagy is induced by both acute and chronic ischemia in the heart, and autophagosome formation is further enhanced after reperfusion. However, either prolonged or severe ischemia rather inhibits autophagy,

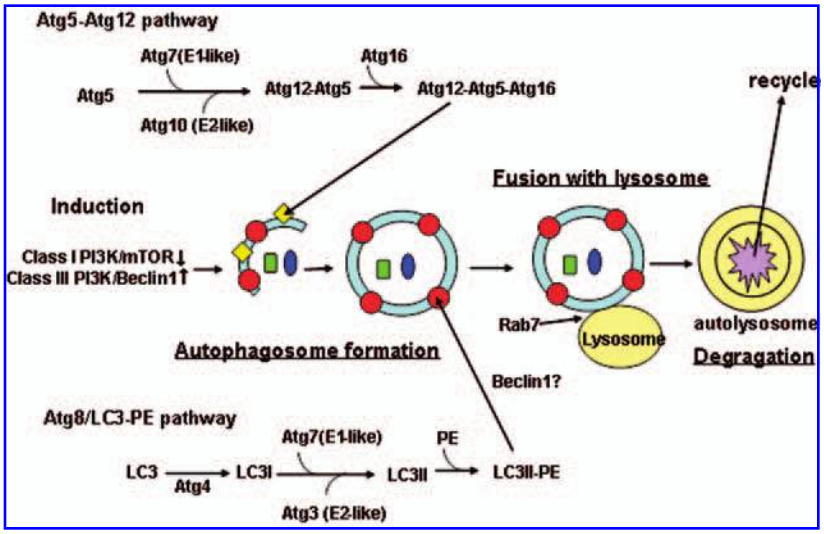

FIG. 1. Scheme of autophagy. Activation of class III P13K or inactivation of class I P13K plays an important role in the induction of autophagy. After induction, old proteins or organelles are sequestered by autophagosomes. Two conjugation cascades lead to autophagosome formation. After completion of autophagosome formation, autophagosomes fuse to lysosomes, thereby forming autolysosomes, where the sequestered proteins are degraded by lysosomal proteases and recycled in the form of amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars)

possibly because ATP-dependent steps are involved in autophagosomal formation. Induction of autophagy by ischemia and reperfusion in cardiac myocytes was confirmed using cultured neonatal and adult cardiac myocytes *in vitro* (90), suggesting that the effect of I/R upon autophagy of cardiac myocytes is cell autonomous. Importantly, however, a recent report showed that autophagic activities, determined by the flux measurement, are attenuated by I/R at the level of both induction and degradation of autophagosomes in an HL-1 cardiac myocyte cell line (32), despite the fact that accumulation of autophagosomes *at steady state* is enhanced by I/R. Whether the flux measurement of autophagy allows the same result in primary cultures of cardiac myocytes or in adult hearts *in vivo* remains to be tested. Although evaluating the flux of autophagy is challenging *in vivo*, the report clearly suggests the importance to evaluate the activity of autophagy at multiple steps.

TRIGGERS OF AUTOPHAGY DURING ISCHEMIA/REPERFUSION: THE ROLE OF OXIDATIVE STRESS, ER STRESS, AND PROTEINASES

Myocardial ischemia causes depletion of intracellular ATP, which would produce a condition similar to nutrient starvation, such as glucose starvation. Interestingly, accumulation of autophagosomes increases during the reperfusion phase, even after the lack of ATP is partially alleviated (18). Theoretically, accumulation of autophagosomes during the reperfusion phase could be caused by decreases in lysosomal degradation of autophagosomes alone (32). However, autophagosomes continue to be made during the reperfusion phase *in vivo* because LC3-GFP dots are significantly less in *beclin1*+/− mice (64). What is the trigger of autophagosomes formation during the reperfusion phase? Reactive oxygen species are produced in mitochondria when the electron transport resumes after suppression by ischemia. Increases in oxidative stress cause mitochondrial permeability transition (MPT) and mitochondrial damage, which could be in part mediated by upregulation of BH3-only proteins, such as Bnip3 (92). Interestingly, both Bnip3 and activation of MPT play an important role in mediating autophagy during I/R in HL-1 cells (33) and hepatocytes (55), respectively, *in vitro*. Since Bnip3 induces extensive damage of the mitochondrial network and the fragmented mitochondria can be found in the autophagosomes in Bnip3 overexpressing HL-1 cells (33), autophagy may be activated to remove damaged mitochondria. Currently, however, molecular mechanisms connecting Bnip3 or MTP and autophagy have not been identified.

The endoplasmic reticulum (ER) is a key organelle mediating posttranslational modifications of nascent proteins and protein folding (86, 96), which are essential for the delivery of proteins to appropriate subcellular localizations and also for the normal function of proteins. I/R impairs the ability of the ER to synthesize, fold, and sort proteins, thereby causing accumulation of misfolded proteins in ER, the response collectively called ER stress (59). ER stress initiates a series of compensatory responses, termed unfolded protein responses, including attenuation of new protein synthesis, upregulation of ER chaperons, and stimulation of apoptosis (59). Unfolded protein responses are activated by I/R and heart failure in the heart (60, 73, 96). Although misfolded proteins accumulated by ER stress can be removed from ER and degraded by proteasomes, excessive unfolded protein might be removed also through autophagy. In fact, ER stress induced by tunicamycin or dithiothreitol triggers autophagy in yeast (102). Phosphorylation of PERK/eIF2α, an important mediator of the unfolded protein response, induces conversion of LC3I to LC3II, an essential step of autophagy in C2C5 cells (52). Furthermore, *atg5* knockout MEF cells are more vulnerable to ER stress than control MEF cells, suggesting that autophagy is protective against ER stress (76). Under starvation, fragmented ER membrane structures are transported to autophagic vacuoles (34). Furthermore, Beclin1, an essential mediator of autophagy, is primarily localized at ER (78). These results suggest that a direct connection may exist between ER stress and autophagosome formation.

I/R activates multiple mechanisms of proteolysis/protein degradation (13). Interestingly, there seems to be complex interplays among them. For example, I/R stimulates calcium-activated proteases, such as calpain (15, 16, 43). Atg5 is cleaved by calpain and the cleaved product of Atg5 stimulates apoptosis rather than autophagy (17, 103). On the other hand, in *calpain*-deficient cells, autophagy is impaired, suggesting that calpain is required for macroautophagy in mammalian cells (20). In another report, impaired proteasome function caused upregulation of autophagy genes in human cells, suggesting that autophagy may compensate for proteasome dysfunction (4). These results raise a possibility that autophagy might be coordinately regulated by other mechanisms of protein degradation during I/R.

