The ubiquitin proteasome system in human cardiomyopathies and heart failure

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Submitted 28 March 2012; accepted in final form 4 March 2013

Day SM. The ubiquitin proteasome system in human cardiomyopathies and heart failure. Am J Physiol Heart Circ Physiol 304: H1283–H1293, 2013. First published March 11, 2013; doi:10.1152/ajpheart.00249.2012.—Maintenance of protein quality control is a critical function of the ubiquitin proteasome system (UPS). Evidence is rapidly mounting to link proteasome dysfunction with a multitude of cardiac diseases, including ischemia, reperfusion, atherosclerosis, hypertrophy, heart failure, and cardiomyopathies. Recent studies have demonstrated a remarkable level of complexity in the regulation of the UPS in the heart and suggest that our understanding of how UPS dysfunction might contribute to the pathophysiology of such a wide range of cardiac afflictions is still very limited. Whereas experimental systems, including animal models, are invaluable for exploring mechanisms and establishing pathogenicity of UPS dysfunction in cardiac disease, studies using human heart tissue provide a vital adjunct for establishing clinical relevance of experimental findings and promoting new hypotheses. Accordingly, this review will focus on UPS dysfunction in human dilated and hypertrophic cardiomyopathies and highlight areas rich for further study in this expanding field.

cardiomyopathies; heart failure; human studies; protein quality control; ubiquitin proteasome system

Introduction

The ubiquitin proteasome system (UPS) is the major pathway for intracellular protein degradation in most organs, including the heart. A functional UPS is critical for normal protein turnover and removes damaged or misfolded proteins in a multistep process (24, 39, 66, 93). Dysfunction of the UPS is rapidly gaining recognition as a potentially important mechanism involved in the pathogenesis of a number of cardiac diseases, including heart failure (38, 71, 89, 93, 96, 101), cardiomyopathies (12, 44, 71, 78), hypertrophy (18, 29, 54, 72), atrophy (2, 72), ischemia-reperfusion (41, 67), and atherosclerosis (30), providing a strong rationale for further study of specific mechanisms of proteasome impairment and identification of specific targets for therapy. In vitro and animal models have proven highly valuable in assessing effects of various stressors on the UPS and, in some cases, suggesting a causal link between defective protein clearance and disease phenotypes (14, 41, 46). Translation of these findings to human disease can be greatly strengthened by corroboration of discoveries from experimental model systems using human heart tissue from well-defined patient populations. However, with the remarkable opportunity that the availability of human heart tissue provides, come inherent limitations that are important to recognize and acknowledge when interpreting data from such studies. These include variable methods of sample acquisition (particularly for appropriate control hearts), heterogeneity of clinical characteristics and medical therapies, and restricted time frames in the course of disease (typically advanced stages). This review will highlight the valuable insights that have been gained from studies of UPS function in human cardiomyopathies, discuss limitations in interpretation, and compare/contrast findings from human hearts to relevant animal and in vitro models.

Regulation of the Cardiac UPS

The ubiquitin protein ligase system coordinates the conjugation of ubiquitin to specific substrates via a cascade of three enzymatic reactions. Irreversible ubiquitination of a substrate targets it for degradation. This system has been extensively reviewed elsewhere (30, 31, 39) and will not be covered in detail in this review. The critical point to emphasize is that the specificity of the ubiquitin protein ligase system is dictated by the numerous E3s that each recognize a specific target protein(s). A few of likely many cardiac E3s identified to date include atrogin-1, the muscle ring finger (MuRF) family (MuRF1, MuRF2, and MuRF3), carboxyl terminus of heat shock protein 70-interacting protein, and murine double minute (62, 97, 99).
The structure and function of the UPS has also been recently reviewed comprehensively elsewhere (24, 39). In the heart specifically, proteolytic degradation by the UPS is highly regulated at multiple levels to affect gating, assembly, and/or activity (37). Heterogeneity of the proteasome subpopulations represents one important mechanism by which proteasome activity and substrate specificity may be modulated. Within the 20S core, the three catalytic β-subunits can exist as constitutive forms (β1, β2, and β5) or immuno forms (β1i, β2i, and β5i), the latter constituting the “immunoproteasome” and differing in catalytic efficiency and substrate specificity from the constitutive proteasome. Classically, the immunoproteasome was thought to function exclusively in antigen presenting cells to regulate the production of major histocompatibility complexes, but more recent work has demonstrated the existence of immune forms in other organs, including the heart (91). Moreover, single 20S cardiac proteasomes may contain a mixture of immuno and constitutive β-subunits (21). Heterogeneity in the molecular composition of the cardiac 19S regulatory particle has also been demonstrated. Two isoforms of regulatory particle non-ATPase 10 (multi-ubiquitin chain binding subunit) due to alternative splicing have been identified in the mouse heart (28). More recently, two distinct subpopulations of 19S complexes that differ in composition and proteolytic activity were isolated from mouse hearts (90). Finally, the 20S proteasome can complex with the 11S regulatory particle or in a hybrid formation with a 19S and 11S on either end (86). Each of these regulatory particles increases access to the proteolytic core by gating of the 20S α-rings, but the 19S regulatory particle contains intrinsic ATPase activity conferred by the regulatory particle triple A (Rpt) subunits in the base ring, whereas the 11S regulator does not, suggesting that only unfolded proteins are substrates for 11S–20S complexes (24). The admixture of these proteasome species within the heart is likely to be an important determinant of proteasome function in different cardiac disease states, but available data, particularly in human heart disease, are limited.

