Autophagy in Load-Induced Heart Disease

Beverly A. Rothermel, Joseph A. Hill

Abstract—The heart is a highly plastic organ capable of remodeling in response to changes in physiological or pathological demand. For example, when workload increases, compensatory hypertrophic growth of individual cardiomyocytes occurs to increase cardiac output. Sustained stress, however, such as that occurring with hypertension or following myocardial infarction, triggers changes in energy metabolism and sarcomeric protein composition, loss of cardiomyocytes, ventricular dilation, reduced pump function, and ultimately heart failure. It has been known for some time that autophagy is active in cardiomyocytes, occurring at increased levels in disease. Now, with recent advances in our understanding of molecular mechanisms governing autophagy, the potential contributions of cardiomyocyte autophagy to ventricular remodeling and disease pathogenesis are being explored. As part of this work, several recent studies have focused on autophagy in heart disease elicited by changes in hemodynamic load. Pressure overload stress elicits a robust autophagic response in cardiomyocytes that is maladaptive, contributing to disease progression. In this context, load-induced aggregation of intracellular proteins is a proximal event triggering autophagic clearance mechanisms. These findings in the setting of pressure overload contrast with protein aggregation occurring in a model of protein chaperone malfunction, where activation of autophagy is beneficial, antagonizing disease progression. Here, we review recent studies of cardiomyocyte autophagy in load-induced disease and address molecular mechanisms and unanswered questions. (Circ Res. 2008;103:1363-1369.)

Key Words: autophagy ■ cardiac hypertrophy ■ heart failure ■ hypertrophy ■ signal transduction

Heart disease is arguably the most important noninfectious health problem ever to confront the human race. It is currently the leading cause of death in industrialized nations, a sad reality that is expected to extend soon to the entire world. In many instances, heart disease culminates in failure, a syndrome characterized by the inability of the heart to meet the metabolic demands of the body. Currently, 5 million Americans experience chronic heart failure, a syndrome with mortality of approximately 50% at 5 years.1

Heart failure is the end result of many different insults to the myocardium. Among them is chronic exposure of the heart to excessive hemodynamic burden. In this context, a hypertrophic growth response develops to compensate for chronic increases in workload. Indeed, this initial growth response is thought to be beneficial, facilitating the response of the organism to exercise, postnatal development, or pregnancy. However, under conditions of sustained load, such as that occurring in patients with uncontrolled hypertension, the myocardium progresses to a state of decompensated heart failure (load-induced heart failure). Here again, myocyte hypertrophy is a hallmark feature, and yet this form of cardiac remodeling is maladaptive, predisposing to arrhythmia and systolic dysfunction. Consistent with this, it is established from clinical, epidemiological, and experimental studies that...
left ventricular hypertrophy is the most important antecedent risk factor for development of heart failure.2,3 And, accordingly, recent work has focused on the hypertrophic phenotype itself as a therapeutic target.4 The public health significance of these diseases is highlighted by the fact that one-third of US adults have hypertension.

Much work has been devoted to elucidating molecular and cellular mechanisms which underlie both physiological and pathological cardiac remodeling; yet, much remains unknown. Among the mechanisms at play, it has been known for 30 years that lysosomal pathways are activated in a variety of models of heart disease.6–10 Consistent with this, increases in lysosomal activity in tissue samples from diseased and failing human hearts have been reported.3,11–16 More recently, with advances in our understanding of the molecular mechanisms governing autophagy,17–19 a lysosomal-mediated pathway of protein and organelle recycling, researchers have begun to dissect the autophagic response of cardiomyocytes and to determine whether this response contributes to disease progression, antagonizes it, or does neither. These studies have revealed an important role for autophagy in the cardiomyocyte reaction to numerous types of stress, and compelling evidence has emerged that this autophagic response participates in the pathogenesis of disease.

