Role of the Ubiquitin Proteasome System in the Heart

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Abstract: **Proper protein turnover is required for cardiac homeostasis and, accordingly, impaired proteasomal function appears to contribute to heart disease. Specific proteasomal degradation mechanisms underlying cardiovascular biology and disease have been identified, and such cellular pathways have been proposed to be targets of clinical relevance. This review summarizes the latest literature regarding the specific E3 ligases involved in heart biology, and the general ways that the proteasome regulates protein quality control in heart disease. The potential for therapeutic intervention in Ubiquitin Proteasome System function in heart disease is discussed. (*Circ Res*. 2013;112:1046-1058.)**

Key Words: heart failure ■ ischemia ■ myocardial remodeling, ventricular ■ proteasome endopeptidase complex ■ ubiquitin

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Cardiovascular diseases are the leading causes of morbidity and mortality worldwide, according to the World Health Organization.[1] They include chronic heart failure, a condition that has reached epidemic proportions, characterized by the inability of the heart to pump enough blood and oxygen to support the body's organs. Heart failure can occur suddenly or, more commonly, can result from chronic conditions, such as hemodynamic overload resulting from chronic hypertension or valvular diseases, or congenital defects.[2] Combined with advances in medical treatments, an awareness of the underlying disease-causing mechanisms has contributed to a slight decrease in levels of cardiovascular disease risk factors in developed countries. Although this has led to a decrease in cardiovascular event rates in developed countries, there has been a steady increase in incidence in low- and middle-income countries. Such disheartening statistics and the huge estimated cost for communities make it imperative to search for novel pathophysiological mechanisms and therapeutic targets capable of slowing disease progression.

Ubiquitin Proteasome System

Over the past several decades, it has become increasingly apparent that protein degradation by the Ubiquitin Proteasome System (UPS) controls many fundamental biological processes. In most mammalian cells, the UPS degrades ≈90% of proteins, ensuring that misfolded, oxidized, or damaged proteins, which possess an intrinsic toxicity, are degraded. As many as 30% of newly synthesized proteins are degraded by the proteasome within a few minutes of their synthesis.[3,4] In addition to its role in removing damaged or misfolded proteins, the UPS targets the degradation of specific substrates in a highly regulated manner. It is now apparent that no part of the cell is out of reach of the UPS; proteins in the nucleus, cytoplasm, endoplasmic reticulum lumen, as well as membrane proteins, are all kept in check by the ubiquitinating enzymes and the proteasome.[5] Aside from the proteasome, other mechanisms of protein degradation include cleavage by nonproteasomal intracellular proteases, found in lysosomes/autophagosomes,[6] which are responsible for the turnover of long-lived proteins and organelles. For a comprehensive description of the role of autophagy in heart biology and disease, see Nemchenko.[7] Although the UPS has been mostly studied in the context of cell and cancer biology, increasing evidence suggests that dysfunction of the UPS plays a role in cardiac pathologies. This review will summarize the latest literature regarding the general role of the UPS in protein quality control in the heart, and the specific E3 ligases known to contribute to cardiac diseases.

The 2004 Nobel Prize in Chemistry was awarded to Aaron Ciechanover, Avram Hersko, and Irwin Rose for their discovery and characterization of the ATP-dependent UPS. Briefly, the proteasome is a large, multisubunit protease complex that functions in an ATP-dependent manner. Proteins are targeted to the proteasome when covalently tagged with chains of the small modifier-protein, ubiquitin. The polyubiquitin chain is assembled on the substrate protein via an enzymatic cascade, in which ubiquitin is activated by covalent linkage to an E1 ubiquitin–activating enzyme and transferred to an E2 ubiquitin–conjugating enzyme, before an E3 ubiquitin ligase mediates transfer to a lysine residue in the substrate or a lysine in the growing polyubiquitin chain[8,9] (Figure 1). With a few exceptions, proteins targeted for degradation are tagged with ubiquitin chains linked through lysine-48 of the

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Nonstandard Abbreviations and Acronyms

CHIP	carboxy terminus of Hsp70-interacting protein
CRL	cullin RING ubiquitin ligase
CSN	COP9 signalosome
CUL	cullin
E1	ubiquitin-activating enzyme
E2	ubiquitin-conjugating enzymes
E3	ubiquitin ligase
ER	endoplasmic reticulum
FOXO	Forkhead box O
HECT	homologous to E6-AP carboxyl terminus (HECT)
HIF	hypoxia-inducible factor
LV	left ventricular
MAFbox	muscle atrophy F-box
MDM2	murine double minute
MR	myocardial remodeling
MuRF1	muscle-specific RING finger 1
RING	Really Interesting New Gene
SCF	Skp1-Cullin-F-box protein complex
Siah2	seven in absentia homolog
SKP2	S-phase kinase-associated protein 2
TAC	transverse aortic constriction
UPS	ubiquitin proteasome system

ubiquitin molecule. The specificity of this reaction is dictated by the E3 protein, which selects the substrate. The complex way in which these 3 enzymes work in concert is reviewed elsewhere.[5,10]