A second mechanism for proteasome regulation involves post-translational modification of proteasome subunits. A series of studies from Ping and colleagues (105) have elegantly characterized endogenous phosphorylation patterns of the 20S in murine hearts and identified novel associating proteins including protein kinase A (PKA) and protein phosphatase 2A. They further showed that exogenous administration of PKA in vitro increased phosphorylation of certain 20S subunits and increased proteolytic activity. Overall, they have identified 52 phosphorylation events in murine heart and liver, with phosphorylation sites on nine of the cardiac 20S subunits (45). Given the central role of dysregulated cAMP-dependent signaling in the diseased heart (56), it is likely that altered PKA-mediated phosphorylation of proteasome subunits provokes proteasome dysfunction under a variety of pathological conditions. Other kinases, such as casein kinase II, protein kinase C, and calcium/calmodulin-dependent protein kinase II, probably also play important roles in modulating proteasome function in the heart, but few data are yet available. Other post-translational modifications to proteasome subunits, including oxidation and acetylation, can modify proteasome function (45, 105). Oxidative damage or modification to proteasome subunits inhibits proteasome function in experimental models of ischemia and ischemia-reperfusion (11, 68, 70) and may constitute an important mechanism for proteasome dysfunction in other forms of chronic heart disease associated with increased oxidative stress.

Human Cardiomyopathies: Etiologies and Clinical Features

The diseases on which this review focuses, cardiomyopathies, are intrinsic heart muscle diseases characterized by adverse ventricular and atrial remodeling and systolic and/or diastolic dysfunction. Disease severity and age at presentation encompass a very broad spectrum and treatment options range from surveillance, to medical therapy, to more advanced therapies for heart failure and associated arrhythmias (26, 35). When studies that have used human heart tissue to study the UPS are considered, it is important to understand the distinct features of different cardiomyopathies in patients from whom tissue was obtained, as well as the stage and chronicity of disease. The vast majority of studies use heart tissue from patients with dilated cardiomyopathy (DCM) and end-stage heart failure at the time of cardiac transplantation or implantation of a left ventricular (LV) assist device (LVAD). These patients uniformly have very advanced disease, usually with protracted courses of heart failure treated medically for many years. Etiologies include genetic (~30–50%), most commonly inherited as autosomal dominant traits, or acquired forms from environmental insults such as toxins, viral myocarditis, or ischemia-infarction (34, 95). The term ischemic cardiomyopathy is used to describe the subset of cardiomyopathies resulting from previous myocardial infarction(s) and/or ongoing coronary ischemia. A few studies have used LV biopsy samples from patients undergoing other surgeries, such as valve replacement or coronary artery bypass and these patients often have less advanced disease than those undergoing LVAD implantation or transplantation.