Many Mechanisms Contribute to Cardiac Remodeling

Hypertrophic growth is a primary mechanism through which the heart normalizes ventricular wall stress. It is characterized by enhanced protein synthesis and an increase in the size and organization of cardiomyocyte sarcomeres.5,20 Remodeling of the heart in response to changes in demand is both robust and rapid, achieving 40% to 60% increases in highly conditioned athletes, with similar degrees of growth seen in the setting of pathological stress.5

In myocardial growth that stems from the physiological loading of exercise or pregnancy, cardiac structure and function are normal and there is no association with clinical heart failure.21,22 By contrast, hypertrophy in patients with hypertension, obesity, valvular heart disease, prior infarction, neurohumoral activation, or mutations in genes coding for contractile proteins is characterized by a shift toward glycolytic metabolism, disorganization of the sarcomere, alterations in calcium handling and contractility, myocyte dropout, fibrosis, systolic and/or diastolic dysfunction, activation of a so-called “fetal gene program,” and “electrical remodeling” (alterations in the expression and/or function of ion transporting proteins). It is important to recognize that physiological and pathological hypertrophy share some common features, and the phenotypes may best be interpreted as existing on a continuum.21

In the setting of prolonged stress, the heart manifests apparently irreversible decompensation, culminating in dilation and contractile dysfunction. This is accompanied by thinning of the ventricular walls through a combination of proteolysis and/or myocyte death. A number of theories have been proposed to explain these events, including blood supply inadequate to meet the demands of the hypertrophied myocardium, alterations in contractile proteins, extracellular matrix remodeling, and alterations in α-adrenergic signaling.5

Remodeling of the adult heart involves changes in the equilibrium between protein synthesis and protein degradation and a delicate life-and-death balance of individual myocytes. Because growth of the heart eventually plateaus even in the setting of persistent stress and is reversible when growth signals abate, pathways that antagonize cellular enlargement must play a role in regulating heart size (as opposed to simple deactivation of hypertrophic pathways). Indeed, in the heart, a number of negative regulators of growth have been identified24–31 that act either through suppression of progrowth pathways or through direct stimulation of protein degradation. As noted above, activation of autophagy and lysosomal pathways has been recognized for many years in human heart failure and in a variety of models of heart disease. At 1 level, it seems paradoxical that a mechanism of protein degradation, and consequent cell shrinkage, would be activated in the setting of hypertrophy. However, pathological hypertrophic remodeling of the heart involves more than simply the addition of proteins; there is a change in the content of numerous sarcomeric components. Thus, although the overall result is an increase in myocyte size, activation of degradative pathways, such as the proteasome and autophagy, may be required to remodel existing sarcomeres.

Previous descriptive studies cast little light on the important questions of whether stress-induced cardiac autophagy occurs in response to the inciting stress, is a secondary response, promotes or antagonizes disease, or exists as an epiphenomenon. This is where several recent studies have stepped in, providing compelling evidence that autophagy participates directly in the pathogenesis of heart disease.32–40 Here, we focus on autophagic mechanisms in load-induced heart disease. A review of autophagy in ischemic heart disease will appear as part of this review series.

Autophagic Activity Increases During Load-Induced Remodeling

Macroautophagy (hereafter termed autophagy) is a highly conserved lysosomal-mediated process of protein and organelle recycling41,42 in which intracellular components are first surrounded by double membrane-bound autophagic vesicles. These vesicles then fuse with lysosomes to deliver their contents for degradation. Aggregates of misfolded proteins too large for processing through the proteasome can be degraded via autophagy. Autophagy is also critical for the degradation and turnover of organelles including mitochondria. Through these processes, autophagy can promote cell survival under conditions of nutrient deprivation by providing substrates to maintain intermediary metabolism and by removing toxic or damaged proteins and organelles. Autophagy carries out both prosurvival and prodeath processes, because unrestrained autophagic activity has been shown to lead to neuronal cell death.14,43,44 Whereas autophagic cell death has not been definitively demonstrated in heart, adult cardiomyocytes, like other terminally differentiated cells, are likely capable of type II autophagic programmed cell death. And removal of a damaged myocyte could be either beneficial or detrimental, depending on the context. Furthermore, cell survival is not the only avenue through which autophagy
could affect cardiac function, because changes in lysosomal content and activity could have a direct physical impact on sarcomere function.

Our group reported that in pressure overload heart failure, a common form of clinical disease, autophagic activity is rapidly induced. Using an established model of surgical constriction of the aorta, we detected significant and prolonged activation of autophagy in cardiomyocytes. Next, we studied mice haploinsufficient for beclin 1, a gene required for early events in autophagy. In these beclin 1 mice, load-induced increases in autophagic activity were blunted, and pathological remodeling of the left ventricle was moderately diminished. Conversely, in mice engineered for forced overexpression of Beclin 1 in cardiomyocytes (αMHC-beclin 1), pressure overload triggered an amplified autophagic response and pathological remodeling of the heart was more severe. Together, these findings led us to conclude that load-induced activation of autophagy is maladaptive.

