Intracellular Protein Aggregation Is a Proximal Trigger of Cardiomyocyte Autophagy

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Background—Recent reports demonstrate that multiple forms of cardiovascular stress, including pressure overload, chronic ischemia, and infarction-reperfusion injury, provoke an increase in autophagic activity in cardiomyocytes. However, nothing is known regarding molecular events that stimulate autophagic activity in stressed myocardium. Because autophagy is a highly conserved process through which damaged proteins and organelles can be degraded, we hypothesized that stress-induced protein aggregation is a proximal trigger of cardiomyocyte autophagy.

Methods and Results—Here, we report that pressure overload promotes accumulation of ubiquitinated protein aggregates in the left ventricle, development of aggresome-like structures, and a corresponding induction of autophagy. To test for causal links, we induced protein accumulation in cultured cardiomyocytes by inhibiting proteasome activity, finding that aggregation of polyubiquitinated proteins was sufficient to induce cardiomyocyte autophagy. Furthermore, attenuation of autophagic activity dramatically enhanced both aggresome size and abundance, consistent with a role for autophagic activity in protein aggregate clearance.

Conclusions—We conclude that protein aggregation is a proximal trigger of cardiomyocyte autophagy and that autophagic activity functions to attenuate aggregate/aggresome formation in heart. Findings reported here are the first to demonstrate that protein aggregation occurs in response to hemodynamic stress, situating pressure-overload heart disease in the category of proteinopathies. (Circulation. 2008;117:3070-3078.)

Key Words: autophagy □ heart failure □ hypertrophy □ protein aggregation □ remodeling

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Under conditions of stress, the heart undergoes a compensatory hypertrophic growth response that serves to normalize wall stress and to diminish myocardial oxygen demand. In the chronic state, cardiac hypertrophy is an independent risk factor for heart failure and lethal arrhythmia, leading causes of morbidity and mortality in Western society. Numerous pathways have been causally implicated in the transition from myocyte hypertrophy to failure, including programmed cell death, cellular atrophy, and very recently, autophagy. However, mechanisms governing the transition from compensated hypertrophy to heart failure are incompletely characterized.

Autophagy is a highly conserved process of protein degradation involved in turnover of mitochondria and peroxisomes and nonsselective degradation of cytoplasmic components during periods of starvation or stress. The autophagic reaction begins with formation of the autophagosome, a double-membrane structure of unknown origin that engulfs cytoplasmic contents without clearly defined substrate specificity. As the autophagosome matures, it fuses with a lysosome to form an autolysosome, leading to proteolysis of engulfed materials.

Autophagic activity is associated with the pathogenesis of various diseases, including neurodegenerative disorders, skeletal myopathy, cancer, and microbial infection. Recent reports demonstrate that multiple forms of cardiovascular stress, including pressure overload, chronic ischemia, ischemia-reperfusion, and diphtheria toxin–induced injury, provoke an increase in autophagic activity in cardiomyocytes. Our group demonstrated recently that in pressure-overload heart failure, a common form of clinical heart failure, induction of autophagic activity is maladaptive. A prominent feature among neurodegenerative diseases associated with autophagy, including Huntington’s disease, amyotrophic lateral sclerosis, parkinsonism, and Alzheimer disease, is deposition of proteins within intracellular aggregates. The prevailing notion is that autophagic pathways serve a salutary function by facilitating removal of aggregates.
too large for efficient proteasome-mediated clearance. In fact, in several neurodegenerative diseases, including Huntington’s disease and amyotrophic lateral sclerosis, a strong association exists between induction of autophagy and the presence of protein aggregates. In the absence of basal levels of autophagic activity in brain, abnormal aggregates of intracellular proteins develop, and pharmacological or genetic induction of autophagy is sufficient to reduce polyglutamine-induced cytotoxicity in animal models of Huntington’s disease. Taken together, these data suggest that intracellular protein aggregates are capable of stimulating autophagic activity, which serves, in turn, to facilitate clearance of the aggregates.

Methods

Pressure-Overload Hypertrophy

Male C57BL6 mice (6 to 8 weeks old) were subjected to pressure overload by severe thoracic aortic banding (sTAB), a model of pressure-overload heart failure.

Primary Culture of Neonatal Rat Ventricular Myocytes

Cardiomyocytes were isolated from the ventricles of 1- to 2-day-old Sprague-Dawley rat pups and plated as described at a density of 1250 cells per 1 mm in medium containing 10% FCS with 100 μmol/L bromodeoxyuridine. Forty-eight hours after plating, cells were transferred to medium supplemented with 1% FBS and 1 μmol/L bromodeoxyuridine, at which point treatment began.

