This Review is part of a thematic series on Autophagy, which includes the following articles:

Crosstalk Between Autophagy and Apoptosis in Heart Disease

Autophagy in Ischemic Heart Disease
Cardiomyocyte Autophagy in Load-Induced Heart Disease
Autophagy and Atherosclerosis
Molecular Mechanisms of Autophagy

Joseph A. Hill, Guest Editor

Crosstalk Between Autophagy and Apoptosis in Heart Disease

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Abstract—Autophagy is a cell survival mechanism that involves degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles. In addition, autophagy mediates cell death under specific circumstances. Apoptosis, a form of programmed cell death, has been well characterized, and the molecular events involved in apoptotic death are well understood. Damaged cardiomyocytes that show characteristics of autophagy have been observed during heart failure. However, it remains unclear whether autophagy is a sign of failed cardiomyocyte repair or is a suicide pathway for the failing cardiomyocytes. Although autophagy and apoptosis are markedly different processes, several pathways regulate both autophagic and apoptotic machinery and autophagy can cooperate with apoptosis. This review summarizes the evidence for crosstalk between autophagy and apoptosis. (Circ Res. 2008;103:343-351.)

Key Words: autophagy ■ apoptosis ■ cell survival ■ cell death ■ heart failure

Autophagy is an evolutionarily conserved process for bulk degradation and recycling of cytoplasmic components, such as long-lived proteins and organelles. In nutrient-deprived cell, autophagy is a cell-survival mechanism.1–3 There are 3 main autophagic pathways: macroautophagy,4,5 microautophagy,6 and chaperon-mediated autophagy.7 The term “autophagy” refers to macroautophagy, unless otherwise specified. Autophagy involves sequestration of cytosolic constituents, including proteins and organelles, in autophagosomes and degradation in lysosomes (Figure 1). Autophagy is controlled by autophagy-related genes, many of which are involved in autophagosome formation. This process features 2 conjugation systems that are well conserved among eukaryotes: Atg12-Atg5 and Atg8 (microtubule-associated protein 1 light chain 3 [LC3])–phosphatidylethanolamine systems.8 In general, autophagy is thought to be a nonsselective degradation system. This feature is in marked contrast to the ubiquitin–proteasome system, which specifically recognizes only ubiquitinated proteins for proteasomal degradation. Recent studies have demonstrated a variety of physiological and pathophysiological roles in autophagy, such as adaptation to nutrient deprivation, intracellular clearance of protein and organelles, development, antiaging, elimination of microorganisms, cell death, tumor suppression, and antigen presentation. Autophagy appears to modulate both cell viability and death.9 However, the role of autophagy in cell death is controversial. The presence of autophagic vacuoles in dying cells may be interpreted in 1 of 2 ways: either cells activate autophagy in an attempt to survive or autophagy is a part of the process of cell death.10

Many stimuli modulate autophagy and numerous upstream signaling pathways (growth factor signaling, phosphatidylinositol [PI]3-kinase/Akt, mitogen-activated protein kinases, AMP-dependent protein kinase [AMPK], small GTPases,
trimeric G proteins, inositol triphosphates, calcium signaling, and others) regulate the process. Many of these pathways work through the mammalian target of rapamycin (mTOR), which is a potent inhibitor of autophagy. mTOR inhibitors such as rapamycin activate autophagy. In addition, autophagy may be induced by mTOR-independent mechanisms. Trehalose is an mTOR-independent autophagy enhancer. The Atg1 ortholog ULK1 (uncoordinated 51-like kinase 1) controls autophagy.

Nucleation of an autophagic vesicle involves a type III PI3-kinase complex that includes the kinase (mammalian Vps34), beclin 1 (Atg6), and the ultraviolet irradiation resistance–associated tumor suppressor gene (UVRAG). Ambr1 stimulates autophagy by interacting with beclin 1, which activates Vps34. Bif-1 binds to UVRAG, and this interaction regulates the PI3-kinase activity and induces autophagic vesicle formation (Figure 2). Two ubiquitin-like conjugation systems (Atg12-Atg5 and LC3–phosphatidylethanolamine) are part of the vesicle-elongation process. Lipid conjugation converts the soluble form of LC3 (LC3-I) to the autophagic vesicle-associated form (LC3-II). LC3-II is used as a marker of autophagy. Autophagosomes fuse with lysosomes to create autolysosomes (Figure 1).