The human genome encodes hundreds of E3 ubiquitin ligases, which are classified into 3 main classes based on structural similarities: the RING-finger proteins (the most abundant class of E3 enzymes), the U-box proteins (the U-box domain shares characteristics with the RING-finger domain), and the HECT-domain (homologous to E6-associated protein C terminus) proteins (there are ≈40 HECT domain proteins in humans). HECT domain proteins directly catalyze the degradation of substrates through their HECT domain, whereas RING finger proteins function as adaptor proteins facilitating the degradation of subunits. Although some RING-domain E3 ligases function independently, many RING-finger type ubiquitin ligases function as part of multi-subunit complexes, such as the cullin RING ubiquitin ligases (CRLs), which cull or sort substrates for degradation.[11]

Cullin proteins are molecular scaffolds that have crucial roles in the posttranslational modification of cellular proteins involving ubiquitin. The mammalian cullin protein family comprises 8 members (CUL1 to CUL7 and PARC), which are characterized by a cullin homology domain. CUL1 to CUL7 assemble multisubunit CRL complexes, the largest family of E3 ligases with >200 members.[12] The prototypical CRL is the SKP1-CUL1-F-box protein (SCF) complex. The CUL1 scaffold binds Rbx1 (the RING protein), an E2 enzyme, and the SKP1 bridging factor. SKP1 interacts with interchangeable substrate-targeting subunits, called F-box proteins, via their F-box domain. There are 69 F-box proteins identified in humans.[13,14] F-box proteins target substrates for degradation via their distinct protein-protein interaction motifs. These domains typically recognize short degradation motifs (degrons) that are often subject to posttranslational modifications, such as phosphorylation. It is possible for each F-box protein to target multiple substrates, allowing the core SCF scaffold to regulate the turnover of an extremely large number of proteins. F-box proteins are involved in diverse processes, including the cell cycle, circadian rhythm, iron metabolism, etc. Of relevance to this review, muscle atrophy F-box (Atrogin/MAFbx/FBXO45) is a key player in the regulation of heart and muscle cell size.

Role of the Proteasome in Heart Disease

Myocardial Remodeling

Myocardial remodeling (MR) occurs after an injury to the myocardium (necrosis, pressure overload, volume overload, or aging) and involves a change in the size, shape, and function of the cardiac chambers.[15] Cardiac hypertrophy is a common form of MR and often precedes overt heart failure. Hypertrophy is viewed as a compensatory response of the heart, through which the heart muscle thickens to normalize ventricular wall stress.[16] Whether cardiac hypertrophy is an adaptive (physiological) or a maladaptive (pathological) process is a matter of debate. Recent work suggests that the nature and the duration of the trigger activate different biochemical pathways responsible for adaptive or maladaptive hypertrophy.[17,18] In general, although cardiac hypertrophy has beneficial effects in the short-term by normalizing wall stress, prolonged cardiac hypertrophy (maladaptive hypertrophy) results in an elevated risk of heart failure and cardiovascular mortality.[18]

Cardiac hypertrophy is characterized by cell enlargement rather than proliferation, because cardiomyocytes are terminally differentiated. This is generally attributed to an increase in cellular protein content. In cardiac hypertrophy, both protein synthesis and, counterintuitively, protein degradation rates are increased. The rate of protein degradation increases as a cotranslational protein quality control mechanism to ensure that any abnormally synthesized proteins are degraded (Figure 2).