Immunohistochemistry

We performed antigen retrieval by microwave heat-induced epitope retrieval using 1X Biogenex Citra (Biogenex, San Ramon, Calif; 10 minutes at 95°C). Primary antibody dilutions were as follows: 1:30 α-β-crystallin (CryAB; Vector Laboratories, Burlingame, Calif; VP-A103), 1:50 anti-MAP-LC3 (Santa Cruz Biotechnology, Inc; Santa Cruz, Calif; sc-16756), 1:50 anti-vimentin (Santa Cruz; sc-5565), 1:50 anti-ubiquitin (Santa Cruz; sc-9133), 1:1000 anti-ubiquitin (Abcam; ab7254), or 1:50 γ-tubulin (Santa Cruz; sc-10732).

Immunocytochemistry

Cultured cardiomyocytes were washed 3 times in PBS supplemented with calcium (CaCl2 0.1g/L) and magnesium (MgCl2 6H2O 0.1g/L). Cells were then fixed in 4% paraformaldehyde, permeabilized for 2 minutes in 0.1% Triton-X 100, and then blocked for 15 minutes in PBS with 3% normal goat serum and 1% BSA.

Transient Transfection of Cultured Cardiomyocytes

Twenty-four hours after plating, neonatal rat ventricular myocytes (NRVMs) were transfected with a green fluorescent protein (GFP)-tagged LC3 construct as previously described.

Chymotrypsin-Like Activity

Cells were harvested in buffer H (20 mmol/L Tris-HCl, 20 mmol/L NaCl, 1 mmol/L EDTA, 5 mmol/L bromocaptoethanol, pH 7.6 at 4°C) and sheared by repeated passage through a 27-gauge needle, and the supernatant was collected after sedimentation of insoluble material (centrifugation at 16,000g for 10 minutes). Then, 10 μg supernatant protein was added to 300 μL activity assay buffer (50 mmol/L Tris-HCl, 5 mmol/L bromocaptoethanol, 50 μmol/L Suc-LLVY-AMC, pH 8.0 at 37°C).

Statistical Methods

Averaged data are reported as mean ± SEM. Statistical significance was analyzed (StatView) with Student’s unpaired, 2-tailed t test (comparison of 2 groups) or 1-way ANOVA (comparison of ≥2 independent groups), followed by Bonferroni’s method for post hoc pairwise multiple comparisons.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Pressure Overload Induces Proteotoxic Stress in Ventricular Myocytes

We have shown previously that cardiomyocyte autophagy contributes to pathological remodeling of the left ventricle (LV) induced by severe afterload stress. To define proximal events that trigger autophagy, we subjected mice to sTAB, a procedure that induces pressure-overload heart failure. As reported previously, 7 days of sTAB was sufficient to induce clinical heart failure as demonstrated by development of robust cardiac hypertrophy, pulmonary edema, and diminished systolic function (Figure 1).

Elevated afterload imposes oxidative, biomechanical, and neurohumoral stress on the heart, each of which is capable of eliciting proteotoxic injury. Given this, we examined pressure-overload-stressed LV for accumulation of damaged, misfolded proteins. Damaged proteins are covalently coupled to ubiquitin, which targets the tagged substrate for proteolysis by the proteasome. If accumulation of ubiquitinated proteins outpaces proteasomal degradation, buildup of intracellular ubiquitinated protein occurs, resulting in formation of protein aggregates, large heterogeneous complexes that are poor substrates for proteasome-mediated proteolysis.

To test for increases in ubiquitinated proteins and protein aggregates, we immunostained sections of pressure-stressed heart for ubiquitin. Here, we detected dramatic accumulation of ubiquitin-positive inclusion bodies distributed throughout the LV 72 hours after sTAB (Figure 2A). No significant ubiquitin-like immunoreactivity was detected in regions not subjected to biomechanical stress such as right ventricle or either atria. Of interest, we detected a preponderance of ubiquitin staining in the basal septum, a region previously reported to be a “hot spot” for load-induced autophagic activity. Western blot analysis confirmed the robust induction of ubiquitinated protein, detected as diffuse, high-molecular-weight bands on immunoblot, in pressure-stressed LV (Figure 2B).