**Regulation of Apoptosis**

Apoptosis is the most thoroughly characterized form of programmed cell death, and the sequence of molecular events involved in apoptotic cell death is well understood. Apoptosis is defined by characteristic changes as follows: altered nuclear morphology including chromatin condensation and fragmentation, minor changes in cytoplasmic organelles, cell shrinkage, plasma membrane blebbing, and apoptotic body formation. There are 2 major apoptotic signaling pathways: the intrinsic pathway and the extrinsic pathway. A wide variety of apoptotic signals, including growth factor deprivation, hypoxia, oxidative stress, and DNA damage, activate the intrinsic pathway, which is regulated by members of the Bcl-2 family. Traditionally, the members of the Bcl-2 family have been grouped into 3 classes: the first class inhibits apoptosis and includes Bcl-2, Bcl-xL, and Mcl-1; the second class promotes apoptosis and includes Bax and Bak; and the third divergent class of BH3-only proteins, such as Bad and Bid, have a conserved BH3 domain that enhances apoptosis via regulation of proapoptotic Bcl-2 proteins. Apoptotic signals can activate the BH3-only proteins, thereby inactivating the proapoptotic Bcl-2 proteins. This relieves inhibition of the proapoptotic Bcl-2 proteins and promotes apoptosis. In contrast, the opposing model postulates direct activation of Bax and Bak by several BH3-only proteins. The proapoptotic members of the Bcl-2 family of proteins enhance the permeability of the mitochondrial outer membrane. An increase in outer membrane permeability results in a protein release from the intermembrane space to the cytoplasm, including apoptogenic molecules such as cytochrome c. In the presence of ATP, cytochrome c binds to apoptotic protease activating factor-1 and triggers oligomerization. This complex, known as an apoptosome, recruits and cleaves procaspase 9 into the active enzyme, which, in turn, activates the enzyme that is directly responsible for cell death: caspase 3. In contrast, the extrinsic pathway is activated when death ligands, such as the Fas ligand or tumor necrosis factor (TNF)-α, bind to cognate receptors on the plasma membrane. These receptors contain an intracellular death domain, which can recruit and activate caspase 8 via the adaptor protein, Fas-associated protein with death domain (FADD), at the cell surface. Recruitment of caspase 8 subsequently activates downstream effector caspases, such as caspase 3, with no involvement of the Bcl-2 family.

**Autophagy as a Cell Survival Mechanism**

Autophagy is a recycling process of cytoplasmic components, such as long-lived proteins and organelles. The prosurvival role of autophagy has been observed in yeast, plants, flies, and mammals. Inhibition of autophagy results in accumulation of cytoplasmic components and promotion of apoptosis. Treatment with pharmacological autophagy inhibitors (3-methyladenine, bafilomycin A1, monensin and hydroxychloroquine) and knockdown of Atg genes (Atg5, Atg10, Atg12, and Beclin 1) increase apoptosis and cell death in nutrient-deprived cells. When autophagy is blocked during the early stages, autophagic vacuoles are not present in the cells. However, serum- and amino acid–starved lysosome-
associated membrane protein (LAMP)2-negative cells accumulate autophagic vacuoles because fusion between autophagic vacuoles and lysosomes is inhibited. Accumulation of autophagic vacuoles leads to cell death with hallmarks of apoptosis. Autophagy delays apoptotic cell death in breast cancer cells following DNA damage.