Human heart tissue can also become available from patients with hypertrophic cardiomyopathy (HCM). HCM is the most common inherited cardiomyopathy (population frequency, 1:500), is characterized by cardiac hypertrophy and diastolic dysfunction that typically develops over many years, and can be complicated by heart failure, arrhythmias, and sudden cardiac death (33, 48, 57). The majority of HCM cases are linked to autosomal dominant mutations in cardiac sarcomere genes, with a small percentage of others associated with mutations in Z-disc, metabolic, and Ca2+-handling genes (1, 15, 32, 47, 49, 50, 64, 73). Roughly, two-thirds of HCM patients develop LV outflow tract obstruction (51), and a small, but important, subset of these patients have symptoms refractory to medical therapy, necessitating surgical resection of muscle in the basal portion of the intraventricular septum, referred to as a septal myectomy (17, 55). Rarely, patients with nonobstructive HCM develop adverse ventricular remodeling accompanied by restrictive diastolic filling and/or systolic dysfunction and progress to needing mechanical support or cardiac transplantation. However, by far, septal myectomy tissue is the primary source of human heart muscle available for research in patients with HCM. Unlike cardiac transplant patients, most HCM patients with HCM undergoing myectomy do not suffer from overt heart failure and may maintain a relatively well-compensated state of ventricular hypertrophy for many years, following surgical relief of LV outflow tract obstruction (59).
**Limitations Inherent in the Use of Human Heart Tissue for Research**

Availability of human heart tissue is primarily limited to major academic centers. Even at such centers, control heart tissue of suitable quality is extremely difficult to obtain. Non-failing hearts, i.e., ones with ejection fraction >50–55%, are generally accepted as appropriate controls. But these hearts often have some coronary artery disease or mild hypertrophy (many organ donors have hypertensive neurological crises). Another limitation in using human heart tissue is the inability to obtain tissue at different time points as can be done in animal studies. This constrains conclusions that can be made about the evolution or causality of any particular pathway over the continuum of a disease process. Finally, it is difficult to control for the potential influence of different clinical variables and pharmacological therapies. Because of this heterogeneity, it is critically important to study a sufficient number of samples and provide relevant demographic and clinical data. Keeping these factors in mind, human heart tissue is still an undeniably valuable resource that can be used to translate important findings from experimental model systems and as a means of establishing clinically relevant hypotheses.

**UPS Function in Human DCM and Heart Failure**

Accumulation of polyubiquitinated proteins is the most commonly used measure in published studies to assess UPS function in human DCM and heart failure. A consistent finding among all of the studies is the observation that polyubiquitinated proteins are increased in DCM/failing hearts compared with control hearts (Table 1). This has been measured by quantitative Western blot analysis using antibodies that recognize ubiquitin with densitometric analysis of high molecular weight proteins (9, 38, 71, 96, 101). Immunolabeling for ubiquitin in fixed tissue sections has also revealed a greater number and size of ubiquitin-positive deposits in failing myocardium compared with controls (25, 29, 38, 60, 88, 101). In one study, double labeling for ubiquitin and myosin showed colocalization in large aggregates (38). In the same study, ubiquitin colocalized with monodansylcadaverine, a specific marker for autophagic vacuoles, suggesting a link between ubiquitin conjugate accumulation and autophagy. Most of the referenced studies used tissue from end-stage heart failure patients undergoing cardiac transplantation or LVAD. However, some used biopsy samples from patients with less advanced disease such as those undergoing aortic valve replacement (29) or coronary artery bypass grafting (25) with or without LV systolic dysfunction in each case, or ventriculoplasty (aneurysm resection) (60), suggesting that accumulation of polyubiquitinated proteins occurs before the development of decompensated heart failure. The reproducibility of this finding provides convincing support for an imbalance in the rate of protein synthesis and polyubiquitination relative to the rate of protein degradation in human DCM. While accumulation of polyubiquitinated protein conjugates is often interpreted as an indicator of UPS function, these assays have been performed using human heart tissue with more variable results (Table 1).