Proteasome (Dys)Function in Chronic Heart Failure

Given the many roles that are played by the UPS in hypertrophy, it is not surprising that a united model linking UPS activity to hypertrophy is not available. Robust evidence coming from analyses of human biopsies of failing hearts supports the concept that defective protein degradation contributes to heart failure. However, although most studies are congruent in showing high total levels of ubiquitinated proteins and ubiquitin-positive aggregates in failing hearts,[19–21] it remains unclear whether this is attributable to impaired proteasome function. Some reports show that the levels and activity of the proteasome are unchanged in failing hearts.[20] In contrast, other reports show that proteasome activity is impaired in failing hearts, possibly as a result of oxidative modifications on proteasome subunits.[22] In these cases, it appears that the

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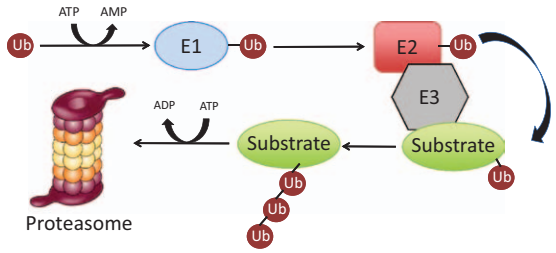

Figure 1. The Ubiquitin Proteasome System. The proteasome degradation of specific substrates and misfolded proteins occurs through an enzymatic cascade of ubiquitination.

decrease in proteasome activity precedes cardiac dysfunction, as shown in an elegant long-term evaluation of proteasomal activity in mice subjected to pressure overload.[23] In turn, it has been demonstrated that left ventricular (LV) unloading in humans with chronic heart failure leads to improved proteasome activity.[24] This was recently recapitulated by Predmore et al,[22] who demonstrated that markedly reduced proteolytic activities in failing human hearts could be partially restored after LV unloading.

Evidence for a Cardioprotective Role of Proteasome Inhibition

A novel and exciting field of interest in this concern is the role of proteasome inhibition in heart disease. It appears that in some cases, pharmacological inhibition of proteasome activity prevents hypertrophy and reverts MR. Depre et al found increased proteasome expression and activity on chronic pressure overload in dogs, particularly in the subendocardial layers that were subjected to higher stress. Notably, the proteasome inhibitor, epoxomicin, completely

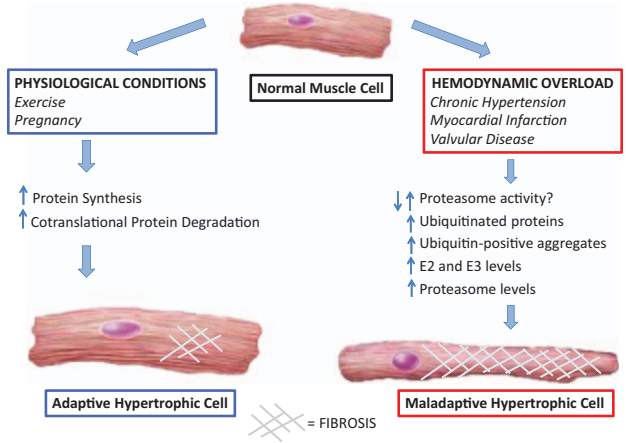

Figure 2. Hypertrophy and proteolysis in the heart. The heart undergoes remodeling in response to changes in physiological demand or disease state, enlarging in an attempt to manage the increased blood flow demanded on excessive heart workload. This hypertrophy is classified as either physiological hypertrophy, such as after exercise or pregnancy, or pathological hypertrophy. It is unclear whether myocardial remodeling involves a decrease or an increase in proteasome activity. There is evidence that impaired proteasomal function is a hallmark of cardiac diseases and is attributable to myocardial stressors such as ischemia, pressure overload, inflammation, or protein aggregates that impair proteasomal function. In contrast, there is evidence that myocardial remodeling is associated with increased proteasome activity and subunit expression.

prevented LV hypertrophy.[25] In another dog model of pace-induced heart failure, several UPS genes were upregulated, including genes encoding proteasome subunits, E2-ubiquitin conjugating enzymes and E3-ligases.[26] Furthermore, Meiners et al[27] demonstrated suppression of cardiomyocyte hypertrophy by low dose inhibition of the UPS with bortezomib, whereas 2 reports by Stansfield et al[28] and Hedhli et al[29] both showed regression of hypertrophy in mice using other proteasome inhibitors.[30]

Evidence Against a Cardioprotective Role of Proteasome Inhibition

However, the concept of proteasome inhibition as a means for cardioprotection has led to conflicting findings. In contrast to the above studies, Tang et al recently showed that chronic proteasome inhibition by bortezomib was sufficient to induce LV hypertrophy to a similar degree as transverse aortic constriction (TAC; an experimental means to induce hypertrophy). Even more importantly, proteasome inhibition by bortezomib worsened the hypertrophy caused by TAC and resulted in heart failure and death in mice,[31] supporting the hypothesis that problems with protein degradation might contribute to MR and chronic heart failure progression.[32]