Inactivation of Atg genes can cause cell death in vivo experiments. Mice deficient in Atg5 express a nearly normal phenotype at birth but die within 1 day of delivery. Because the transplacental nutrient supply is suddenly interrupted at birth, neonates face severe nutrient deprivation until lactation is fully established. During embryogenesis, the level of autophagy in mice remains low. However, after birth, autophagy is immediately upregulated in various tissues and remains elevated for 3 to 12 hours before returning to basal levels within 1 to 2 days. Atg7-deficient mice also die within 1 day after birth for the same reason as Atg5-deficient mice; except for lower birth weights, Atg7-deficient mice appear normal at birth with no apparent developmental defect. Homozygous beclin 1–deficient (beclin 1−/−) mice are embryonic lethal at 7.5 to 8.5 days of embryogenesis. Beclin 1−/− embryonic stem cells have a severely altered autophagic response, although their apoptotic response to serum withdrawal or ultraviolet light is normal. Heterozygous beclin 1–deficient (beclin 1−/+ ) mice experience a high incidence of spontaneous tumors that express wild-type beclin 1 mRNA and protein, suggesting that beclin 1 is a haploinsufficient tumor suppressor gene. In beclin 1−/− mice, autophagy is decreased. In contrast, depletion of Beclin 1 during Caenorhabditis elegans development increases caspase-dependent apoptosis. LAMP2-deficient mice have an increased rate of mortality between 20 and 40 days of age probably because of extensive accumulation of autophagic vacuoles in many tissues including heart. The ultrastructure of cardiac myocytes from LAMP2-deficient mice is abnormal and heart contractility is severely reduced, indicating that LAMP2 deficiency causes Danon’s disease.

To examine the role of autophagy under normal conditions, mice with tissue-specific Atg-deficiency were developed. Basal autophagy, which removes protein aggregates in non-stressed cells, is essential to the viability and function of neurons. Mutant mice with neuron-specific depletion of Atg5 or Atg7 developed neurodegeneration with cytoplasmic inclusion bodies that contain protein aggregates. Also, T cell–specific deficiency of Atg5 increases apoptosis of mature T cells in peripheral organs.

When autophagy is enhanced, cell death mediated by apoptosis is inhibited. Glyceraldehyde-3-phosphate dehydrogenase enhances autophagy and preserves survival after apoptotic cytochrome c release in the absence of caspase activation. Nuclear GAPDH promotes Atg12 expression to induce autophagy. When autophagy is induced by inhibition of mTOR, toxicity of polyglutamine expansions is reduced in fly and mouse models of Huntington’s disease. The accumulated mutant huntingtin protein recruits beclin 1 and impairs the beclin 1–mediated long-lived protein turnover, thereby reducing autophagic degradation in neuronal cells. This sequence of intracellular events may cause the neurodegenerative disorder, Huntington’s disease.

Because many types of cellular stresses trigger apoptotic cell death, the prosurvival role of autophagy is evident in cells that lack the apoptotic pathway. In Bax/Bak double-knockout murine cells, growth factor withdrawal by interleukin 3 deprivation induces autophagy that is essential to cell survival during the weeks following growth-factor deprivation. Prevention of autophagy by short interfering RNA silencing of autophagy-related genes or treatment with chemical inhibitors leads to rapid cell death. During this time, repletion of growth factors results in recovery of cell size, proliferative potential, and the ability to take up and metabolize glucose. Camptothecin-induced apoptosis in breast cancer MCF-7 cells is associated with activation of cathepsin B and aggregation of Bax and Bid on mitochondria. Bid knockdown protects cancer cells against apoptosis and induces autophagy.

Autophagy removes not only harmful protein aggregates but also harmful organelles, such as mitochondria and endoplasmic reticulum (ER). Recent reports suggest that mitochondrial autophagy (mitophagy) is particularly important for inhibition of cell death. Depolarization of mitochondria during the mitochondrial permeability transition induces autophagy, which selectively removes damaged mitochondria. Thus, autophagy is a cytoprotective mechanism.

Autophagy as a Cell Death Mechanism

Under specific circumstances, autophagy not only protects cells against death but also mediates cell death. The morphological features of autophagy, which are distinct from apoptosis, have been observed in dying cells. If autophagy destroys the cytosol and organelles beyond a certain threshold, autophagic cell death will occur. In many of these cases, the morphological features, either of autophagic and apoptotic cell death, or of autophagic and necrotic cell death, are observed in the same cells. Autophagic cell death occurs during development, in diseased mammalian tissues, and in tumor cell lines treated with chemotherapeutic agents. These suggest a crosstalk between autophagy and apoptosis. Pharmacological or genetic inhibition of autophagy prevents cell death in vitro settings under specific circumstances. In human leukemic cells, Bcl-2 downregulation increases autophagy, leading to cell death. Inhibition of calpain-like proteinase increases autophagic cell death. The autophagic response to stress stimuli may trigger either apoptotic or necrotic cell death. For example, autophagy is specifically triggered after HIV-1 envelope glycoproteins bind to C-X-C receptor (CXCR4) in CD4/CXCR4-expressing cells, leading to apoptotic cell death.