Autophagy can be stimulated when caspase is inhibited in L929 cells (104, 105). Interestingly, autophagy activated by caspase inhibitors targets specific proteins, such as catalase, for degradation, thereby stimulating oxidative stress and cell death (105). Caspase inhibitors reduce myocardial apoptosis after I/R and thus are assumed to be protective (101). However, it is important to clarify whether caspase inhibitor treatment increases autophagy after I/R, and if so, whether increased autophagy is protective for the heart under I/R.

SIGNALING MECHANISMS MEDIATING AUTOPHAGY DURING ISCHEMIA—A SIMILARITY TO NUTRIENT STARVATION

During myocardial ischemia, a rapid drop in intracellular ATP concentrations and a concomitant increase in AMP/ATP lead to activation of AMP activated protein kinase (AMPK) (21). Since AMPK is one of the most sensitive sensors of the energy status in cells (37), it appears quite reasonable to postulate that AMPK participates in ischemia-induced induction of autophagy (67, 81, 91) (Fig. 2). In cultured cardiac myocytes, glucose deprivation-induced autophagy was inhibited in the presence of dominant negative AMPK (64). Furthermore, autophagosome formation in response to myocardial ischemia was also decreased in mice overexpressing dominant negative AMPK (64). Interestingly, during myocardial ischemia, the ATP content in the heart was lower, whereas LV end-diastolic pressure was higher in the dominant negative AMPK mice than in control mice (95). These results suggest that AMPK plays an important role in mediating autophagy during myocardial ischemia. Furthermore, induction of autophagy during myocardial ischemia could be protective, namely preserving both ATP production and cardiac function. Activation of AMPK causes phosphorylation of TSC2 and subsequent inhibition of Rheb, thereby leading to inhibition of mTOR (37), a negative regulator of autophagy (74, 82). Interestingly, it has been shown that, in anoxic cardiac myocytes, activation of AMPK leads to inhibition of protein synthesis through phosphorylation of eukaryotic elongation factor-2 (eEF2) rather than inhibition of mTOR (36). Together with recent evidence that eEF2 kinase, which phosphorylates eEF2, regulates autophagy (31, 94), ischemia-induced autophagy may be mediated through the AMPK–eEF2 kinase pathway rather than AMPK-induced inhibition of mTOR, although this hypothesis needs to be tested experimentally (Fig. 2).

BECLIN1 ROLE IN MEDIATING AUTOPHAGY DURING REPERFUSION

Since AMPK is rapidly inactivated upon reperfusion, it is unlikely that the further increase in autophagosome formation during the reperfusion phase is mediated by AMPK. Interestingly, in the mouse model of I/R, expression of Beclin1 is dramati-

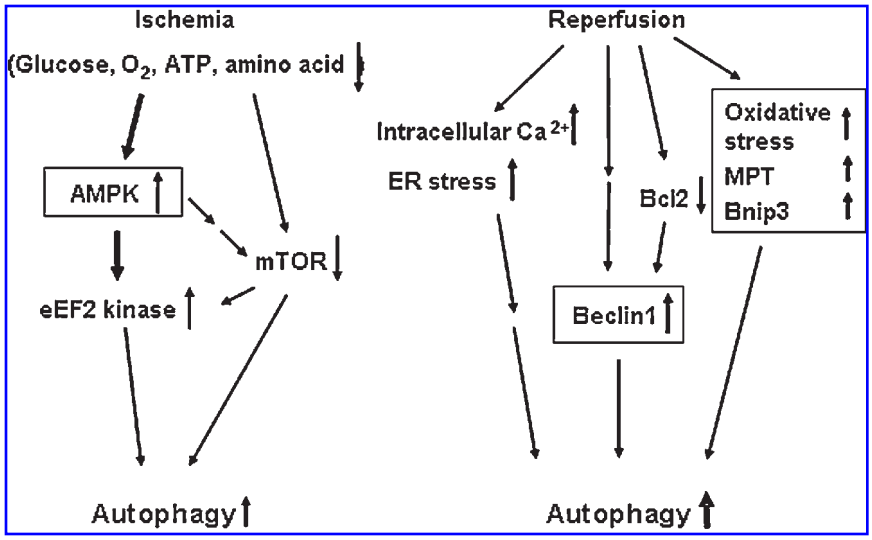

FIG. 2. Hypothetical schemes describing the mechanism of formation of autophagy under ischemia and reperfusion. In ischemia, oxygen and nutrient supplies are decreased, and the intracellular ATP level is reduced, causing activation of AMPK and inactivation of mTOR. These stimuli lead to autophagy for cell survival. On the other hand, upon reperfusion, activation of AMPK and inactivation of mTOR are no longer observed. Instead, expression of Beclin 1 is markedly upregulated. Simultaneously, increases in intracellular calcium, subsequent activation of the calcium-activated protease, oxidative stress, and ER stress occur. These stimuli induce autophagy, which may not be necessarily cytoprotective.

cally upregulated in the reperfusion phase but not in the ischemic phase (64), raising a possibility that Beclin1 may play an important role in mediating autophagy during the reperfusion phase (Fig. 2). In fact, autophagosome formation during the reperfusion phase was significantly inhibited in *beclin 1*+/− mice (64). In cultured cardiac myocytes, urocortin, an endogenous cardioprotective peptide, can reduce the expression of Beclin1, thereby suppressing autophagy and cell death by I/R. The authors proposed that inhibition of Beclin1 expression is mediated by the P13K/AKt pathway (90). Thus, it is possible that class I PI3K negatively affects autophagy through suppression of Beclin1 expression during the reperfusion phase. It has been shown recently that TNFα-induced upregulation of Beclin1 is mediated by JNK in vascular smooth muscle cells (40). Since JNK is activated by reperfusion (5, 12, 23, 69), JNK might be involved in upregulation of Beclin1 during the reperfusion phase in the heart as well.

IS AUTOPHAGY PROTECTIVE OR DETRIMENTAL FOR THE HEART DURING ISCHEMIA/REPERFUSION?