The first study to use this assay in human hearts found no difference in chymotrypsin-like activity in DCM compared with that in control hearts (38). One possible explanation is that the use of previously frozen extracts and absence of ATP in the assay buffer contributed to the high degree of variability among samples. Another study found increased chymotrypsin-like activities in DCM compared with control hearts (9), using a single concentration of ATP (28 μM) in freshly prepared extracts. A more recent study showed the opposite, an ~50% decrease in chymotrypsin-like and caspase-like activities in failing compared with control hearts with a marked inhibition of activity at low concentrations of ATP in failing hearts (71). The differences between the two latter studies are that Birks et al. (9) used biopsy samples from donor hearts, whereas Predmore et al. (71) used tissue obtained from whole donor hearts not used for transplant. The sample size was also considerably larger in the latter study. Differences in patient demographic and clinical characteristics and proteasome assay conditions including concentration of ATP and use of different inhibitors to control for proteasome-independent activities could also account for the discrepant findings. It is also important to recognize the limitations of the assay, even under ideal conditions. Fluorogenic substrates are small molecules that are probably capable of diffusing into the proteolytic core without the need for ATP-dependent processing by the 19S, at least to some extent, and so this assay may not accurately reflect in vivo activity of the assembled 26S proteasome. Support for this comes from animal studies in which proteasome peptidase activities are increased, despite impaired ability of the UPS to degrade a surrogate substrate in vivo (14). In fact, accumulation of polyubiquitinated proteins in failing hearts was observed by Birks et al. (9), despite increased proteasome peptidase activities.

**Effects of Mechanical Unloading on UPS Function**

The increasing use of LVADs as a bridge to transplantation has enabled the examination of effects of mechanical unloading of the LV and accompanying biochemical and molecular changes in paired samples from the same patients. Three studies have looked at the effects of mechanical unloading on proteasome abundance and/or activity. One study found that chymotrypsin-like activity significantly increased after LVAD (n = 7 paired samples) but without a concomitant decrease in accumulation of polyubiquitinated proteins (36). Increased chymotrypsin-like activity in failing hearts after LVAD implant was corroborated in six paired samples in the study by Predmore et al. (71). Polyubiquitinated proteins were not assessed in the latter study. The third study used immunohistochemical techniques to quantify 20S proteasome and ubiquitin content in 23 paired samples (101). Abundance of 20S proteasome was decreased in failing samples compared with controls but partially restored post-LVAD. Correspondingly, ubiquitin-positive cardiac myocytes were increased in failing hearts compared with control hearts but decreased after unloading. These data collectively suggest that one of many benefits of mechanical unloading is activation of the proteasome, analogous to findings in skeletal muscle (3), which may reduce accumulation of damaged proteins and contribute to LV reverse remodeling.
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<tr>
<th>Tissue Source</th>
<th>Number of Samples</th>
<th>Ubiquitinated Proteins (Method)</th>
<th>Enzymes in Ubiquitination/De-ubiquitination</th>
<th>Proteasome Abundance</th>
<th>Proteasome Activity</th>
<th>Autophagy Markers</th>
<th>PTMs</th>
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<td>Weekes et al., 2003 (96) Transplant, unused donor hearts</td>
<td>DCM (12)</td>
<td>Increased in failing (Western)</td>
<td>Increased E1, E2 (protein)</td>
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<td>Control (3 donor hearts, 4 biopsies)</td>
<td>Increased in failing (Western and IF)</td>
<td>Increased ubiquitin (mRNA)</td>
<td>No difference α-5 (Western)</td>
<td>No difference chymotrypsin-like activity (highly variable)</td>
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<td>Birks et al., 2008 (9) Transplant, biopsies from donor hearts</td>
<td>Control (17)</td>
<td>Increased in DCM (Western)</td>
<td>Increased E1 (protein)</td>
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<td>Increased chymotrypsin-like activity in DCM</td>
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<td>Kassiotis et al., 2009 (36) LVAD and transplant</td>
<td>DCM paired samples (7)</td>
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<td>Decreased autophagy mediators Atg5, beclin 1, LC3-II post-LVAD</td>
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<td>Increased in EF &gt;40 (Western)</td>
<td>Increased atrogin-1 (E3) in EF &gt;40 (mRNA and protein)</td>
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<td>CABG EF &lt;40 (10)</td>
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<td>Wohlschlaeger et al., 2010 (101)</td>
<td>Controls (4)</td>
<td>Increased in failing versus controls (IF)</td>
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<td>Decreased 20S in failing</td>
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<td>LVAD/transplant (23 paired)</td>
<td>Decreased post-LVAD (IF)</td>
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<td>Otsuka et al., 2010 (60)</td>
<td>DCM (26) Controls (10)</td>
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<td>Increased 26S in DCM (IF)</td>
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<td>Predmore et al., 2010 (71)</td>
<td>DCM (33)</td>
<td>Increased in DCM versus control (Western)</td>
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<td>No differences in 20S, 19S or 11S subunits (Western)</td>
<td>Decreased chymotrypsin-like and caspase-like activities in DCM and HCM versus control</td>
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<td>Increased oxidative modification of Rpt5 in DCM versus control</td>
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<td>HCM (29) Control (8)</td>
<td>No difference in HCM versus control (Western)</td>
<td>No differences in β5 active site availability</td>
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<td>Increased activity post-LVAD</td>
<td>Lower activity in sarcomere mutation positive HCM</td>
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<td>Day et al., 2013 (16a)</td>
<td>DCM (11)</td>
<td>—</td>
<td>—</td>
<td>No difference in 20S, 19S, or 11S subunits (proteomic profiling, mass spectroscopy)</td>
<td>Decreased ATPase activity of Rpt subunits</td>
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<td>Decreased phosphorylation of serine250 of α7</td>
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UPS, ubiquitin proteasome system; PTMs, post-translational modifications; DCM, idiopathic dilated cardiomyopathy; ICM, ischemic cardiomyopathy; HCM, hypertrophic cardiomyopathy; UCH, ubiquitin carboxyl-terminal hydrolase; HAUSP, herpes virus-associated ubiquitin-specific protease; AS, aortic stenosis; MS, mitral stenosis; UFD1, ubiquitin-fusion degradation system-1; MDM2, murine double minute 2; CABG, coronary artery bypass grafting; LVAD, left ventricular assist device; EF, ejection fraction; IF, immunofluorescence; ELISA, enzyme-linked immunospecific absorbance; Rpt, regulatory particle triple A.
Potential Mechanisms for UPS Dysfunction in Human Heart Failure