Convergence between the apoptotic and autophagic pathways has been reported. It has been observed that inducers of apoptosis (ceramide, TNF-related apoptosis-inducing ligand, FADD, death-associated protein [DAP] kinase, and DAP kinase–related protein kinase-1) regulate autophagy. Inhibition of autophagy by 3-methyladenine abrogates TNF–α-induced apoptosis in human T-lymphoblastic leukemic cells, suggesting that the early stages of autophagy are required for TNF–α-induced apoptosis. A physiological signal mediated by the TNF-related, apoptosis-inducing ligand kills normal epithelial cells via the novel endogenous FADD death do-
main pathway, which activates both apoptosis and autophagy.\textsuperscript{34} Downregulation of Atg5 expression in HeLa cells suppresses cell death and vacuole formation induced by interferon-γ. Conversely, ectopic expression of Atg5 using adenoviral delivery induces autophagic cell death. Atg5-dependent cell death is blocked in FADD-deficient cells, indicating that Atg5 contributes to autophagic cell death by interacting with FADD.\textsuperscript{45} DAP kinase and DAP kinase–related protein kinase-1 proteins are Ca\textsuperscript{2+}/calmodulin-regulated Ser/Thr death kinases. DAP kinase and DAP kinase–related protein kinase-1 mediate membrane blebbing and the formation of autophagic vesicles during programmed cell death.\textsuperscript{46} The TNF-related, apoptosis-inducing ligand mediates induction of autophagic processes associated with lumen formation in mammary epithelial cells.\textsuperscript{47}

Autophagic cell death is more likely to occur in cells that cannot die by apoptosis. A caspase 8 inhibitor, benzoylcarbonyl-valyl-alanyl-aspartic acid (O-methyl)-fluoro-methylketone (zVAD), arrests apoptosis and promotes autophagic cell death. Cells that were no longer viable after treatment with the caspase 8 inhibitor contained autophagic vacuoles. Knockdown of either Atg7 or beclin 1 inhibits zVAD-induced cell death in murine L929 cells. Knockdown of receptor-interacting protein or c-Jun N-terminal kinase decreases autophagy and cell death in cells treated with zVAD, indicating that autophagic cell death depends on the receptor-interacting protein–c-Jun N-terminal kinase pathway.\textsuperscript{39} zVAD treatment directly induces catalase degradation, reactive oxygen species accumulation, and cell death in murine L929 cells, all of which can be blocked by autophagy inhibitors.\textsuperscript{48} Bax/Bak double-knockout murine embryonic fibroblasts (MEFs) fail to undergo apoptosis and instead manifest significant autophagy, followed by delayed cell death. Reactive oxygen species and oxidative stress produced by hypericin-induced photodamage, induce ER stress, and apoptosis. In Bax/Bak double-knockout cells, a nonapoptotic pathway that requires sustained autophagy commits the oxidatively damaged cells to death.\textsuperscript{49} These results suggest that in the oxidative stress paradigm, the commitment to cell death occurs upstream of the Bax-dependent increase in outer mitochondrial membrane permeability and that the irreversible photodamage incurred by the ER after hypericin photodynamic treatment triggers autophagic cell death in apoptosis-deficient cells. Knockdown of either Atg5 or beclin 1 blocks the death of Bax/Bak double-knockout MEFs treated with either staurosporine or etoposide.\textsuperscript{40} However, apoptosis, but not autophagy, is involved in the death of etoposide-treated wild-type MEFs. If apoptosis is blocked in these cells, which preferentially die by apoptosis, the cells may die by any available alternative mechanism, including autophagy. In contrast, autophagy is not induced when apoptosis is inhibited in Apaf 1–deficient or caspase 9–deficient MEFs or in wild-type MEFs treated with caspase inhibitors. These results indicate that accumulation of autophagic vacuoles precedes apoptotic cell death. Moreover, autophagy induces cell death when apoptosis is inhibited. However, there remains the question of whether autophagy is a death mechanism in cells with intact apoptotic machinery.\textsuperscript{50}