Autophagy plays an important role in mediating cell survival during stress conditions such as nutrient starvation. However, either "nonphysiological" or "inappropriate" activation of autophagy can lead to cell death. The causative role of autophagy in mediating either survival or death of cardiac myocytes during stressed conditions remains to be elucidated. Thus far, the functional significance of autophagy in the heart has been best defined in autophagy induced by nutrient starvation. For example, autophagy is required for normal heart function and survival during perinatal starvation (53). Although less autophagy is induced in *atg5* −/− mice in response to starvation, they exhibit more severe cardiac dysfunction and die earlier than control mice (53). We have also shown that induction of autophagy in response to glucose deprivation is essential for survival of cardiac myocytes (64). Thus, activation of autophagy is generally essential for survival of cardiac myocytes in response to nutrient starvation. It should be noted, however, that in the H9c2-transformed cardiac myocyte cell line, inhibition of glucose starvation-induced autophagy by 3-methyladenine (3-MA), an inhibitor for class III PI3K (3), reduced cell death, suggesting that autophagy can be detrimental in some experimental conditions. The basis for the differences between these two studies is not yet clear.

Similarly, autophagy either stimulates or inhibits cell survival in cultured cardiac myocytes subjected to ischemia (hypoxia)/reperfusion (reoxygenation). For example, in cardiac cell line HL-1cells, I/R-induced increases in Bax-positive cells were increased by inhibition of autophagy through knockdown of either Beclin1 or Atg5, whereas Bax positive cells were decreased by Beclin1 overexpression (32). Similarly, the viability of cardiac myocytes after anoxia–reoxygenation was decreased when autophagy is suppressed by 3-MA (22). These results suggest that autophagy during I/R could be protective. Consistently, the following independent observations support the notion that autophagy could be protective against modest myocardial ischemia *in vivo*. First, we have shown that in pig hearts with chronic ischemia, less apoptosis was observed in the LV myocardium with autophagy, which exhibited nearly a full recovery of cardiac function when coronary blood flow was restored (100). Second, in the rabbit isolated perfused heart preparation, reperfusion after up to 40 min of ischemia stimulated a cellular repair process with a dramatic increase in autophagy and restored cardiac function (18). Third, fetal mouse hearts in organ cultures tolerate without residual injury up to 4 h incubation with glucose-free medium by upregulating autophagy (85). The causative role of autophagy in mediating the protective effects should be demonstrated in each case. Contrary to the cardioprotective effects of autophagy during I/R, some studies report that autophagy during I/R, especially at the reperfusion phase, may not be necessarily protective. For example, Valentim *et al.* demonstrated that both autophagy and myocyte death induced by I/R in neonatal and adult cardiac myocytes were blocked in the presence of either 3-MA or knockdown of Beclin1 *in vitro* (90). We have shown recently that in GFP-LC3/*beclin1* +/− mice, the number of autophagosomes after I/R was significantly reduced, which was accompanied by decreases in both the infarct size and the number of TUNEL positive cells in the area at risk (64). Consistent with these results, inhibition of cathepsin, a lysosomal enzyme responsible for degradation of autophagosomes, inhibits I/R injury (7, 8) and reperfusion-induced apoptosis (87). These data suggest that activation of autophagy during I/R may be rather detrimental in some cases. Thus, it is important to elucidate what will make autophagy protective or detrimental in the heart under I/R.

Several possibilities may explain why autophagy could be both protective and detrimental in the heart. First, hyperactivation of autophagy could cause cell death (Fig. 2). Hyperactivation of autophagy could take place through several mechanisms. For example, our results suggest that expression of Belcin1 is dramatically upregulated in the reperfusion phase in the mouse heart *in vivo* (64). Furthermore, the ability of Beclin1 to promote autophagy is negatively regulated by interaction with Bcl-2. Whether autophagy induces cell death or cell survival may be determined by the balance between Bcl-2 and Beclin1 (78). Thus, either expression or posttranslational modification of Bcl-2 would affect the activity of Beclin1 and subsequent occurrence of autophagy. Theoretically, downregulation of Bcl-2 not only stimulates apoptosis but also activates autophagy through activation of Beclin1, thereby stimulating cell death. Thus, the combination of upregulation of Beclin1 (64) and downregulation of Bcl-2 (29) during the reperfusion phase would dramatically stimulate the activity of Beclin1. Inappropriate activation of the lysosomal protein degradation system (autophagy), when the cell is dying by apoptosis, may be detrimental and even facilitate necrosis.

Second, the functional significance of autophagy may be determined by the nature of the triggering stress (Fig. 2). For example, since nutrient stress is a physiological stress for autophagy (57), cells may simply enjoy the protective environment without having much to lose. On the other hand, if autophagy is activated without nutrient/energy starvation, since autophagy may simply work as a destruction box, cells jeopardize themselves to commit suicide. Autophagy can be activated without starvation, using mTOR-inactivation-independent signaling mechanisms (97). If cardiac myocytes inappropriately activate autophagy even in the absence of en-

ergy starvation, autophagy in the reperfusion phase could be detrimental.

Finally, it has been shown that Atg5, which is essential for autophagosome formation, is cleaved by calpain in response to apoptotic stimuli and translocated to mitochondria, where it associates with the anti-apoptotic molecule Bcl-xL and induces cytochrome c release (17, 103). These results suggest that Atg5 is involved in apoptosis. Interestingly, Atg5 was originally described as an apoptosis-specific protein (28), which is upregulated during apoptosis in neurons (62). These studies suggest that some of the autophagy-related proteins might be involved in both autophagy and apoptosis, raising a possibility that autophagy and apoptosis could be interconnected by common mediators. Thus, it is possible that intervention to either stimulate or inhibit autophagy secondarily affects other mechanism of cell death, thereby affecting survival of cardiac myocytes during I/R. The functional role of autophagy regulating cell survival/death during myocardial I/R should be carefully evaluated using experimental maneuvers selectively regulating autophagy.

AUTOPHAGY IS ACTIVATED IN HEART FAILURE

Accumulation of autophagosomes or vacuole formation is also observed in other pathologic conditions in the heart, including cardiomyopathy (2, 35, 49). It remains to be clarified, however, whether autophagosome formation in the heart in these pathological conditions is salutary or detrimental. It also remains unclear whether the increase in autophagosomes in the pathologic heart is caused by either increased formation of autophagy or impaired breakdown of autophagosomes. In a cardiomyopathic hamster model, autophagic vacuolar degeneration was accompanied by increased expression of Rab7 and cathepsin D, which should stimulate lysosomal fusion and degradation of autophagosomes (68). In dilated cardiomyopathy patients, expression of cathepsin D was decreased, which could induce accumulation of autophagosomes due to inadequate degradation (51). In mice deficient with lysosome-associated membrane protein-2 (*lamp-2*), an important constituent of the lysosomal membrane (14, 25), extensive accumulation of autophagic vacuoles was observed in many tissues, including the heart. Lamp-2 exists in the late phase autophagic vacuole, and thus the degradation rate of autophagosomes is slowed down in hepatocytes of *lamp-2* deficient mice. (24). Cardiac myocytes in *lamp-2*-deficient mice are ultrastructurally abnormal, and contractility is severely reduced(24, 84, 88), suggesting that impaired break-down of autophagosomes alone could lead to cardiac dysfunction.