In the context of cellular stress, the abundance of chaperone proteins, molecules that play a critical role in protein targeting and stability, increases as an adaptive response. Consistent with this notion, we detected significant increases in the abundance of multiple heat shock proteins (HSPs) in pressure-stressed myocardium 7 days after surgery (Figure 2C). Steady-state abundances of CryAB, HSP25, and GADD45 were modestly increased and that of BiP was dramatically increased. Together, these findings are consistent with a model in which the intracellular milieu within load-stressed cardiomyocytes is conducive to protein damage and misfolding.

Accumulation of ubiquitinated proteins is generally believed to occur as a consequence of 1 of 3 mechanisms: an increase in protein ubiquitination, a decrease in deubiquitina-
tion, or a decrease in proteasome-mediated clearance. To determine whether the accumulation of ubiquitinated proteins in pressure-stressed LV was due to a decrease in proteasome activity, we assayed ventricular lysates for chymotrypsin-like activity (CTL-A). One week after sTAB, basal septum CTL-A was increased 35% compared with sham-operated controls (P<0.05; n=4; each lysate run in triplicate; Figure 2D). To test for a component of ATP-dependent proteasome activity, CTL-A was measured in lysates supplemented with ATP. In these experiments, CTL-A was not significantly different from that measured without the addition of exogenous ATP (data not shown). Together, these data suggest that accumulation of ubiquitinated protein is not a consequence of diminished proteasome activity but rather is due to increased flux that exceeds proteasome capacity.

**Autophagic Activity Increases in Pressure-Stressed Ventricle**

Autophagic activity was monitored by morphological and biochemical means. In sTAB hearts, we detected an increased abundance of multilamellar autophagosomes by 72 hours after surgery (Figure 3A), findings indicative of autophagic activity. Additionally, during the autophagic response, LC3 (microtubule-associated protein 1 light chain 3), an 18-kDa homologue of Atg8 in yeast, is processed and lipid conjugated. The resulting 16-kDa active isoform migrates from the cytoplasm to isolation membranes and autophagosomes. Recentely, intracellular migration of LC3 to vesicular membranes has emerged as a reliable marker of autophagic activity. Consistent with increased autophagic activity, we detected robust increases in autophagosome-localized LC3 in pressure-stressed cardiomyocytes (Figure 3B). These findings were corroborated by immunoblot analysis that demonstrated increases in LC3 processing and elevated levels of Beclin 1 protein, 2 markers of autophagic activity (Figure 3C, data not shown).

**Intracellular Aggresome Formation in Pressure-Stressed Ventricle**

In certain contexts, protein aggregates coalesce as perinuclear structures called aggresomes. Aggresomes are cytoplasmic inclusion bodies located in the perinuclear region near the microtubule-organizing center that result from active, dynein-
dependent transport of small aggregates from other parts of the cell. Given our observations of robust increases in chaperone protein levels in the setting of accumulation of ubiquitin-positive high-molecular-weight proteins, we hypothesized that aggresome formation was occurring. To test for their presence, we stained pressure-stressed LV tissues for CryAB and vimentin, a structural component of the aggresome.9 After 7 days of severe pressure-overload stress, we detected juxtanuclear organization of CryAB-associated proteins with an adjacent vimentin-containing shell (Figure 4A, arrow), a finding consistent with aggresome formation. Further evidence of aggresome formation was found when we coimmunostained for CryAB and ubiquitin. Again, after 7 days of pressure stress, we observed an increase in both ubiquitin and CryAB staining, with colocalization of signal in the perinuclear region (Figure 4B, arrow). Thus, ubiquitin-like immunoreactivity in pressure-stressed cardiac myocytes accumulated as large perinuclear aggregates that colocalize with CryAB and vimentin (additional images in Figure I of the online Data Supplement). From these findings, we conclude that severe pressure overload triggers protein damage, protein ubiquitination, and formation of perinuclear aggresomes.

Protein Aggregation Triggers Autophagy

These findings led us to hypothesize that accumulation of ubiquitinated proteins is a proximal step leading to protein aggregation and subsequent activation of autophagic clearance mechanisms. To test this, we treated NRVMs with a proteasome inhibitor (MG-132, 5 μmol/L), isolated protein from the insoluble fraction, and probed for ubiquitin. In these studies, we found that insoluble, ubiquitin-tagged protein accumulation was detectable as early as 4 hours after proteasome inhibition (Figure 5A). At subsequent time points, accumulation of ubiquitin-tagged protein within the insoluble fraction was progressive and robust. No evidence of cytotoxicity was detected out to 16 hours (Figure 5B), although a modest degree of cell death was detected on exposure to MG-132 for ≥24 hours. These findings suggest that the cardiomyocyte cytosol has a limited capacity to retain ubiquitinated protein in the soluble fraction, with excessive accumulation resulting in protein precipitation as an insoluble matrix.