\textbf{Figure 3.} Roles of autophagy in cell survival. Under basal conditions, autophagy functions as a cell survival mechanism and maintains homeostasis. If autophagic activity is insufficient, long-lived proteins and defective organelles accumulate and apoptotic cell death occurs. In contrast, if autophagy destroys the cytosol and organelles beyond a certain threshold, autophagic cell death will occur.

\textbf{Molecular Mechanisms of Crosstalk Between Autophagy and Apoptosis}

Under basal conditions, long-lived proteins and defective organelles are recycled. Cytoplasmic bacteria are also targets for autophagy.\textsuperscript{51} Autophagy functions as a cell survival mechanism and maintains homeostasis. If autophagic activity is insufficient, long-lived proteins and defective organelles accumulate and apoptotic cell death occurs. In contrast, if autophagy destroys the cytosol and organelles beyond a certain threshold, autophagic cell death will occur, especially in apoptosis-insufficient cells (Figure 3).

Several signaling pathways that are induced by common cellular stressors regulate both autophagy and apoptosis. Reactive oxygen species not only trigger apoptosis\textsuperscript{52} but also are essential for autophagy and specifically regulate Atg4 activity.\textsuperscript{53} Ceramide is associated with cell growth arrest and cell death induction, whereas sphingosine 1-phosphate stimulates cell proliferation and promotes survival in numerous cell types.\textsuperscript{54} Ceramide not only is a prominent inducer of apoptosis but also triggers autophagic cell death in malignant glioma cells via activation of BNIP3, a proapoptotic member of the Bcl-2 family.\textsuperscript{55} Sphingosine 1-phosphate–induced autophagy protects cells from death with apoptotic features during nutrient deprivation.\textsuperscript{56} These results suggest that ceramide and sphingosine 1-phosphate constitute a rheostat system that controls cell death. An increase of endogenous ceramide promotes a robust accumulation of Beclin 1 and an autophagic response associated with cell death. An increase of sphingosine 1-phosphate level after starvation induces a mild accumulation of Beclin 1 and promotes cell survival by inhibiting the induction of apoptosis.\textsuperscript{54} Increases in the cytosolic free Ca\textsuperscript{2+} concentration not only activate proapoptotic signals\textsuperscript{57} but also potently induce autophagy by activating calmodulin-dependent kinase kinase.\textsuperscript{58} p53 is a tumor suppressor that regulates both autophagy and apoptosis. Also,
p53 can initiate apoptosis by inducing expression of PUMA (p53-upregulated modulator of apoptosis), which leads to release of cytochrome c from mitochondria and apoptotic cell death. The p53 target, DRAM (damage-regulated autophagy modulator), may regulate autophagy by participating in the fusion of autophagosomes and lysosomes. DRAM is required for p53-induced apoptosis, suggesting that DRAM-dependent autophagy operates upstream of apoptosis.

Mitochondria function as a switch between apoptosis and autophagy. Depolarization of mitochondria during the mitochondrial permeability transition in response to low-intensity stress leads to the induction of autophagy, which, in turn, selectively removes damaged mitochondria as a cytoprotective mechanism. With increasing stress, proapoptotic factors are released from mitochondria undergoing the mitochondrial permeability transition. An overburdened autophagic apparatus may release lysosomal enzymes and possibly other factors to promote cell death. Under extreme stress, the mitochondrial permeability transition occurs in all mitochondria and the intracellular supply of ATP is exhausted. This bioenergetic failure results in necrotic cell death (Figure 4). In yeast, an outer membrane protein, Uth1p, is required for efficient mitochondrial autophagy. DRAM is an outer membrane protein that maintains autophagy at levels that are compatible with cell survival rather than cell death. Bcl-2–interacting protein may regulate autophagy by participating in the mitochondrial permeability transition in response to low-intensity stress, the mitochondrial permeability transition occurs in all mitochondria. Under moderate stress, autophagy is induced. With increasing stresses, apoptosis begins to occur because of cytochrome c release from mitochondria. Under extreme stresses, necrosis occurs because of ATP depletion.