CONCLUSIONS

Autophagy has both protective and detrimental effects for the heart subjected to I/R. Autophagy in the ischemic phase and that in the reperfusion phase may be mediated by different cellular mechanisms and possess distinct functional significance. However, elucidating the causative role of autophagy in mediating cell survival or cell death has been hampered by the difficulty in manipulating autophagy without affecting other cellular responses. For example, even evolutionarily conserved autophagy genes may possess autophagy-independent functions (26, 103). Thus, it seems essential to compare cardiac phenotypes in multiple mouse models of autophagy to identify which cardiac phenotype is specifically regulated by autophagy. In any case, we speculate that induction of well-controlled autophagy as well as suppression of inappropriate autophagy should protect cardiac myocytes from cell death during both acute and chronic phase of I/R in the heart. Thus, elucidating the specific cellular mechanism, allowing activation of autophagy at an appropriate level and appropriate timing may lead to better treatment for ischemia heart diseases.

ACKNOWLEDGMENTS

This work was supported in part by United States Public Health Service Grants HL59139, HL67724, HL67727, HL69020, and HL73048, and by American Heart Association Grant 0340123N.

ABBREVIATIONS

AMPK, AMP activated kinase; Atg, autophagy related gene; eEF2, eukaryotic elongation factor-2; eIF2α, alpha-subunit of the eukaryotic initiation factor-2; ER, endoplasmic reticulum; Hsc73, heat shock cognate protein of 73 kD; I/R, ischemia-reperfusion; LAMP, lysosome associated membrane protein; LC3, microtubule associated protein light chain 3; 3-MA, 3-methyladenine; MEF, mouse embryonic fibroblasts; MPT, mitochondrial permeability transition; mTOR, mammalian target of rapamycin; PE, phosphatidylethanolamine; PERK, PKR-like ER kinase; PI3K; phosphatidylinositol 3-kinase; Rheb, Ras homologue enriched in brain; TSC2, tuberous sclerosis complex.

REFERENCES

1. Adhami F, Liao G, Morozov YM, Schloemer A, Schmithorst VJ, Lorenz JN, Dunn RS, Vorhees CV, Wills–Karp M, Degen JL, Davis RJ, Mizushima N, Rakic P, Dardzinski BJ, Holland SK, Sharp FR, and Kuan CY. Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. *Am J Pathol* 169: 566–583, 2006.
2. Akazawa H, Komazaki S, Shimomura H, Terasaki F, Zou Y, Takano H, Nagai T, and Komuro I. Diphtheria toxin-induced autophagic cardiomyocyte death plays a pathogenic role in mouse model of heart failure. *J Biol Chem* 279: 41095–41103, 2004.
3. Aki T, Yamaguchi K, Fujimiya T, and Mizukami Y. Phosphoinositide 3-kinase accelerates autophagic cell death during glucose deprivation in the rat cardiomyocyte-derived cell line H9c2. *Oncogene* 22: 8529–8535, 2003.
4. Alemi M, Prigione A, Wong A, Schoenfeld R, Dimauro S, Hirano M, Taroni F, and Cortopassi G. Mitochondrial DNA deletions inhibit proteasomal activity and stimulate an autophagic transcript. *Free Radic Biol Med* 42: 32–43, 2007.
5. Armstrong SC. Protein kinase activation and myocardial ischemia/reperfusion injury. *Cardiovasc Res* 61: 427–436, 2004.