Next, we set out to confirm that autophagy is activated in cardiac myocytes under conditions of protein aggregation. To do this, we induced protein aggregation in NRVMs by proteasome inhibition (MG-132 or epoxomicin); autophagic capacity was reduced pharmacologically (3-methyladenine...
of ubiquitin-conjugated proteins (Figure 5E) similar to that seen in pressure-stressed LV in vivo. To test whether autophagic activity can reduce the abundance of aggregated proteins, we cultured cells in medium supplemented with 3-MA (5 mmol/L). As expected, we detected no change in ubiquitin levels or subcellular organization of aggregates in control cells treated with a short course of 3-MA alone (Figure 5E, image 3). However, in cells in which proteasome activity was inhibited (MG-132) and autophagy was blocked (3-MA), we observed a dramatic increase in the size of perinuclear aggresomes (Figure 5E, image 4). These data suggest that autophagy antagonizes aggresome formation by providing an alternative clearance pathway.

This model was further tested by suppressing autophagy by siRNA-mediated knockdown of Beclin 1. First, robust knockdown of Beclin 1, a protein required for recruitment of Atg12-Atg5 conjugates to preautophagosomal membranes, was confirmed in control experiments (supplemental Figure II). Knockdown of Beclin 1 led to significant decreases in constitutive autophagic activation in NRVMs, and it was not associated with cytotoxicity (supplemental Figure II). Next, we tested the effects of Beclin 1 knockdown on autophagic activity. First, it is important to note that Beclin 1 is not required for LC3-I to LC3-II conversion (ie, lipidation) but is required for LC3 association with membranes. Given this, tracking LC3 processing is not a reliable means of quantifying autophagy in the setting of Beclin 1 knockdown. Thus, we tracked p62 abundance because this protein is degraded in autophagosomes. As expected, Beclin 1 knockdown at both 24 hours (Figure 6) and 48 hours (not shown) elicited increases in the abundance of p62. Proteasome inhibition with MG-132 decreased p62 levels, consistent with degradation of p62 by activated autophagy (Figure 6). Thus, these data lend further credence to the notion that protein aggregation is sufficient to induce cardiomyocyte autophagy.

Parallel Activation of Proteasomal and Autophagic Clearance Pathways

In neurons, protein aggregates, similar to those reported here, are capable of inducing autophagy. Thus, we tested whether the presence of aggregates in cardiomyocytes is sufficient to trigger autophagy. To do this, we monitored autophagic activity in vitro by tracking the localization of GFP-tagged LC3 in transiently transfected cardiac myocytes. With this approach, autophagic activity can be detected as punctate, autophagosome-localized GFP signal, as opposed to the diffuse, cytosolic distribution seen under resting conditions. In cells treated with a proteasome inhibitor (MG-132, 5 μmol/L for 16 hours), we detected an abundant increase in autophagosome-localized LC3 (Figure 7A). In these experiments, autophagic activity, quantified as the number of cells with autophagic vacuoles divided by the total number of transfected cells, was increased 1.8-fold (P<0.05; Figure 7B). Of note, induction of cardiomyocyte autophagy in response to proteasome inhibition was similar in magnitude to that triggered by rapamycin (10 nmol/L), a powerful activator of autophagy (Figure 7B). Together, these data are consistent with the induction of autophagy in response to
accumulation of protein aggregates and argue strongly against nonspecific effects resulting from MG-132 toxicity.

Given our in vivo findings demonstrating that both proteasomal and autophagic clearance pathways are activated in response to pressure-overload stress, combined with our in vitro data demonstrating that inhibition of the proteasome leads to an increase in autophagic activity, we set out to determine whether the converse pertains, i.e., whether inhibition of autophagy results in an increase in proteasome activity. NRVMs were cultured for 7 days in the presence of 3-MA or vehicle, with proteasome activity determined by measuring CTL-A. In these experiments, we observed a $73\pm17\%$ increase ($n=3$; each lysate run in triplicate; $P<0.05$) in CTL-A after 7 days of 3-MA treatment relative to control (Figure 7C). These results are consistent with our finding of coordinated regulation of proteasomal and autophagic mechanisms in pressure-stressed heart. Furthermore, they lend support to a model in which autophagic and proteasomal clearance mechanisms function in parallel in cardiac myocytes.