Exposure to proapoptotic stimuli activates calpain, which inhibits beclin-1–dependent autophagy in yeast and mammalian cells. Cardiac Bcl-2 transgenic expression also inhibits autophagy in murine heart cells. Bcl-2 inhibits the formation of the beclin 1/Vsp34 PI3-kinase complex and beclin 1–associated class III PI3-kinase activity. Endogenous beclin 1 localizes primarily in the trans-Golgi network, mitochondria, and ER. Bcl-2 is found in the ER and mitochondria. However, Bcl-2 inhibits starvation-induced autophagy in the ER but not in the mitochondria. The interaction between Bcl-2 and beclin 1 might be a rheostat that maintains autophagy at levels that are compatible with cell survival rather than cell death. Recent structural and biochemical studies demonstrate that the interaction between Bcl-2 and beclin 1 involves the BH3 domain of beclin 1. Disruption of the interaction between the beclin 1 BH3 domain and Bcl-2 increases autophagy, ER-localized Bcl-2 also affects Ca2+ handling in the ER and inhibits Ca2+–mediated autophagy. UVRAG is a positive regulator and Bcl-2 is a negative regulator of the beclin 1–class III PI3-kinase complex. Their interaction may fine-tune beclin 1 activity to modulate autophagy within a homeostatic range under normal conditions. Under stressed conditions, Bcl-2 weakly associates with the beclin 1–class III PI3-kinase complex, whereas UVRAG remains in the complex, resulting in amplified autophagy (Figure 2). HSpin1, the human ortholog of Drosophila Spin, binds to Bcl-2 and Bcl-xL. Overexpression of Bcl-xL inhibits HSpin1-induced cell death. A necrosis inhibitor, pyrrolidine dithiocarbonate, blocks the HSpin1-induced cell death, but the pancaspase inhibitors carbobenzoxy-VAD-fluoromethyl ketone and p35 do not. HSpin1-induced cell death is characteristic of autophagic cell death.

The proapoptotic members of the Bcl-2 family can also activate autophagy and cell death. BNIP3, a member of Bcl-2 family, is expressed in mitochondria and induces cell death without caspase activation or cytochrome c release. In addition, cells transfected with BNIP3 exhibit permeability transition pore-opening, mitochondrial dysfunction, and mitophagy, yielding a necrotic-like cell death. BNIP3 increases the steady-state levels of autophagic vacuoles, which correlated with increased cell death. In contrast, autophagy protects against BNIP3-mediated cell death in response to myocardial ischemia/reperfusion injury. In human fibroblasts exposed to the apoptosis-inducing drug staurosporine, Bax localizes to lysosomes, where it mediates lysosomal membrane permeabilization and possibly promotes release of cathepsin D to the cytosol. Cathepsin D triggers Bax activation in T lymphocytes. Bid is activated by lysosomal cathepsin proteases. Cleavage of Bid by lysosomal proteases results in cytochrome c release and subsequent caspase activation.

Atg5 also constitutes a point of crosstalk between autophagic and apoptotic pathways. Inactivation of Atg5 in mice demonstrated that basal autophagy is important for survival during neonatal nutrient deprivation and prevention of heart failure. Inhibition of autophagy by Atg5 knockdown triggers apoptosis. These results indicate that Atg5 promotes autophagy, which is cytoprotective. However, Atg5 also has proapoptotic effects. Atg5 overexpression sensitizes tumor cells to various apoptotic stimuli. Exposure to proapoptotic stimuli activates calpain, which...
cleaves Atg5. The truncated Atg5 translocates from the cytosol to the mitochondria, where it associates with the antiapoptotic molecule Bcl-X₁ and triggers apoptosis. However, another study demonstrated that depletion of calpain impairs induction of autophagy in response to rapamycin treatment and amino acid deprivation, thereby increasing apoptosis. Furthermore, Atg5 plays a crucial role in interferon-γ–induced autophagic cell death by interacting with FADD, which is an adaptor molecule that mediates receptor-mediated apoptosis. These results suggest that Atg5 is a molecular switch factor between autophagy and apoptosis.