6. Baehrecke EH. Autophagic programmed cell death in Drosophila. *Cell Death Differ* 10: 940–945, 2003.
7. Baskin–Bey ES, Canbay A, Bronk SF, Werneburg N, Guicciardi ME, Nyberg SL, and Gores GJ. Cathepsin B inactivation attenuates hepatocyte apoptosis and liver damage in steatotic livers after cold ischemia-warm reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 288: G396–402, 2005.
8. Ben–Ari Z, Mor E, Azarov D, Sulkes J, Tor R, Cheporko Y, Hochhauser E, and Pappo O. Cathepsin B inactivation attenuates the apoptotic injury induced by ischemia/reperfusion of mouse liver. *Apoptosis* 10: 1261–1269, 2005.
9. Bergamini E. Autophagy: a cell repair mechanism that retards ageing and age-associated diseases and can be intensified pharmacologically. *Mol Aspects Med* 27: 403–410, 2006.
10. Bergamini E, Cavallini G, Donati A, and Gori Z. The role of macroautophagy in the ageing process, anti-ageing intervention and age-associated diseases. *Int J Biochem Cell Biol* 36: 2392–2404, 2004.
11. Blommaart EF, Luiken JJ, Blommaart PJ, van Woerkom GM, and Meijer AJ. Phosphorylation of ribosomal protein S6 is inhibitory for autophagy in isolated rat hepatocytes. *J Biol Chem* 270: 2320–2326, 1995.
12. Bogoyevitch MA, Gillespie–Brown J, Ketterman AJ, Fuller SJ, Ben–Levy R, Ashworth A, Marshall CJ, and Sugden PH. Stimulation of the stress-activated mitogen-activated protein kinase subfamilies in perfused heart. p38/RK mitogen-activated protein kinases and c-Jun N-terminal kinases are activated by ischemia/reperfusion. *Circ Res* 79: 162–173, 1996.
13. Bolli R and Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79: 609–634, 1999.
14. Cha Y, Holland SM, and August JT. The cDNA sequence of mouse LAMP-2. Evidence for two classes of lysosomal membrane glycoproteins. *J Biol Chem* 265: 5008–5013, 1990.
15. Chen M, He H, Zhan S, Krajewski S, Reed JC, and Gottlieb RA. Bid is cleaved by calpain to an active fragment in vitro and during myocardial ischemia/reperfusion. *J Biol Chem* 276: 30724–30728, 2001.
16. Chen M, Won DJ, Krajewski S, and Gottlieb RA. Calpain and mitochondria in ischemia/reperfusion injury. *J Biol Chem* 277: 29181–29186, 2002.
17. Codogno P and Meijer AJ. Atg5: more than an autophagy factor. *Nat Cell Biol* 8: 1045–1047, 2006.
18. Decker RS and Wildenthal K. Lysosomal alterations in hypoxic and reoxygenated hearts. I. Ultrastructural and cytochemical changes. *Am J Pathol* 98: 425–444, 1980.
19. Del Roso A, Vittorini S, Cavallini G, Donati A, Gori Z, Masini M, Pollera M, and Bergamini E. Ageing-related changes in the in vivo function of rat liver macroautophagy and proteolysis. *Exp Gerontol* 38: 519–527, 2003.
20. Demarchi F, Bertoli C, Copetti T, Tanida I, Brancolini C, Eskelinen EL, and Schneider C. Calpain is required for macroautophagy in mammalian cells. *J Cell Biol* 175: 595–605, 2006.
21. Dolinsky VW and Dyck JR. Role of AMP-activated protein kinase in healthy and diseased hearts. *Am J Physiol Heart Circ Physiol* 291: H2557–2569, 2006.
22. Dosenko VE, Nagibin VS, Tumanovska LV, and Moibenko AA. Protective effect of autophagy in anoxia-reoxygenation of isolated cardiomyocyte? *Autophagy* 2: 305–306, 2006.
23. Engelbrecht AM, Niesler C, Page C, and Lochner A. p38 and JNK have distinct regulatory functions on the development of apoptosis during simulated ischaemia and reperfusion in neonatal cardiomyocytes. *Basic Res Cardiol* 99: 338–350, 2004.
24. Eskelinen EL, Illert AL, Tanaka Y, Schwarzmann G, Blanz J, Von Figura K, and Saftig P. Role of LAMP-2 in lysosome biogenesis and autophagy. *Mol Biol Cell* 13: 3355–3368, 2002.
25. Fukuda M. Lysosomal membrane glycoproteins. Structure, biosynthesis, and intracellular trafficking. *J Biol Chem* 266: 21327–21330, 1991.
26. Furuya D, Tsuji N, Yagihashi A, and Watanabe N. Beclin 1 augmented cis-diamminedichloroplatinum induced apoptosis via enhancing caspase-9 activity. *Exp Cell Res* 307: 26–40, 2005.
27. Gozuacik D and Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. *Oncogene* 23: 2891–2906, 2004.
28. Grand RJ, Milner AE, Mustoe T, Johnson GD, Owen D, Grant ML, and Gregory CD. A novel protein expressed in mammalian cells undergoing apoptosis. *Exp Cell Res* 218: 439–451, 1995.
29. Grunenfelder J, Miniati DN, Murata S, Falk V, Hoyt EG, Kown M, Koransky ML, and Robbins RC. Upregulation of Bcl-2 through caspase-3 inhibition ameliorates ischemia/reperfusion injury in rat cardiac allografts. *Circulation* 104: I202–1206, 2001.
30. Gutierrez MG, Munafo DB, Beron W, and Colombo MI. Rab7 is required for the normal progression of the autophagic pathway in mammalian cells. *J Cell Sci* 117: 2687–2697, 2004.
31. Hait WN, Wu H, Jin S, and Yang JM. Elongation factor-2 kinase: its role in protein synthesis and autophagy. *Autophagy* 2: 294–296, 2006.
32. Hamacher–Brady A, Brady NR, and Gottlieb RA. Enhancing macroautophagy protects against ischemia/reperfusion injury in cardiac myocytes. *J Biol Chem* 281: 29776–29787, 2006.
33. Hamacher–Brady A, Brady NR, Logue SE, Sayen MR, Jinno M, Kirshenbaum LA, Gottlieb RA, and Gustafsson AB. Response to myocardial ischemia/reperfusion injury involves Bnip3 and autophagy. *Cell Death Differ* 14: 146–157, 2007.
34. Hamasaki M, Noda T, Baba M, and Ohsumi Y. Starvation triggers the delivery of the endoplasmic reticulum to the vacuole via autophagy in yeast. *Traffic* 6: 56–65, 2005.
35. Hein S, Arnon E, Kostin S, Schonburg M, Elsasser A, Polyakova V, Bauer EP, Klovekorn WP, and Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 107: 984–991, 2003.
36. Horman S, Beauloye C, Vertommen D, Vanoverschelde JL, Hue L, and Rider MH. Myocardial ischemia and increased heart work modulate the phosphorylation state of eukaryotic elongation factor-2. *J Biol Chem* 278: 41970–41976, 2003.
37. Inoki K, Corradetti MN, and Guan KL. Dysregulation of the TSC-mTOR pathway in human disease. *Nat Genet* 37: 19–24, 2005.
38. Ishikawa T, Furuno K, and Kato K. Ultrastructural studies on autolysosomes in rat hepatocytes after leupeptin treatment. *Exp Cell Res* 144: 15–24, 1983.
39. Jager S, Bucci C, Tanida I, Ueno T, Kominami E, Saftig P, and Eskelinen EL. Role for Rab7 in maturation of late autophagic vacuoles. *J Cell Sci* 117: 4837–4848, 2004.
40. Jia G, Cheng G, Gangahar DM, and Agrawal DK. Insulin-like growth factor-1 and TNF-alpha regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells. *Immunol Cell Biol* 84: 448–454, 2006.
41. Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y, and Yoshimori T. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 19: 5720–5728, 2000.
42. Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, and Ohsumi Y. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. *J Cell Biol* 150: 1507–1513, 2000.
43. Khalil PN, Neuhof C, Huss R, Pollhammer M, Khalil MN, Neuhof H, Fritz H, and Siebeck M. Calpain inhibition reduces infarct size and improves global hemodynamics and left ventricular contractility in a porcine myocardial ischemia/reperfusion model. *Eur J Pharmacol* 528: 124–131, 2005.
44. Kihara A, Kabeya Y, Ohsumi Y, and Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. *EMBO Rep* 2: 330–335, 2001.
45. Kihara A, Noda T, Ishihara N, and Ohsumi Y. Two distinct Vps34 phosphatidylinositol 3-kinase complexes function in autophagy and carboxypeptidase Y sorting in Saccharomyces cerevisiae. *J Cell Biol* 152: 519–530, 2001.
46. Kim J, Dalton VM, Eggerton KP, Scott SV, and Klionsky DJ. Apg7p/Cvt2p is required for the cytoplasm-to-vacuole targeting, macroautophagy, and peroxisome degradation pathways. *Mol Biol Cell* 10: 1337–1351, 1999.
47. Klionsky DJ. The molecular machinery of autophagy: unanswered questions. *J Cell Sci* 118: 7–18, 2005.