Proteasome Inhibition in Pathological Versus Physiological Hypertrophy

Another outstanding issue of clinical value is whether proteasome inhibition can specifically prevent or reverse pathological hypertrophy. Pathological and physiological hypertrophy are caused by different stimuli and are associated with distinct phenotypes. Most of the experimental data have suggested that proteasome inhibition reduces pathological myocardial hypertrophy, while preserving LV function, in spite of increased wall stress, as previously described with inhibition of maladaptive signaling pathways.[33,34] To complicate this scenario, Hedhli et al[30] have reported that proteasome inhibition also reverse adaptive hypertrophy, although hypertrophy was not induced by physiological challenges in this case but rather through genetic manipulation of H11K. Even with regard to other key components of the UPS, there are conflicting data on their role on pathological versus physiological hypertrophy. For example, Atrogin/MAFbx, an E3 ligase discussed below, regulates pathological hypertrophy through its role in the turnover of calcineurin, a mediator of maladaptive hypertrophy.[35] However, Atrogin/MAFbx overexpression has also recently been shown to inhibit physiological hypertrophy.[36] Noteworthy, another E3 ligase discussed below, muscle-specific RING finger 1 (MuRF1), only inhibits pathological hypertrophy.

So how is it possible to reconcile these apparent discrepancies? Differences in dose, duration, and types of proteasome inhibitors may influence whether they function as poisons or remedies to explain the conflicting results.[32,37] Partial or short-term proteasome inhibition may mediate a protective effect in the heart, whereas complete and sustained proteasome inhibition may be toxic because overall protein turnover is inhibited, inducing apoptotic cell death. Future studies involving proteasome inhibition in different models of physiological and

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pathological hypertrophy with more robust readouts, such as lifespan, are needed to clarify these findings.

Familial Cardiomyopathies

Familial cardiomyopathies exemplify how myocardial UPS dysfunction leads to heart remodeling and failure.[38–40] Familial dilated cardiomyopathies represent a subset of dilated cardiomyopathies that are usually inherited in an autosomal dominant manner. Myocardial samples obtained from patients with these pathologies contain increased levels of ubiquitinated proteins, along with increased levels of E1 and E2 enzymes.[21] The best-studied example of familial cardiomyopathy is desmin-related myopathy, which is caused by mutations in either the gene encoding desmin, desmin binding proteins, or in the small heat shock protein αB-crystallin, required for proper folding of desmin.[41,42] Desmin is a type III intermediate filament protein, which is critical for cytoskeletal organization and maintaining cardiomyocyte structure. αB-crystallin is a chaperone protein that triages misfolded proteins for proteasomal degradation or repair and is found abundantly in the heart.[43] Mutations in desmins or αB-crystallin lead to accumulation of aggregation-prone proteins that eventually impair the ubiquitin system and proteasome activity.[38–40] A transgenic model characterized by gain-of-function of mutant (R120G) αB-crystallin develops a dilated cardiomyopathy closely resembling human desmin-related myopathy, characterized by disruption of myofibril alignment, diffuse desmin-positive aggregates, and global impairment of the UPS, indicating proteasomal insufficiency.[44] Furthermore, expression of human R120GcryAB mutation causes oxido-reductive stress and protein aggregation cardiomyopathy in mice.[45] Supporting the role of mutant αB-crystallin in the induction of UPS dysfunction, overexpression of the small heat shock proteins prevented αB-crystallin–induced UPS impairment, presumably by compensating for defective αB-crystallin function.[46,47] Interestingly, in this model, the clinical appearance of hypertrophy and subsequent heart failure is preceded by detectable UPS dysfunction. αB-crystallin functions as a cofactor for the F-box protein, FBXO4, a component of the SCF type E3 ligase, where FBXO4-dependent ubiquitination of substrates is promoted by the interaction between αB-crystallin and FBXO4. Interestingly, the R120G form of αB-crystallin found in desmin-related myopathy displays an increased interaction with FBXO4. It is unknown whether FBXO4 plays a role in familial cardiomyopathies at this time.[48]