As good support for defective protein degradation in human cardiomyopathies has been established, recent studies have begun to address potential mechanisms. Several investigators have examined pathways involved in ubiquitination and deubiquitination in human cardiomyopathies. Increased E1 (9, 96) and E2 protein (38, 96), ubiquitin mRNA (38), and ubiquitin carboxyl-terminal hydrolase mRNA (96) and decreased protein expression of deubiquitinating enzymes isopeptidase T and ubiquitin-fusion degradation system (38) have been observed. Expression of E3s murine double minute 2 (9) and atrogin-1 (25) is also increased in heart failure, and MuRF1 protein expression is increased following mechanical unloading with LVAD (98). The overall observed increase in expression of ubiquitination machinery in failing hearts may be in response to an increased protein burden, from increased protein synthesis that accompanies the hypertrophic response or an excess of damaged or modified proteins to be targeted for proteasomal degradation.

One potential mechanism for defective protein clearance is a decreased overall abundance of proteasomes. Another possibility would be a shift in the distribution of proteasome subpopulations that may have distinct differences in peptidase activity and/or selectively degrade certain proteins or classes of proteins (21, 69). Several studies have begun to examine these possible layers of regulation in human cardiomyopathies. One study observed decreased expression of 20S proteasomes in human failing hearts compared with controls by immunohistochemistry, which was partially restored after LVAD (101) (Table 1). However, by more quantitative methods, no significant differences in the expression of representative subunits from 20S, 19S, or 11S proteasomes in human failing heart compared with control hearts have been found (38, 71). Comprehensive proteomic profiling by mass spectroscopy of enriched proteasome fractions has also shown no difference in protein content of any constitutive or inducible proteasome subunits (16a). However, in the latter study, docking of 19S to 20S was decreased in failing hearts compared to controls (16a). A possible explanation for this was the additional finding of reduced ATPase activity of the Rpt subunits, which are critical for the assembly of complete 26S (or 30S) proteasomes (27, 82). Since most ubiquitinated proteins require ATPase activity of the 19S for recognition, unfolding, and translocation to the α-subunit chamber of the 20S core, defective assembly of 19S with 20S could significantly impair the degradative capacity of the UPS.

Post-translational modifications, such as phosphorylation, acetylation, or oxidation, may be an alternative explanation for proteasome dysfunction in human heart failure. Predmore et al. (71) observed an increase in the oxidative modification of the proteasome dysfunction in human heart failure. Predmore et al. (71) observed an increase in the oxidative modification of the proteasome dysfunction in human heart failure. Predmore et al. (71) observed an increase in the oxidative modification of the proteasome dysfunction in human heart failure. Predmore et al. (71) observed an increase in the oxidative modification of the proteasome dysfunction in human heart failure.