48. Klionsky DJ and Ohsumi Y. Vacuolar import of proteins and organelles from the cytoplasm. *Annu Rev Cell Dev Biol* 15: 1–32, 1999.
49. Knaapen MW, Davies MJ, De Bie M, Haven AJ, Martinet W, and Kockx MM. Apoptotic versus autophagic cell death in heart failure. *Cardiovasc Res* 51: 304–312, 2001.
50. Komatsu M, Waguri S, Ueno T, Iwata J, Murata S, Tanida I, Ezaki J, Mizushima N, Ohsumi Y, Uchiyama Y, Kominami E, Tanaka K, and Chiba T. Impairment of starvation-induced and constitutive autophagy in Atg7-deficient mice. *J Cell Biol* 169: 425–434, 2005.
51. Kostin S, Pool L, Elsasser A, Hein S, Drexler HC, Arnon E, Hayakawa Y, Zimmermann R, Bauer E, Klovekorn WP, and Schaper J. Myocytes die by multiple mechanisms in failing human hearts. *Circ Res* 92: 715–724, 2003.
52. Kouroku Y, Fujita E, Tanida I, Ueno T, Isoai A, Kumagai H, Ogawa S, Kaufman RJ, Kominami E, and Momoi T. ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation. *Cell Death Differ* 14: 230–239, 2007.
53. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, and Mizushima N. The role of autophagy during the early neonatal starvation period. *Nature* 432: 1032–1036, 2004.
54. Lang T, Schaeffeler E, Bernreuther D, Bredschneider M, Wolf DH, and Thumm M. Aut2p and Aut7p, two novel microtubule-associated proteins are essential for delivery of autophagic vesicles to the vacuole. *EMBO J* 17: 3597–3607, 1998.
55. Lemasters JJ, Nieminen AL, Qian T, Trost LC, Elmore SP, Nishimura Y, Crowe RA, Cascio WE, Bradham CA, Brenner DA, and Herman B. The mitochondrial permeability transition in cell death: a common mechanism in necrosis, apoptosis and autophagy. *Biochim Biophys Acta* 1366: 177–196, 1998.
56. Levine B and Klionsky DJ. Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev Cell* 6: 463–477, 2004.
57. Levine B and Yuan J. Autophagy in cell death: an innocent convict? *J Clin Invest* 115: 2679–2688, 2005.
58. Majeski AE and Dice JF. Mechanisms of chaperone-mediated autophagy. *Int J Biochem Cell Biol* 36: 2435–2444, 2004.
59. Marciniak SJ and Ron D. Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 86: 1133–1149, 2006.
60. Martindale JJ, Fernandez R, Thuerauf D, Whittaker R, Gude N, Sussman MA, and Glembotski CC. Endoplasmic reticulum stress gene induction and protection from ischemia/reperfusion injury in the hearts of transgenic mice with a tamoxifen-regulated form of ATF6. *Circ Res* 98: 1186–1193, 2006.
61. Marzella L, Ahlberg J, and Glaumann H. Autophagy, heterophagy, microautophagy and crinophagy as the means for intracellular degradation. *Virchows Arch B Cell Pathol Incl Mol Pathol* 36: 219–234, 1981.
62. Masaki R, Saito T, Yamada K, and Ohtani–Kaneko R. Accumulation of phosphorylated neurofilaments and increase in apoptosis-specific protein and phosphorylated c-Jun induced by proteasome inhibitors. *J Neurosci Res* 62: 75–83, 2000.
63. Massey A, Kiffin R, and Cuervo AM. Pathophysiology of chaperone-mediated autophagy. *Int J Biochem Cell Biol* 36: 2420–2434, 2004.
64. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B, and Sadoshima J. Distinct role of autophagy in the heart during ischemia and reperfusion: roles of AMPK and Beclin 1 in mediating autophagy. *Circ Res* 100: 914–922, 2007.
65. Meijer AJ and Codogno P. Signalling and autophagy regulation in health, aging and disease. *Mol Aspects Med* 27: 411–425, 2006.
66. Melendez A, Talloczy Z, Seaman M, Eskelinen EL, Hall DH, and Levine B. Autophagy genes are essential for dauer development and life-span extension in C. elegans. *Science* 301: 1387–1391, 2003.
67. Meley D, Bauvy C, Houben–Weerts JH, Dubbelhuis PF, Helmond MT, Codogno P, and Meijer AJ. AMP-activated protein kinase and the regulation of autophagic proteolysis. *J Biol Chem* 281: 34870–34879, 2006.
68. Miyata S, Takemura G, Kawase Y, Li Y, Okada H, Maruyama R, Ushikoshi H, Esaki M, Kanamori H, Li L, Misao Y, Tezuka A, Toyo–Oka T, Minatoguchi S, Fujiwara T, and Fujiwara H. Autophagic cardiomyocyte death in cardiomyopathic hamsters and its prevention by granulocyte colony-stimulating factor. *Am J Pathol* 168: 386–397, 2006.
69. Mizukami Y, Yoshioka K, Morimoto S, and Yoshida K. A novel mechanism of JNK1 activation. Nuclear translocation and activation of JNK1 during ischemia and reperfusion. *J Biol Chem* 272: 16657–16662, 1997.
70. Mizushima N, Kuma A, Kobayashi Y, Yamamoto A, Matsubae M, Takao T, Natsume T, Ohsumi Y, and Yoshimori T. Mouse Apg16L, a novel WD-repeat protein, targets to the autophagic isolation membrane with the Apg12-Apg5 conjugate. *J Cell Sci* 116: 1679–1688, 2003.
71. Mizushima N, Noda T, and Ohsumi Y. Apg16p is required for the function of the Apg12p-Apg5p conjugate in the yeast autophagy pathway. *EMBO J* 18: 3888–3896, 1999.
72. Mizushima N, Yamamoto A, Hatano M, Kobayashi Y, Kabeya Y, Suzuki K, Tokuhisa T, Ohsumi Y, and Yoshimori T. Dissection of autophagosome formation using Apg5-deficient mouse embryonic stem cells. *J Cell Biol* 152: 657–668, 2001.
73. Montie HL, Haezebrouck AJ, Gutwald JC, and DeGracia DJ. PERK is activated differentially in peripheral organs following cardiac arrest and resuscitation. *Resuscitation* 66: 379–389, 2005.
74. Noda T and Ohsumi Y. Tor, a phosphatidylinositol kinase homologue, controls autophagy in yeast. *J Biol Chem* 273: 3963–3966, 1998.
75. Obara K, Sekito T, and Ohsumi Y. Assortment of phosphatidylinositol 3-kinase complexes–Atg14p directs association of complex I to the pre-autophagosomal structure in *Saccharomyces cerevisiae*. *Mol Biol Cell* 17: 1527–1539, 2006.
76. Ogata M, Hino S, Saito A, Morikawa K, Kondo S, Kanemoto S, Murakami T, Taniguchi M, Tanii I, Yoshinaga K, Shiosaka S, Hammarback JA, Urano F, and Imaizumi K. Autophagy is activated for cell survival after endoplasmic reticulum stress. *Mol Cell Biol* 26: 9220–9231, 2006.
77. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. *Nat Rev Mol Cell Biol* 2: 211–216, 2001.
78. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, and Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* 122: 927–939, 2005.
79. Petiot A, Ogier–Denis E, Blommaart EF, Meijer AJ, and Codogno P. Distinct classes of phosphatidylinositol 3′-kinases are involved in signaling pathways that control macroautophagy in HT-29 cells. *J Biol Chem* 275: 992–998, 2000.
80. Saijo M, Takemura G, Koda M, Okada H, Miyata S, Ohno Y, Kawasaki M, Tsuchiya K, Nishigaki K, Minatoguchi S, Goto K, and Fujiwara H. Cardiomyopathy with prominent autophagic degeneration, accompanied by an elevated plasma brain natriuretic peptide level despite the lack of overt heart failure. *Intern Med* 43: 700–703, 2004.
81. Samari HR and Seglen PO. Inhibition of hepatocytic autophagy by adenosine, aminoimidazole-4-carboxamide riboside, and N6-mercaptopurine riboside. Evidence for involvement of amp-activated protein kinase. *J Biol Chem* 273: 23758–23763, 1998.
82. Sarbassov DD, Ali SM, and Sabatini DM. Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 17: 596–603, 2005.
83. Shintani T, Mizushima N, Ogawa Y, Matsuura A, Noda T, and Ohsumi Y. Apg10p, a novel protein-conjugating enzyme essential for autophagy in yeast. *EMBO J* 18: 5234–5241, 1999.
84. Stypmann J, Janssen PM, Prestle J, Engelen MA, Kogler H, Lullmann–Rauch R, Eckardt L, von Figura K, Landgrebe J, Mleczko A, and Saftig P. LAMP-2 deficient mice show depressed cardiac contractile function without significant changes in calcium handling. *Basic Res Cardiol* 101: 281–291, 2006.
85. Sybers HD, Ingwall J, and DeLuca M. Autophagy in cardiac myocytes. *Recent Adv Stud Cardiac Struct Metab* 12: 453–463, 1976.
86. Szabadkai G and Rizzuto R. Participation of endoplasmic reticulum and mitochondrial calcium handling in apoptosis: more than just neighborhood? *FEBS Lett* 567: 111–115, 2004.