Another familial cardiomyopathy, hypertrophic cardiomyopathy, is characterized by inappropriate myocardial hypertrophy, LV dysfunction, and increased risk for sudden death. Its prevalence, although previously thought to be rare, is as high as 0.2% in the general population.[49] Hypertrophic cardiomyopathy is inherited as an autosomal dominant disorder with variable penetrance. Mutations in the cardiac myosin-binding protein C, MYBPC3, are the most common cause of hypertrophic cardiomyopathy, accounting for about half of the identified mutations,[50] although >200 mutations in 14 genes encoding sarcomeric proteins contribute to the disease. Identified mutations frequently result in a truncated protein, which are preferentially degraded by the UPS and compete with other substrates, leading to UPS overload and impaired proteasomal activity.[51] The clinical phenotype may depend in part on the defect of sarcomere integrity (haploinsufficiency) and in part on UPS dysfunction. In support of causation between UPS impairment and specific clinical phenotypes of hypertrophic cardiomyopathy, Bahrudin et al[52] have shown that a missense mutation in the MYBPC3 protein (E334K) causes destabilization of the protein, which leads to UPS impairment and subsequent LV dilation and dysfunction. Furthermore, a recent study by Chen et al[53] demonstrated loss-of-function effects of TRIM63 mutations on E3 ligase activity that contribute to hypertrophic cardiomyopathy, supporting the hypothesis that impaired protein degradation is implicated in the pathogenesis of hypertrophic cardiomyopathy.

Ischemia-Reperfusion Injury

The absence of blood flow to tissues, termed ischemia, results in a shortage in available oxygen and glucose and is followed by cell death. Early recovery of the ischemic myocardium is essential to minimize tissue injury associated with acute myocardial infarction. However, myocardial ischemia-reperfusion can also induce complex and deleterious effects, called ischemia-reperfusion injury,[54] caused by increased levels of intracellular calcium, reactive oxygen species, and altered cell metabolism. Indeed, experimental studies suggest that myocardial ischemia-reperfusion injury accounts for up to 50% of the final size of a myocardial infarct.[55] To prevent further tissue injury, clinical strategies, like primary percutaneous coronary intervention or reperfusion therapies, have been developed to provide early and rapid restoration of blood flow to the heart, representing treatments to reduce infarct size and improve clinical outcomes.

The UPS is a promising potential therapeutic target in ischemia-reperfusion injury. However, as with MR, several pertinent studies have yielded conflicting findings. On one hand, the experimental use of proteasome inhibitors limits ischemia-reperfusion injury, leading to a decrease of infarct size up to 50% in some studies,[56–59] probably by reducing inflammation in the myocardium.[56–58] On the other hand, ischemia-reperfusion injury has been shown to inhibit proteasome function,[60–63] and it has been shown that improving proteasome function protects against ischemia-reperfusion injury in mice.[64] Such impairment of the UPS after ischemia-reperfusion is attenuated by ischemic preconditioning, probably through inhibition of oxidative damage to proteasome subunits.[65] Dysfunctional proteasome function was first observed in brain ischemia,[66] and subsequently described by Bulteau et al[60] in an in vivo rat model of ischemia-reperfusion. Such findings were confirmed and expanded in other studies, which demonstrated that the reduced proteasome function is associated with increased levels of ubiquitinated proteins.[61] Recently, it has been shown that this is attributable to selective, rather than global, inhibition of proteasome activity,[63] where some proteasome substrates are still degraded (Nrf2), whereas others are not (p-IκB).

Several hypothetical mechanisms have been proposed to explain how ischemia-reperfusion impairs proteasome function (Figure 3). First, it is thought that the production of oxidative species during ischemia may result in the modification of proteasome subunits, directly impairing proteasome function.

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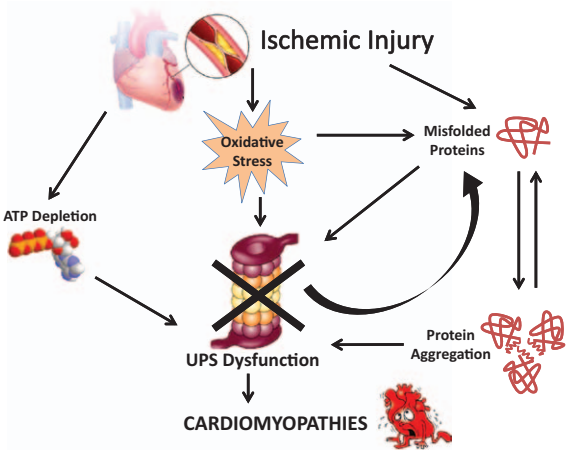

Figure 3. Hypothetical model of Ubiquitin Proteasome System (UPS) dysfunction after ischemia-reperfusion injury. Ischemia-reperfusion injury impairs proteasome function through 3 main mechanisms. First, the production of oxidative species during ischemia may result in the modification of proteasome subunits; second, ischemia induces ATP depletion, thereby inhibiting proteasome function; finally, ischemic and oxidative stress lead to damaged, misfolded proteins that impair UPS function; UPS dysfunction in turn favors the accumulation of misfolded proteins creating a vicious cycle of increased protein production and reduced degradation. Furthermore, misfolded and oxidized proteins have a proclivity to form aggregates that make them resistant to proteolysis. UPS dysfunction secondary to the accumulation of misfolded and aggregated proteins finally leads to the development of cardiomyopathies.