In a study published around the same time, Nakai et al addressed the same questions via inactivation of a different gene required for autophagy, viz., atg5. In this elegant study, cardiac-specific inactivation of atg5 early in cardiogenesis was not associated with an abnormal cardiac phenotype, similar to our results with beclin 1 mice. However, pressure overload in adult atg5 mutant animals triggered rapid and dramatic declines in cardiac function. Intriguingly, inactivation of atg5 in the heart after the mice had reached maturity elicited rapid hypertrophic growth and profound heart failure even the absence of exogenous pressure stress. Based on these studies, Nakai et al concluded that basal autophagy is required for normal cardiac function and that upregulation of autophagy is an adaptive response to hemodynamic stress.

Is Cardiomyocyte Autophagy Adaptive or Maladaptive?

Whereas both of these studies demonstrated load-induced activation of cardiomyocyte autophagy, the experimental outcomes led to opposing conclusions as to whether autophagy is adaptive or maladaptive in this setting. Indeed, the dual nature of autophagy is a recurring theme in other organ systems and disease states. However, careful comparison of these 2 studies provides an intriguing opportunity to probe further into mechanism.

First, we postulate that the physiological impact of autophagy exists as a continuum, where either too much or too little autophagic activity can be detrimental (Figure 1). First, several lines of evidence suggest that basal levels of autophagy are adaptive, whereas stress-related increases in autophagy can be maladaptive. Nakai et al used a model in which atg5 inactivation presumably led to near-complete elimination of constitutive autophagy. In contrast, the beclin 1 mice have only a 50% reduction in autophagic flux. Thus, important functions carried out by basal levels of constitutive autophagy were lost in ATG5-deficient hearts but not in beclin 1 hearts. We suggest that in the setting of pressure overload, wild-type mice mount an autophagic response that is sufficiently robust as to effect a maladaptive response. The ATG5-deficient mice cannot increase autophagy and remain in the maladaptive range. In beclin 1 mice, the increase in autophagic activity is blunted, maintaining activity closer to the adaptive range. In αMHC-beclin 1 transgenic mice, load-induced autophagic activity may shift even farther into the maladaptive range. Also, some evidence suggests that increased Beclin 1 expression can be indicative of maladaptive autophagic activity. We find that Beclin 1 levels increase in the hearts of mice subjected to pressure overload, consistent with this being a pathological response.

Second, these 2 studies may lead to insights into the nature of cardiomyocyte death. Molecular links between autophagy and apoptosis are well established, and ATG5 and Beclin 1 activation of cardiomyocyte death. Molecular links between autophagy and apoptosis are well established, and ATG5 and Beclin 1 link myocardial dysfunction to apoptosis. However, careful analysis of autophagy must be considered. Although cardiac-specific knockouts may be most instructive for probing precise molecular mechanisms, systemic changes in autophagy may more closely mimic clinical outcomes from drug therapy. Thus, both approaches targeting multiple nodal points in the autophagic process are essential and informative. Finally, atg5 inactivation in adulthood elicited a phenotype distinct from that observed in animals where atg5 was inactivated early in cardiogenesis, suggesting activation of undefined compensatory mechanisms; similar mechanisms may exist in beclin 1 hearts. Also, a host of additional questions remain to be answered, including determining the molecular pathways that...
activate autophagy in response to hemodynamic stress and the nature of the cellular components being degraded.

**Is Proteotoxicity a Trigger of Autophagy in Load-Induced Failure?**

Many different cellular events can trigger autophagic activity, including nutrient depletion, accumulation of damaged or misfolded proteins, accumulation of reactive oxygen species (ROS), calcium overload, and opening of the mitochondrial permeability transition (MPT). Also, some evidence suggests that amyloid proteins are capable of triggering MPT by inserting into mitochondrial membranes. Of these processes, each has been observed in the failing heart. We recently examined the possibility that an accumulation of damaged protein aggregates contributes to the activation of autophagy in load-induced failure.