87. Takuma K, Kiriu M, Mori K, Lee E, Enomoto R, Baba A, and Matsuda T. Roles of cathepsins in reperfusion-induced apoptosis in cultured astrocytes. *Neurochem Int* 42: 153–159, 2003.
88. Tanaka Y, Guhde G, Suter A, Eskelinen EL, Hartmann D, Lullmann–Rauch R, Janssen PM, Blanz J, von Figura K, and Saftig P. Accumulation of autophagic vacuoles and cardiomyopathy in LAMP-2-deficient mice. *Nature* 406: 902–906, 2000.
89. Tanida I, Ueno T, and Kominami E. LC3 conjugation system in mammalian autophagy. *Int J Biochem Cell Biol* 36: 2503–2518, 2004.
90. Valentim L, Laurence KM, Townsend PA, Carroll CJ, Soond S, Scarabelli TM, Knight RA, Latchman DS, and Stephanou A. Urocortin inhibits Beclin1-mediated autophagic cell death in cardiac myocytes exposed to ischaemia/reperfusion injury. *J Mol Cell Cardiol* 40: 846–852, 2006.
91. Wang Z, Wilson WA, Fujino MA, and Roach PJ. Antagonistic controls of autophagy and glycogen accumulation by Snf1p, the yeast homolog of AMP-activated protein kinase, and the cyclin-dependent kinase Pho85p. *Mol Cell Biol* 21: 5742–5752, 2001.
92. Webster KA, Graham RM, Thompson JW, Spiga MG, Frazier DP, Wilson A, and Bishopric NH. Redox stress and the contributions of BH3-only proteins to infarction. *Antioxid Redox Signal* 8: 1667–1676, 2006.
93. Winchester BG. Lysosomal membrane proteins. *Eur J Paediatr Neurol* 5 Suppl A: 11–19, 2001.
94. Wu H, Yang JM, Jin S, Zhang H, and Hait WN. Elongation factor-2 kinase regulates autophagy in human glioblastoma cells. *Cancer Res* 66: 3015–3023, 2006.
95. Xing Y, Musi N, Fujii N, Zou L, Luptak I, Hirshman MF, Goodyear LJ, and Tian R. Glucose metabolism and energy homeostasis in mouse hearts overexpressing dominant negative alpha2 subunit of AMP-activated protein kinase. *J Biol Chem* 278: 28372–28377, 2003.
96. Xu C, Bailly–Maitre B, and Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 115: 2656–2664, 2005.
97. Yamamoto A, Cremona ML, and Rothman JE. Autophagy-mediated clearance of huntingtin aggregates triggered by the insulin-signaling pathway. *J Cell Biol* 172: 719–731, 2006.
98. Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, and Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. *Cell Struct Funct* 23: 33–42, 1998.
99. Yan L, Sadoshima J, Vatner DE, and Vatner SF. Autophagy: a novel protective mechanism in chronic ischemia. *Cell Cycle* 5: 1175–1177, 2006.
100. Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, and Vatner SF. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci USA* 102: 13807–13812, 2005.
101. Yaoita H, Ogawa K, Maehara K, and Maruyama Y. Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. *Circulation* 97: 276–281, 1998.
102. Yorimitsu T, Nair U, Yang Z, and Klionsky DJ. Endoplasmic reticulum stress triggers autophagy. *J Biol Chem* 281: 30299–30304, 2006.
103. Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, Brunner T, and Simon HU. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. *Nat Cell Biol* 8: 1124–1132, 2006.
104. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, Baehrecke EH, and Lenardo MJ. Regulation of an ATG7-beclin 1 program of autophagic cell death by caspase-8. *Science* 304: 1500–1502, 2004.
105. Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, and Lenardo M. Autophagic programmed cell death by selective catalase degradation. *Proc Natl Acad Sci USA* 103: 4952–4957, 2006.
106. Zeng X, Overmeyer JH, and Maltese WA. Functional specificity of the mammalian Beclin-Vps34 PI 3-kinase complex in macroautophagy versus endocytosis and lysosomal enzyme trafficking. *J Cell Sci* 119: 259–270, 2006.