Such a hypothesis is supported by in vitro experiments showing that proteasome subunits are sensitive to a broad variety of oxidants, particularly in the 26S complex.[67,68] Moreover, antioxidants such as α-tocotrienol act as powerful cardioprotective agents, shown to preserve proteasome function in experimental models of ischemia reperfusion.[69] Second, it is well known that ischemia induces ATP depletion, thereby inhibiting proteasome function, which depends on ATP. An intriguing hypothesis posits that physiological ATP levels may negatively regulate proteasome function under normal circumstances.[70] Powell et al[62] reported that the optimal ATP concentration for in vitro proteasome activity is <100 μmol/L and that higher ATP concentrations inhibit proteasome peptidase activities. On the other hand, it is well known that the physiological intracellular ATP concentration in many cell types is greatly higher (by a factor >10) than such values.[71] Finally, protein aggregates have been proposed to inhibit the UPS. Although the UPS is involved in the degradation of oxidized proteins, heavily oxidized proteins have a proclivity to form aggregates that make them resistant to proteolysis. As pointed out in previous sections, it is well established that the accumulation of misfolded and abnormal proteins may impair UPS function and lead to the development of cardiomyopathies. Ischemia thus results in the accumulation of misfolded and mutated proteins, which aggregate in a vicious cycle characterized by increased protein production and reduced degradation.

A recent study by Li et al[64] has elegantly confirmed the working hypothesis that proteasome functional insufficiency (ie, impairment of proteasome function or increased demand above the functional capacity of the proteasome) plays a pivotal role in myocardial ischemia-reperfusion injury. In mice, cardiomyocyte-restricted overexpression of the 11S proteasome (a model with enhanced proteasome function) resulted in significantly reduced infarct size and postreperfusion myocardial dysfunction. Moreover, when crossed with mice suffering from desmin-related cardiomyopathy, animals with 11S overexpression displayed a marked decrease of aberrant protein aggregation, associated with reduced myocardial hypertrophy and prolonged lifespan. Interestingly, Tian et al[72] recently found that genetically induced moderate inhibition of the proteasome in cardiomyocytes aggravated myocardial ischemia-reperfusion injury, suggesting that the protective effect of proteasome inhibition might result from its action on the noncardiomyocyte compartment.

As with MR, it is possible that proteasome inhibition may be beneficial under some experimental conditions although

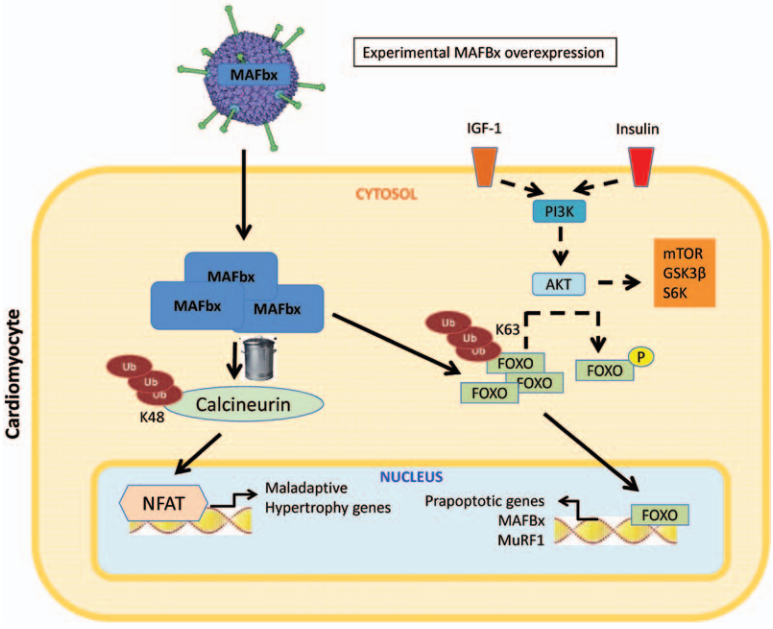

Figure 4. Atrogin/MAFbx overexpression, through adenoviral gene delivery, inhibits in cardiomyocytes both physiological and pathological hypertrophy by different mechanisms. To attenuate pathological hypertrophy, Atrogin/MAFbx mediates the degradation of Calcineurin, a phosphatase that positively regulates hypertrophy, through K48 linked chains of ubiquitin. To attenuate physiological hypertrophy, Atrogin/MAFbx activates the activity of FOXO transcription factors through mediating the ubiquitination of K63 linked chains.