**Relationship Between Autophagy and Apoptosis in Heart Disease**

Autophagy recycles long-lived proteins and organelles, especially mitochondria and maintains cellular energy homeostasis and bioenergetics in the heart. Impairment of autophagy plays a causal role in cardiomyopathy. LAMP2-deficient mice, which have extensive accumulation of autophagic vacuoles, show depressed cardiac contractile function without significant changes in calcium handling. In green fluorescent protein–LC3 transgenic mice, cardiac myocytes from starved mice display high numbers of autophagosomes, many of which contained engulfed mitochondria. Autophagy is responsible for mitochondrial turnover, and incomplete autophagic turnover of old mitochondria may be the source of lipofuscin.

Autophagy has been observed in both hypertrophied myocardium and failing myocardium caused by dilated cardiomyopathy, valvular disease, and ischemic heart disease. In patients with terminal heart failure secondary to ischemic cardiomyopathy or dilated cardiomyopathy, cellular degeneration with granular cytoplasmic ubiquitin inclusion is detected in 0.3% of the cardiomyocytes. These cardiomyocytes are TUNEL– and activated caspase–3–negative but are also negative for C9, a marker of necrosis, implicating autophagic cell death as the cause of cellular degeneration. Cardiomyocytes from failing hearts exhibit unique morphological features. In human failing hearts with idiopathic dilated cardiomyopathy, the prevalence of autophagic, apoptotic, and necrotic cells is 0.08%, 0.002%, and 0.06%, respectively. In human hibernating myocardium, degenerated cardiomyocytes with autophagic vacuoles and nuclear disassembly are also observed. In animal models, dead and dying cardiomyocytes showing characteristics of autophagy have been observed. In the UM-X7.1 hamster model of human dilated cardiomyopathy, heart failure progresses, resulting in 50% mortality by 30 weeks of age. Cardiomyocytes obtained from UM-X7.1 hamsters contain typical autophagic vacuoles, including degraded mitochondria, glycogen granules, and myelin-like figure. In diphtheria toxin receptor transgenic mice, degenerated cardiomyocytes expressing beclin 1 are detected in beclin 1 Tg left ventricles under pressure overload. In wild-type mice, pressure overload attributable to transverse aortic constriction (TAC) induces hypertrophy 1 week after TAC and heart failure 4 weeks after TAC. Cardiac-specific Atg5 deficiency causes cardiac dysfunction and left ventricular dilatation 1 week after TAC. Polyubiquitinated proteins accumulate, ER stress is increased, and apoptosis is promoted in the Atg5-deficient hearts. Four weeks after TAC, autophagy is upregulated in failing wild-type hearts. These results indicate that constitutive autophagy in the heart under baseline conditions is a homeostatic mechanism for maintaining cardiomyocyte size and global cardiac structure and function and that upregulation of autophagy in failing hearts is an adaptive response that protects cells from hemodynamic stress.

In contrast, it has been reported that autophagy during pressure overload plays a detrimental role in mice with genetic alterations in the beclin 1 gene. Beclin 1−/− mice are embryonic lethal at 7.5 to 8.5 days after embryogenesis, but beclin 1−/− mice are viable, manifesting diminished autophagy in multiple tissues. In beclin 1−/− hearts, autophagy is decreased 48 hours after severe TAC, which constricts the aorta to a slightly greater degree than TAC, and pathological remodeling is diminished 3 weeks after severe TAC. No evidence for apoptosis is detected in beclin 1−/− left ventricles 48 hours after severe TAC. However, it is unclear whether apoptosis plays a role in the later stages of pathological remodeling in beclin 1−/− hearts following severe TAC, because there are no data regarding apoptosis 3 weeks after severe TAC. In mice that overexpress beclin 1 in cardiomyocytes (beclin 1 Tg), autophagic activity is augmented. Although cardiac performance is normal under baseline conditions, pressure overload created by TAC leads to cardiac dysfunction in beclin 1 Tg mice. Increased levels of apoptotic cell death are not detected in beclin 1 Tg left ventricles under
TAC conditions. These findings implicate autophagy in the pathogenesis of load-induced heart failure.\textsuperscript{92}