Potential Consequences of Proteasome Dysfunction in Human Heart Failure

No matter what the mechanism, impairment of protein clearance by the UPS would be expected to have several consequences. For example, increased steady-state expression of prohypertrophic and proapoptotic factors could result from impaired degradation. Two examples of this are Akt (71) and p53 (9, 71), which both demonstrate increased protein content in human failing compared with control hearts in the context of UPS impairment in the same samples. The clearance of cytotoxic, oxidized proteins is highly dependent on the 20S proteasome (16, 81). More recent evidence indicates that the immunoproteasome and the 11S regulator also play major roles in degradation of oxidized proteins (65). Mild protein oxidation causes rearrangement of tertiary protein structure with exposure of hydrophobic residues that can directly bind to the hydrophobic binding domain of the α-subunits, making their clearance independent of ubiquitin and ATP (16, 81). UPS inhibition could therefore also contribute to the increased burden of oxidized proteins in heart failure (71).

Formation of toxic protein aggregates is another important ramification of proteasome dysfunction, resulting from impaired clearance of misfolded proteins. Sanbe et al. (76) used an antibody that recognizes a conformation-dependent epitope on soluble amyloid oligomers to examine the accumulation of these toxic intermediates in human autopsy specimens from patients with DCM and HCM (76). Marked immunoreactivity in diseased versus control samples was demonstrated, indicating that protein aggregation was a common feature of a diverse spectrum of human cardiomyopathies. To prove that preamyloid oligomers are proteotoxic in the heart, Pattison et al. (63) engineered a transgenic mouse that expressed a long polyglutamine repeat under control of the α-myosin heavy chain promoter (63). The authors found that expression of an exogenous peptide that was prone to the formation of preamyloid oligomers resulted in cardiomycyte cell death, DCM with reduced systolic function, and premature death at 8 mo of age. The formation of protein aggregates can also cause proteasome inhibition, although the mechanisms are not well understood (7, 8). UPS inhibition occurs before formation of mature fibrillar aggregates or inclusion bodies, suggesting that perhaps an intermediate soluble form could bind to and sequester or “clog” the proteasome (8, 13). However, recent work in a cellular model of Huntington’s disease suggested that mutant aggregate-prone proteins do not directly inhibit the 26S proteasome. Instead, an excess burden of misfolded proteins may preoccupy chaperones, limiting the pool available for the proper folding of other cellular proteins, diverting them to the UPS for degradation, and eventually overwhelming the system by competitive inhibition (23).

Activation of autophagy is another potential consequence of UPS inhibition (40, 104). While the mechanisms by which these two pathways interact are not fully understood, multiple lines of evidence implicate p62 as a key linker protein and sensor of proteotoxic stress. p62 is a multifunctional adaptor protein that binds K63-linked ubiquitin chains and also directly binds to microtubule-associated protein 1A/1B-light chain 3,
which could promote autophagosome formation (84, 92). Autophagic vacuoles have been demonstrated in failing human hearts (29), and these contain with ubiquitin (38). Furthermore, the expression of autophagic mediators including Atg5, beclin 1, and microtubule-associated protein 1A/1B-light chain 3-II was decreased after LVAD (101). Taken together, proteasome dysfunction in human heart failure may have profound downstream effects on diverse pathways that actively contribute to disease progression.

**UPS Function in HCM**

Far less is known about UPS function in human HCM compared with DCM associated with heart failure, primarily because of limited tissue availability. Hein et al. (29) included tissue from a group of patients with cardiac pressure overload hypertrophy from aortic stenosis, but with preserved systolic function, in their analysis of ubiquitin-related autophagy. There was no significant increase in ubiquitin labeling in myocyte sections from patients in this group compared with control as there was for the groups with depressed ejection fraction. This may be explained by the relatively modest degree of hypertrophy present (wall thickness, 15 mm; no difference in LV mass compared with control group). As mentioned in the previous section, in patients with more severe hypertrophy associated with inherited HCM, toxic soluble intermediates to protein aggregates were abundant in cardiac myocyte sections, suggesting generalized failure of protein clearance but not necessarily implicating a particular pathway.