Address reprint requests to
Junichi Sadoshima
Cardiovascular Research Institute
University of Medicine and Dentistry of New Jersey
New Jersey Medical School
185 South Orange Avenue
Medical Science Building G-609
Newark, NJ 07103

E-mail: Sadoshju@umdnj.edu

Date of first submission to ARS Central, April 5, 2007; date of acceptance, April 5, 2007.

This article has been cited by:

1. Claudia Penna , Maria-Giulia Perrelli , Pasquale Pagliaro . Mitochondrial Pathways, Permeability Transition Pore, and Redox Signaling in Cardioprotection: Therapeutic Implications. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Feng XIE, Wei LIU, Lin-Xi CHEN. 2012. The Progress of Autophagy Involved in Heart Disease*. *PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS* **39**:3, 224-233. [CrossRef]
3. R. Troncoso, J. M. Vicencio, V. Parra, A. Nemchenko, Y. Kawashima, A. del Campo, B. Toro, P. K. Battiprolu, P. Aranguiz, M. Chiong, S. Yakar, T. G. Gillette, J. A. Hill, E. D. Abel, D. LeRoith, S. Lavandero. 2011. Energy-preserving effects of IGF-1 antagonize starvation-induced cardiac autophagy. *Cardiovascular Research* . [CrossRef]
4. Nirmala Hariharan , Peiyong Zhai , Junichi Sadoshima . 2011. Oxidative Stress Stimulates Autophagic Flux During Ischemia/Reperfusion. *Antioxidants & Redox Signaling* **14**:11, 2179-2190. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. B. Loos, S. Genade, B. Ellis, A. Lochner, A.-M. Engelbrecht. 2011. At the core of survival: Autophagy delays the onset of both apoptotic and necrotic cell death in a model of ischemic cell injury. *Experimental Cell Research* **317**:10, 1437-1453. [CrossRef]
6. Pasquale Pagliaro , Francesca Moro , Francesca Tullio , Maria-Giulia Perrelli , Claudia Penna . 2011. Cardioprotective Pathways During Reperfusion: Focus on Redox Signaling and Other Modalities of Cell Signaling. *Antioxidants & Redox Signaling* **14**:5, 833-850. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
7. Agnese Viganò, Michele Vasso, Anna Caretti, Valentina Bravatà, Laura Terraneo, Chiara Fania, Daniele Capitanio, Michele Samaja, Cecilia Gelfi. 2011. Protein modulation in mouse heart under acute and chronic hypoxia. *PROTEOMICS* n/a-n/a. [CrossRef]
8. James S.C. Gilchrist, Tom Cook, Bernard Abrenica, Babak Rashidkhani, Grant N. Pierce. 2010. Extensive autolytic fragmentation of membranous versus cytosolic calpain following myocardial ischemia–reperfusion. *Canadian Journal of Physiology and Pharmacology* **88**:5, 584-594. [CrossRef]
9. Patrice Codogno, Alfred J. MeijerSignaling in Autophagy Related Pathways 2583-2588. [CrossRef]
10. Narasimman Gurusamy , Dipak K. Das Autophagy and Redox Signaling 207-212. [Abstract] [Summary] [Full Text PDF] [Full Text PDF with Links]
11. N. Gurusamy, Dipak K. Das. 2009. Is autophagy a double-edged sword for the heart?. *Acta Physiologica Hungarica* **96**:3, 267-276. [CrossRef]
12. Narasimman Gurusamy , Dipak K. Das . 2009. Autophagy, Redox Signaling, and Ventricular Remodeling. *Antioxidants & Redox Signaling* **11**:8, 1975-1988. [Abstract] [Full Text PDF] [Full Text PDF with Links]
13. Xingchun Gou, Qiang Ru, Hongxin Zhang, Yanke Chen, Ling Li, Hushan Yang, Jinliang Xing, Zhinan Chen. 2009. HAb18G/CD147 inhibits starvation-induced autophagy in human hepatoma cell SMMC7721 with an involvement of Beclin 1 down-regulation. *Cancer Science* **100**:5, 837-843. [CrossRef]
14. Narasimman Gurusamy, Istvan Lekli, Nikolai V. Gorbunov, Mihaela Gherghiceanu, Lawrence M. Popescu, Dipak K. Das. 2009. Cardioprotection by adaptation to ischaemia augments autophagy in association with BAG-1 protein. *Journal of Cellular and Molecular Medicine* **13**:2, 373-387. [CrossRef]
15. T SHIN, S KUBOKI, N HUBER, T EISMANN, E GALLOWAY, R SCHUSTER, J BLANCHARD, T PRITTS, A LENTSCH. 2008. Activation of Peroxisome Proliferator-Activated Receptor-# During Hepatic Ischemia Is Age-Dependent. *Journal of Surgical Research* **147**:2, 200-205. [CrossRef]
16. Chiao-Po Hsu, Ibrahim Odewale, Ralph R. Alcendor, Junichi Sadoshima. 2008. Sirt1 protects the heart from aging and stress. *Biological Chemistry* **389**:3, 221-231. [CrossRef]
17. Christopher C. Glembotski. 2008. The role of the unfolded protein response in the heart. *Journal of Molecular and Cellular Cardiology* **44**:3, 453-459. [CrossRef]
18. Keith A. Webster . 2007. Hypoxia: Life on the Edge. *Antioxidants & Redox Signaling* **9**:9, 1303-1308. [Abstract] [Full Text PDF] [Full Text PDF with Links]
19. Dipak K. Das Methods in Redox Signaling . [Citation] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]