The role of autophagy in the heart during ischemia/reperfusion has also been examined.\textsuperscript{93} Autophagy is induced by ischemia and is further enhanced by reperfusion. Autophagy resulting from ischemia is accompanied by AMPK activation and is inhibited by dominant-negative AMPK. In contrast, autophagy during reperfusion is accompanied by upregulation of beclin 1 but not by activation of AMPK. Induction of both autophagy and cardiac injury during ischemia/reperfusion are attenuated in beclin 1\textsuperscript{−/−} mice, accompanied by a decrease in apoptosis.\textsuperscript{93} These results suggest that autophagy may be protective during ischemia, whereas it may be detrimental during reperfusion.

There are several explanations for the phenotypes described above. First, autophagy induced by mild and sustained pressure overload inhibits apoptosis and plays a protective role in wild-type mice. However, when autophagy is insufficient in cardiac-specific Atg5-deficient mice, apoptosis is promoted, leading to cell death (Figure 3). Accumulation of polyubiquitinated proteins and ER stress can lead to apoptosis.\textsuperscript{94,95} Severe TAC causes excessive mitochondrial damage that may exceed the threshold for autophagic cell death. Thus, autophagic cell death is reduced in beclin 1\textsuperscript{−/−} mice and promoted in beclin 1 Tg mice. Under conditions of extreme stress, autophagic cell death may cooperate with apoptotic cell death, although apoptosis was not detected in beclin 1\textsuperscript{−/−} mice. In addition to autophagy and apoptosis, necrosis also is induced under such extreme conditions (Figure 4). Extreme induction of autophagy induces cardiac dysfunction. Thus, the cell death is prevented in beclin 1\textsuperscript{+/−} mice. Second, cardiac-specific Atg5-deficient mice exhibited altered autophagy in cardiomyocytes, whereas conventional beclin 1\textsuperscript{−/−} mice have reduced autophagic capacity in all kinds of cell types, some of which may influence cardiac function, ie, blood cells and fibroblasts. Third, Atg5 and beclin 1 may have a molecule-specific function. Beclin 1 is a haplosufficient tumor suppressor gene. Beclin 1\textsuperscript{−/−} mice develop neoplastic mammary lesions and display an increased incidence of spontaneous malignancies,\textsuperscript{23,24} Considering that conventional beclin 1−deficient mice die at 7.5 to 8.5 days of embryogenesis,\textsuperscript{23} whereas conventional Atg5- and Atg7-deficient mice survive until birth,\textsuperscript{21,22} beclin 1 may have additional functions beyond autophagy. As mentioned above, both beclin 1 and Atg5 have functions that are related to apoptosis. Fourth, the basal level of autophagy may influence the resultant phenotypes. The basal levels of autophagy in Atg5-deficient mice are abolished, whereas basal autophagy is not altered in Beclin 1\textsuperscript{−/−} mice. Basal autophagy may carry out functions distinct from induced autophagy.

**Conclusions**

Under normal or mild stressed conditions, autophagy degrades and recycles cytoplasmic components, such as long-lived proteins and organelles. The balance between autophagy and apoptosis maintains homeostasis. Inactivation of autophagy may cause abnormal proteins and organelles to accumulate, thereby promoting apoptosis or necrosis. Excessive autophagic activity induced by severe stimuli can destroy a large fraction of the cytosol and organelles, especially mitochondria and ER, leading to the complete loss of all cellular functions and abnormal cell morphology, apoptosis, and necrosis. Thus, the dead and dying cells can simultaneously show characteristics of autophagy, apoptosis, and necrosis. The small chemicals that regulate autophagy can tell us the precise role of autophagy during various stages of cardiac remodeling.

**Sources of Funding**

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to K.O.).

**Disclosures**

None.

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Circ Res. 2008;103:343-351
doi: 10.1161/CIRCRESAHA.108.175448
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
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