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not in others. Whereas partial or short-term proteasome inhibition may by beneficial by limiting the inflammatory response, total proteasome inhibition, particularly under circumstances in which the UPS is already dysfunctional, may lead to cell death.[32] Pending a definitive answer, caution in the use of proteasome inhibitors in ischemia-reperfusion is sensible, especially because several clinical studies describe cardiotoxicity secondary to bortezomib treatment for multiple myeloma.[73,74]

Cardiac-Specific Proteasomes

The 26S proteasome comprises the catalytic 20S core particle and the regulatory 19S cap particle. The 19S cap is composed of 6 ATPases (Rpt1-6), providing access to the catalytic core, and 12 non-ATPase (Rpn) subunits. PA28, otherwise known as the 11S particle, is an ATP-independent activator of the 20S particle. The different 11S complexes have different subcellular localizations. The catalytic complex contains caspase-like, trypsin-like, and chymotrypsin-like peptidase activities within its β1, β2, and β5 subunits, respectively. These catalytic subunits can be substituted with inducible β-subunits, called β1i, β2i, and β5i, in response to immune stimulation. Interestingly, in many forms of cardiomyopathies, including ischemia-reperfusion injury and diabetic cardiomyopathy, inducible subunit expression is significantly increased.[75–77]

Table. Specific UPS Components Identified in the Heart

Name	Substrates and Function	Reference
E1, ubiquitin activating enzyme		20
E2, ubiquitin conjugating enzymes		20
E2N		141
E2I		142
E2D		143
E2G2		144
E3, ubiquitin ligases		
LNX, ligand of Numb protein	Numb, (↑Notch signaling)	118
Itch	Notch, (↓inhibition, c-Jun)	123,124
FBW7/Sel-10	JNK signaling	119,120
MuRF1	JNK signaling	96,145
MIB	Notch signaling	121,122
pVHL E3 complex	HIF-1α	131,146
CHIP	p53, ERα	107–110,147
Cullins	CRL functioning	111
MDM2	FoxO signaling	19,104–106
Atrogin1/MAFbx	calcineurin, p53, JNK, FoxO signaling	35,36,90
Nedd4	VEGF, Na^+ and K^+ channels	126–130
Skp2	FoxO signaling	113–116
Siah2	Fis1/Drp1, mitochondrial fission	134

In the last decade, multiple components of the UPS have been identified in the heart. Most of the available data come from human biopsies and have identified the presence of the E1, several E2s, and several E3 enzymes. The exact role of the various E3s is far from being fully elucidated.

In fact, the mammalian cardiac proteasome is molecularly heterogeneous,[78] and its functional regulation differs from proteasomes in other tissues and from yeast cells.[79] This relates mainly to tissue specific expression of several proteasome components (eg, an alternatively spliced isoform of Rpn10 within the 19S complex).[76] A recent study by Kloss et al[80] has classified different proteasomal subpopulations based on their susceptibility to proteasome inhibitors, categorizing rat heart proteasomes into their spectrum of subtypes. It has been suggested that the 11S and PA200 complexes do not play a major regulatory role in the heart under basal conditions (differing from other organs), but do in conditions of stress, such as experimental diabetes mellitus.[76,77] Furthermore, the β1i-subunit of the 20S subcomplex plays an important role in the heart, because its deficiency exacerbates ischemia-reperfusion damage in mice.[75] Lastly, the β1i-knockout results in an accumulation of oxidized proteins and a reduction in proteasome activity, suggesting β1i may influence the cardiac proteasome's response to oxidative stress.

Degradation Pathways Involved in Heart Biology

Given the complexity of the UPS system, it is reasonable to expect that in the upcoming years, many new substrates and UPS components will be identified in the heart. This section will discuss several of the currently known cardiac E3 ligases and substrate pathways. The Table summarizes the UPS components (E1, E2, E3, and substrates) that have been identified in the heart at this time.