More recently, Predmore et al. (71) interrogated UPS activity in human HCM samples from patients with defined genotypes. There was a marked reduction in chymotrypsin-like and caspase-like activities in HCM compared with control hearts. The magnitude reduction in proteasome activity in HCM hearts was comparable with failing hearts, although not associated with a significant increase in polyubiquitinated proteins. Whether this is due to a lesser increase in protein synthesis and polyubiquitination or a lesser burden of damaged proteins in HCM compared with failing hearts is unclear. Proteasome activities were lower in the HCM samples from patients with sarcomere gene mutations than in those without mutations, despite the younger age of the former group. Handling of mutant sarcomere proteins may overwhelm the protein quality control system and compromise the processing of other proteins. Recently published experimental data support this hypothesis and will be discussed in the following section.

**Comparing Human Disease with Experimental Model Systems**

UPS function has been interrogated in a variety of in vitro and animal models and comprehensively reviewed elsewhere (40, 53, 66, 67, 78, 85, 91, 94, 100, 103). Published studies most relevant for comparison to human cardiomyopathies are the 1) mouse models of desmin-related cardiomyopathies (DRCs), 2) cardiac hypertrophy models (i.e., thoracic aortic banding, isoproterenol induced), and 3) HCM-associated myosin binding protein C (MYBPC3) mutant knock in mice.

First, the DRC mouse model provides compelling data for impaired UPS function in cardiomyopathies. Expression of desmin or αβ-crystallin mutants results in cardiomyocyte accumulation of soluble protein aggregates similar to those seen in human end-stage cardiomyopathies of diverse causes (76). In the animal model, proteasome function is severely impaired, as assessed by the in vivo surrogate UPS substrate green fluorescent protein containing a degron sequence to target to UPS, and occurs before detectable gross cardiac pathology or heart failure (14, 43). Furthermore, protein aggregation is required for UPS inhibition (44), and reversal of aggregate formation by voluntary exercise prolongs survival in αβ-crystallin mutant mice (46). Together, these data strongly support a pathogenic and causal role for defective protein clearance in DRC that may extrapolate more broadly to other cardiomyopathies associated with abnormal protein aggregation.

Second, modulation of UPS activity has been reported in different hypertrophic experimental models, although not consistently in the same direction. In one study, polyubiquitinated proteins were increased commensurate with decreased UPS activity after thoracic aortic constriction in mice and before the development of heart failure (88). However, in a chronic hypertrophy canine model, proteasome activation was observed in the LV subendocardium (18). In the same study, short-term administration of a proteasome inhibitor to mice following thoracic aortic banding prevented the development of hypertrophy. Two subsequent reports showed similar reductions in the hypertrophic response to in vitro and in vivo stimuli with proteasome inhibition (54, 83). Most recently, Drews et al. (20) also reported increased 26S proteasome activity after 7 days of continuous isoproterenol infusion to induce cardiac hypertrophy in mice. This was associated with increased expression and incorporation of inducible β-subunits and several 19S subunits into the proteasome. Together, the bulk of the evidence in animal and in vitro hypertrophy models would appear conflicting with findings in human HCM (71, 76) where protein aggregation and impaired UPS function have been demonstrated. There are a number of potential explanations that include the obvious species difference, but also the nature of the stress, and magnitude, duration, or rate of progression of disease. In experimental models the stress is much more acute and of short duration (days/weeks), whereas in humans, the stress imposed by pressure overload (hypertension or valvular heart disease) or genetic perturbations (HCM) is insidious and of much longer duration (years/decades). The important question that has been raised by animal and in vitro models, whether proteasome inhibition is a viable strategy to treat hypertrophic heart disease is therefore in jeopardy in light of recent data in humans. Supporting the contention that suppressing proteasome activity would be deleterious in cardiac hypertrophy is a recent study showing that pharmacological proteasome inhibition is sufficient to induce cardiac hypertrophy and maladaptive remodeling after thoracic-aortic banding, to stimulate prohypertrophic signaling via calcineurin-dependent nuclear factor of activated T-cells nuclear translocation, and to increase mortality (87).