Strict regulation of protein degradation is especially critical in long-lived postmitotic cells such as cardiac myocytes and neurons, where the ability to replace cells is limited, yet intracellular proteins and organelles turnover continuously. In fact, protein conformation disease, characterized by toxic aggregations of misfolded proteins, is a growing family of diseases, which includes Alzheimer disease, Parkinsonism, amyotrophic lateral sclerosis, and both polyglutamine and polyalanine expansion disorders. In many of these disease states, autophagy is activated as a mechanism to clear misfolded proteins. The current thinking is that expression of a dominant-negative, aggregate-prone protein in terminally differentiated postmitotic cells triggers autophagic pathways that facilitate removal of aggregates too large for efficient clearance by the proteasome, thus acting in a salutary fashion. Failure of this system leads to aggregation of misfolded proteins, which are toxic to the cell. Some evidence suggests that increased autophagic activity does not directly clear aggregates themselves but rather clears aggregate precursors, shifting the equilibrium away from aggregate formation.

In the absence of basal levels of autophagic activity in heart and brain, abnormal aggregates of intracellular proteins develop, pointing to the importance of constitutive autophagic clearance pathways in these tissues. Complete loss of this constitutive turnover system may be a factor contributing to the pathology of the cardiac-specific atg5 knockout. In heart disease, there is clinical evidence of proteotoxicity because abnormal protein aggregation and accumulation of ubiquitinated proteins can be detected in human hearts with idiopathic or ischemic cardiomyopathies.

In a recent study, we explored the possible role of protein aggregation as a trigger of autophagy in a mouse model of load-induced heart failure. In cultured cardiomyocytes, we found that accumulation and aggregation of ubiquitinated proteins is capable of activating autophagy to levels comparable to pharmacological induction. In load-stressed failing hearts in vivo, we detected accumulation of ubiquitinated protein aggregates with the same spatial and temporal pattern as increases in autophagic activity. In other words, in the setting of an increase in hemodynamic load, regions of the heart showing maximal autophagic activity harbor the highest density of ubiquitinated protein aggregates. Proteasomal degradation pathways were similarly activated in these same hearts. Thus, proteasomal and autophagic activities increase in parallel, and the accumulation of ubiquitinated aggregates is not attributable to ineffective proteasome-mediated degradation but rather result from an increase in protein damage.

**Autophagy Is Adaptive in a Proteotoxic Model of Desmin-Related Cardiomyopathy**

To determine whether protein aggregation alone is sufficient to activate a pathological autophagic response, we turned to a model of desmin-related cardiomyopathy (DRCM). DRCM is a severe and progressive disease for which there are limited therapeutic options. This class of disease arises from mutations in several different proteins, including the intermediate filament protein desmin, myotilin, dystrophin, and the small heat shock protein αB-crystallin (CryAB). Mutations that disrupt the interaction between desmin and CryAB elicit a phenotype of myofibrillar disarray, protein aggregation, contractile dysfunction, and sudden death. Transgenic mice expressing a CryABR120G mutation, which is responsible for one of the most severe forms of this disease in humans, accumulate intermyofibrillar protein aggregates and manifest progressive deterioration of cardiac function. Here, CryABR120G mutation results in protein aggregation and aggresome formation, mitochondrial toxicity, disruption of proteasome function, and a state of “reductive stress.” Here, CryABR120G-associated DRCM derives from chronic expression of an aggregate-inducing protein in terminally differentiated, nondividing cells, a paradigm similar to neurodegenerative diseases.

Given the clear analogy with neurodegenerative diseases, where autophagy is activated prominently and serves to clear toxic proteins, we evaluated autophagic flux in CryABR120G transgenic mice. Here, we found that autophagy was activated even before the formation of detectable protein aggregates or the emergence of cardiac pathology, suggesting that the accumulation of misfolded proteins in the myocardium is sufficient to activate autophagy. Importantly, we found that in this context, increasing autophagy was beneficial, antagonizing disease progression. These findings suggest that autophagic clearance mechanisms in DRCM are directly comparable to autophagic pathways in proteotoxic neurodegenerative disorders, in both cases serving to slow the evolution of disease.

If accumulation of protein aggregates and induction of autophagy are common features in DRCM and load-induced failure, why do our studies indicate that autophagy is adaptive in the former and maladaptive in the latter? To address this question, it is important to examine how misfolded proteins trigger autophagy and what additional factors may influence the kinetics, duration, and magnitude of autophagic flux in pressure overload failure.