**Discussion**

Recent reports reveal that cardiomyocyte autophagy is activated in response to multiple forms of cardiac injury. However, proximal triggers of autophagic activity in the heart are unknown. The major findings of this study are that (1) pressure-overload hemodynamic stress elicits changes in the cardiomyocyte milieu conducive to protein damage and misfolding with consequent accumulation of polyubiquitinated proteins and increases in chaperone protein levels; (2) protein aggregation takes place, leading to formation of perinuclear aggresomes; (3) clearance is activated via both autophagic and proteasomal protein quality control pathways; (4) protein aggregate and aggresome formation are sufficient to trigger cardiomyocyte autophagy, and (5) autophagic activity in the cardiomyocyte participates in clearance of aggregated proteins. Finally, the findings reported here are the first to demonstrate that protein aggregation occurs in response to hemodynamic stress, situating pressure-overload heart disease in the category of proteinopathies.
Stress-Induced Cardiomyocyte Proteinopathy

Proteinopathy, toxic aggregations of misfolded proteins, is a growing family of human disorders that includes Alzheimer disease, parkinsonism, atrophied lateral sclerosis, and both polyglutamine and polyalanine expansion disorders. In heart disease, abnormal protein aggregation and accumulation of ubiquitinated proteins have been detected in human hearts with idiopathic or ischemic cardiomyopathies. In both brain and heart, however, relatively little is known regarding whether these intracellular inclusions are toxic themselves or whether they represent a compensatory mechanism that sequesters harmful, soluble proteins within the cytoplasm.

Insoluble protein aggregates are processed by pathways that are just now being deciphered. First, misfolded proteins are delivered to the microtubule-organizing center by dynein-dependent retrograde transport along microtubules. When the degradative capacity of the proteasome is exceeded, protein aggregates accumulate in perinuclear inclusions called aggresomes, organized structures surrounded by vimentin filaments that recruit chaperones, ubiquitin, proteasomes, and mitochondria. In pressure-stressed myocardium, we have detected an abundance of large perinuclear inclusions marked by ubiquitin that colocalize with chaperone proteins and vimentin, fulfilling the definition of aggresomes. In liver, aggresomes (Mallory bodies) form in response to a variety of stresses, including proteasome inhibition. Aggresomes also have been detected in a model of desmin-related cardiomyopathy, a disorder characterized by abnormal amyloid deposition in the heart that occurs when proteasome capacity is exceeded by the production of aggregation-prone misfolded proteins. We inhibited the proteasome in cultured cardiomyocytes to test directly for a causal link between aggregate formation and cardiomyocyte autophagy. In these experiments, we found that accumulation and aggregation of ubiquitinated protein is a powerful inducer of autophagy, capable of robust activation of autophagy to levels comparable to pharmacological induction. Together, these findings are consistent with a model in which autophagic activity is a general response to protein aggregation in the heart and point to a potential role for autophagy in cardiomyopathies of diverse origin.

Interestingly, we and others do not detect inclusions that are membrane bound. The inclusions we detect are much larger than mammalian autophagosomes. Together, these findings suggest that autophagy serves to clear monomeric and oligomeric precursors of aggregates rather than the large inclusions themselves.

To test the involvement of proteasomal activity, we measured chymotryptic activity acting on a small fluorogenic peptide substrate Suc-LLVY-AMC. Owing to its small size, this peptide diffuses easily into the proteolytic chamber of the 20S proteasome; hence, it provides a readout of 20S proteasome activity. Consistent with this, the addition of exogenous ATP to stimulate 26S proteasome activity had no significant effect on CTL-A (<10%). In these experiments, we find that total proteasome function is increased in heart failure, consistent with similar findings in a model of desmin-related cardiomyopathy, suggesting increased flux through proteasomal degradation pathways. Our data demonstrate that proteasomal and autophagic activities increase in parallel.

α1-Antitrypsin deficiency is another proteinopathy in which autophagy is activated as a clearance mechanism. In this disease, proteolytic destruction of elastic connective tissue matrix occurs in lung because of unrestrained proteolysis. In liver, however, toxicity resulting from the accumulation of misfolded mutant α1-antitrypsin leads to chronic liver inflammation and hepatocellular carcinoma. Recent studies have revealed dramatic increases in autophagic activity in liver, which serves to prevent toxic accumulation of mutant α1-antitrypsin by selectively targeting insoluble aggregates.