Third, expression of mutant sarcomere proteins may directly interfere with UPS function. In vitro studies have shown that the expression of truncated (77) or missense (4, 5) MYBPC3 can inhibit the proteasome. These findings were translated to a mouse knock-in model, MYBPC3 Glu264Lys (89). This mutation is in the last base of exon 6 in a conserved splice site and analogous to Glu258Lys in humans. Three different mRNAs resulted from this single nucleotide substitution in the mouse.
One transcript that led to a premature termination codon was present at a low level because of susceptibility to nonsense-mediated RNA decay. The other two created full length proteins and were degraded by the UPS. More recent work by this group demonstrates reduced clearance of a surrogate UPS substrate in MYBPC3 mutant knock-in mice and decreased proteasome activity (79). Heterozygous knock-in mice have a milder phenotype, but continuous infusion of isoproterenol and phenylephrine unmasked UPS impairment (80). Taken together with the observation of lower proteasome activity in human HCM in hearts from patients with sarcomere gene mutations than in those without mutations (71), data are beginning to accrue that HCM-associated mutant sarcomere proteins may interfere with normal protein quality control in the cardiac myocyte. While no data are yet available in human HCM as to the role of E3s on clearance of mutant MYBPC3 proteins, in vitro atrogin-1 has been shown to target a truncated MYBPC3 for UPS-mediated degradation, but not wild-type MYBPC3 (52). This is perhaps due to different localization of truncated and full-length MYBPC3 within the sarcomere (Z band vs. A band) since atrogin-1 was shown to physically interact with wild-type MYBPC3. Conversely, while MuRF1 interacts with MYBPC3, it does not appear to have E3 activity but instead may have an indirect effect on transcriptional regulation of MYBPC3 and myosin heavy chain (MYH7) (52).

Conclusions and Future Directions

Most studies observe some level of proteasome impairment in human cardiomyopathies. Relevant animal models provide strong support for a causal role of UPS dysfunction and protein aggregation in disease pathogenesis. A model for dysregulation of protein quality control in human cardiomyopathies is proposed (Fig. 1).

There is substantial interest in developing novel therapeutics that target specific pathways or components of the ubiquitin conjugation and proteasome systems (6). Proteasome inhibitors have demonstrated efficacy for hematologic malignancies, particularly multiple myeloma, with one, Bortezomib, approved by the Food and Drug Administration, and others in various stages of clinical development. Bortezomib is a reversible inhibitor of β5, the 20S proteasome subunit with chymotrypsin-like proteolytic activity. The target range for human proteasome inhibition is 60–80% for optimal efficacy while limiting toxicity (75). Plasma cells are inherently sensitive to proteasome inhibition because of their large volume of immunoglobulin production, which requires the constitutive expression of physiological unfolded protein response genes (58). The excessive endoplasmic reticulum stress response that results from proteasome inhibition in multiple myeloma cells leads to proapoptotic signaling (19). Whether global proteasome inhibition will have a role for treatment of certain human heart conditions, such as ischemia-reperfusion or hypertrophy, is uncertain. Bortezomib has been experimentally shown to cause cardiac dysfunction (74, 87), and a subset (~6–12%) of patients receiving bortezomib have experienced cardiac complications, including heart failure, LV dysfunction, bradyarrhythmias, and atrial fibrillation (22, 102). Some of these patients have cardiac amyloidosis associated with their underlying malignancy and, therefore, may be more susceptible to cardiac complications. Nevertheless, in light of these data...
coupled with evidence for already impaired proteasome function in human cardiomyopathies, global proteasome inhibition is liable to be harmful, at least as a long-term therapy for cardiomyopathies. However, the converse, global activation of the proteasome, may be a more promising strategy worth pursuing. Recent cellular (42) and animal data (41) show that overexpression of proteasome activator 28α (11S activator) is cardioprotective against various challenges, without disturbing normal protein turnover or cardiac function. These data raise the possibility that pharmacologic or small molecule activators of 11S in the heart could be advantageous for enhancing proteasome function in cardiomyopathic hearts.

To design more precise targets, a better understanding of the mechanisms for proteasome dysfunction in human cardiomyopathies will be critical. These mechanisms may include alterations in ubiquitination pathways upstream of the proteasome including E3 activity, shifting of proteasome subpopulations to modify substrate specificity or meet higher cellular demands, post-translational modifications to proteasome subunits, and/or interference by misfolded, truncated, oxidized, or otherwise damaged proteins. Furthering knowledge on multiple fronts, using a combination of cellular and animal models, along with human heart tissue, will ultimately lead to development of a more specific direction within the complexity of the UPS for therapeutic benefit.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
S.M.D. conception and design of research; S.M.D. prepared manuscript; S.M.D. drafted manuscript; S.M.D. edited and revised manuscript; S.M.D. approved final version of manuscript.

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