**Multiple Autophagic Pathways May Be Active in Load-Induced Failure**

Misfolded proteins are processed by multiple pathways within the cell. First, they can be degraded by proteasomes throughout the cytoplasm. Second, misfolded proteins can aggregate, and as they do, they are delivered to the MTOC (microtubule organizing center) by dynein-dependent retro-
grade transport along microtubules (Figure 2). When the degradative capacity of the proteasome is exceeded, protein aggregates accumulate in perinuclear inclusions called aggresomes.72 Perinuclear, aggresome-like structures are readily detected in both pressure overload heart failure38 and DRCM,69 indicative of protein aggregation as a feature of both diseases. Current evidence suggests that intracellular inclusions of insoluble protein represent a compensatory mechanism that sequesters harmful, soluble proteins, thereby diminishing their negative effects on the cell. In some instances, of course, proteotoxicity is protein-specific, a result of loss- or gain-of-function of the mutated or misfolded protein. However, mounting evidence suggests that early, still-soluble aggregates are the most harmful species,60,73 and sequestration of these toxic aggregates in aggresomes or degrading toxic proteins via autophagy can protect the host cell. Thus, activation of autophagy at early stages of heart disease may be a protective mechanism to scavenge and eliminate misfolded, polyubiquitinated proteins that escape the overwhelmed proteasome pathway. We postulate that in the case of DRCM, autophagy is adaptive because its primary role is to remove toxic protein aggregates, whereas in load-induced failure, protein aggregation is not the sole inducer of autophagy and the extent of autophagic flux has risen to maladaptive levels.

Numerous processes can trigger autophagy in a variety of settings.17–19,41 As mentioned earlier, many of these are features characteristic of load-induced heart failure. For instance, accumulation of ROS and Ca\(^{2+}\) overload can each elicit MPT, causing mitochondria to depolarize, uncouple, swell, and release proapoptotic proteins such as cytochrome c. Furthermore, uncoupled mitochondria cease to generate ATP; worse, they become an ATP sink owing to futile ATPase activity. Thus, mitochondria that have undergone MPT must be repaired or removed to prevent a catastrophic loss of ATP and consequent necrotic death or triggering of apoptotic death. A selective form of autophagy called mitophagy74,75 carries out this critical protective function (Figure 2). Taken too far, however, elimination of mitochondrial content may be a key element of autophagic cell death.

Another pathway that can induce autophagic activity which may be activated during load-induced heart failure is a generalized response to nutrient deprivation (Figure 2). On an organism level, skeletal muscle and liver are thought to be the primary sites of starvation-induced autophagy. It remains to be seen whether, under extreme conditions, a cardiac-localized flux through this pathway is mobilized. Also, at this time, there is no clear separation between the different pathways of autophagic flux outlined in Figure 2. Rather, there are substantial opportunities for crosstalk.

Although accumulation of protein aggregates in cardiomyocytes occurs in response to either hemodynamic stress or genetic mutation of critical proteins, experimental evidence indicates that autophagy is maladaptive in one context34,38 and beneficial in the other.35,39 Thus, important questions for the future include whether there are inherent differences in the types of protein aggregates that form and/or the associated autophagic response. Is the pathophysiological outcome determined by severity and/or duration of the autophagic response or the nature of the autophagic substrate?

**Perspective**

Enormous effort has been directed at identifying novel therapeutic strategies with long-term efficacy in heart failure. Currently, many groups seek to decipher mechanisms governing disease-related remodeling of the heart and consequent systolic dysfunction, clinical heart failure, and arrhythmic sudden death to improve treatment and prevention. Now, accumulating evidence links autophagy with the pathogenesis of multiple forms of heart disease, suggesting that autophagy may be a novel target of therapy. However, the role of autophagy in heart disease is dose- and context-dependent, which poses new questions and special challenges. As new advances continue to emerge, it may soon be possible to enhance or inhibit autophagic activity selectively with meaningful impact on heart disease.
Sources of Funding
This work was supported by the Donald W. Reynolds Cardiovascular Clinical Research Center (to J.A.H.); NIH grants HL-72016 (to B.A.R.), HL-075173 (to J.A.H.), and HL-080144 (to J.A.H.); and American Heart Association grants 065520Y (to B.A.R.) and 0640084N (to J.A.H.).

Disclosures
None.

References


Autophagy in Load-Induced Heart Disease
Beverly A. Rothermel and Joseph A. Hill

Circ Res. 2008;103:1363-1369
doi: 10.1161/CIRCRESAHA.108.186551
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/103/12/1363

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/