Thus, a growing body of evidence implicates autophagy as a protective response in genetic diseases associated with cytoplasmic aggregation-prone proteins. Here, we extend this to disease triggered by environmental stress. Importantly, recent studies demonstrating that pharmacological upregulation of autophagy is protective in a wide variety of disease models associated with intracellular protein aggregation raise the exciting prospect of autophagic activation as a novel therapeutic strategy. Our findings here extend this prospect to the myocardium.
Role in Heart Failure Progression

The presence of increased autophagic activity in animal models of heart failure and in samples from human failing hearts provides no insight into whether cardiomyocyte autophagy is beneficial or pathological. Indeed, it is possible that autophagic activity carries out different functions, depending on disease stage and severity or the nature of the components being degraded. For example, we have shown previously that genetic amplification of the autophagic response to acute-onset pressure stress leads to increased hypertrophic growth of the heart. On the other hand, if the protein aggregates themselves are toxic, increased autophagic removal could be beneficial. Furthermore, recent evidence strengthens the assertion that dysregulated autophagic activity induces a caspase-independent programmed cell death program (type II programmed cell death). Nakai et al. reported that cardiac-specific inactivation of atg5 early in cardiogenesis was not associated with an abnormal cardiac phenotype. However, subjecting these animals to pressure stress during adulthood triggered rapid and dramatic declines in cardiac function. Intriguingly, inactivation of atg5 in the heart after the mice had reached maturity led rapidly to cardiac hypertrophy, LV dilation, contractile dysfunction, and a syndrome consistent with heart failure. Clearly, to exploit autophagy as a therapeutic target, it is essential to identify when it is protective and when it contributes to the pathogenesis of disease.

Evidence presented here indicates that autophagy is activated to eliminate damaged proteins that accumulate within the cardiomyocyte. Several reports point to functional defects in the ubiquitin-proteasome system in heart failure, resulting in accumulation of misfolded proteins. Consistent with this, we have observed increased ubiquitin staining in pressure-stressed ventricle, which is most prevalent in the basal septum. Therefore, activation of autophagy at early stages of the disease may be a protective mechanism to scavenge and eliminate misfolded, polyubiquitinated protein aggregates that have overwhelmed the degradative capacity of the proteasomal system.

Perspective

Cardiac hypertrophy is a major predictor of heart failure, a prevalent disorder with high mortality, and there is urgent need for novel therapies to prevent or reverse pathological cardiac remodeling. Our data point to a progression of protein damage, aggregation, and coalescence into aggregates as a previously unrecognized mechanism of disease. Furthermore, our findings suggest that autophagic activity in cardiomyocytes is a general response to diverse stressors and contributes to disease pathogenesis in multiple contexts. As new advances in deciphering molecular regulation of autophagy emerge, it may soon be possible to enhance or inhibit autophagic activity selectively to affect heart disease meaningfully. In addition, given that some drugs already in clinical use alter the process of autophagy and organ systems in which increased autophagic activity is associated with disease, advances in this field are all the more urgent.

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Disclosures

None.

References

Chronic hemodynamic stress such as poorly controlled hypertension induces hypertrophic growth of the heart. This pathological growth response, with time, leads to ventricular dilation, systolic dysfunction, and clinical heart failure, which is a leading cause of morbidity and mortality in Western society. Whereas numerous signaling pathways have been implicated in the stress response of the myocardium, mechanisms governing the transition from compensated hypertrophy to heart failure are poorly understood. In this study, we report that pressure overload promotes accumulation of ubiquitinated protein aggregates in left ventricular myocytes that are processed into structures called aggresomes. We also demonstrate that these protein aggregates activate an evolutionarily conserved process of protein sequestration and removal ubiquitinated protein aggregates in left ventricular myocytes. We also demonstrate that these protein aggregates activate an evolutionarily conserved process of protein sequestration and removal.

**Clinical Perspective**

Pressure overload–induced protein aggregation is a proximal trigger of cardiomyocyte autophagy and that autophagic activity functions to attenuate aggregate/aggresome formation in heart. Findings reported here are the first to demonstrate that protein aggregation occurs in response to hemodynamic stress, situating load-induced heart disease in the expanding category of proteinopathic diseases.
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