Structural Proteins: The Sarcomere

In addition to protein quality control by the UPS, recent evidence has uncovered an unprecedented role for the UPS in the turnover of long-lived sarcomeric proteins, although very little specific information is available. Studies using proteasome inhibitors[81] and graded steady-state reduction of intracellular ATP content[82] showed that the UPS mediates the degradation of myofibrillar proteins in mammalian cells. Soluble myofibrillar proteins, including myosin, actin, and troponin, are degraded by the UPS, whereas their association in a multi-component complex protects them from entering the UPS cycle.[83] More specifically, myosin heavy chain is degraded by the proteasome in cultured neonatal myocytes,[84] and cardiac troponin I is degraded by muscle-specific RING finger 1 (MuRF1; see below), which also reduces contractility in vitro.[85] More recently, Haghikia et al[86] linked the downregulation of Ube2i and Ube2g1 to the disruption of the cardiomyocyte sarcomere structure after STAT3 disruption. Very recently, the previously uncharacterized cardiac-specific F-box protein, Fbxl22, was shown to promote the proteasome-dependent degradation of key sarcomeric proteins, such as α-actinin and filamin C.[87]

Atrogin/MAFbx

Atrogin (also known as muscle atrophy F-box, MAFbox) is a muscle and heart-specific F-box E3 ligase, which appears to be a fundamental regulator of myocardial remodeling. It was

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initially shown to have a pivotal role in skeletal muscle atrophy, induced during skeletal muscle atrophy and in other catabolic states.[88,89] It is part of a family of biochemically related molecules, named atrogenes, encoding proteins involved in protein degradation, which are regulated during the process of skeletal muscle atrophy. Pioneering studies coming from the Patterson laboratory first showed the role of Atrogin/MAFbx in MR; high levels of Atrogin/MAFbx suppressed cardiomyocyte hypertrophy induced both by adrenergic stimulation in vitro and by pressure overload in vivo.[35] Atrogin/MAFbx inhibits pathological hypertrophy through its regulation of the ubiquitin-dependent degradation of calcineurin, a calcium-activated phosphatase that is required for pathological hypertrophy[35] (Figure 4). Calcineurin is activated by stimuli such as pressure overload, and adrenergic agents, resulting in pathological hypertrophy, along with fibrosis, chamber dilation, and heart failure.

Atrogin/MAFbx also inhibits physiological, adaptive hypertrophy. Physiological hypertrophy does not depend on calcineurin, but instead depends on the activation of Akt signaling, which increases protein synthesis and transcription of cardiac genes that promote hyptertrophy.[36] Atrogin/MAFbx inhibits physiological hypertrophy by enhancing the activity of the forkhead transcription factors, FoxO1 and FoxO3a, members of a family of transcription factors that activate genes involved in muscle wasting, including Atrogin/MAFbx itself. Intriguingly, Atrogin/MAFbx mediates the ubiquitination of K63-linked chains of ubiquitin onto the FoxO transcription factors, rather than canonical K48-linked ubiquitin chains, which typically regulate protein degradation.[36] It will be interesting to determine how Atrogin/MAFbx regulates these 2 distinct types of ubiquitination chains. Furthermore, it will be interesting to determine the mechanism by which K63-linked ubiquitin chains can stimulate the transcriptional activity of FoxO transcription factors.

Another substrate regulated by Atrogin/MAFbx is IκB-α. In contrast to other reports showing that Atrogin/MAFbx inhibits hypertrophy, Usui et al[90] recently reported that endogenous Atrogin/MAFbx mediates pressure overload–induced (maladaptive) cardiac hypertrophy through regulation of nuclear factor κB. Specifically, an Atrogin/MAFbx knockout mouse displayed attenuated MR after pressure overload, thereby improving the clinical phenotype with less lung congestion, interstitial fibrosis, and better LV function. This was associated with the stabilization of IκB-α and inactivation of nuclear factor κB.

Several outstanding issues remain regarding the function of Atrogin/MAFbx.[91] First, how is it possible to reconcile the observation that both the knock-out and the overexpression of Atrogin/MAFbx lead to MR and attenuation of progression to heart failure? Second, how can the same ubiquitin ligase lead to atrophy in skeletal muscle, while leading to hypertrophy in cardiac muscle? Third, does Atrogin/MAFbx actually mediate both adaptive and maladaptive growth? Lastly, it is unknown which transcription factor(s) triggers Atrogin/MAFbx expression during hypertrophy; FoxO3 is not a candidate because its expression does not coincide with Atrogin/MAFbx induction.[91]

MuRF1

MuRF1 is a sarcomere-associated protein present in cardiac and skeletal muscle whose levels, like those of Atrogin/MAFbx, increase with muscle atrophy. MuRF1 levels increase in human cardiac tissue isolated from patients undergoing therapeutic atrophy after placement of a LV assist device as treatment to decrease hemodynamic overload.[92] MuRF1 activity is also upregulated in failing hearts, and high levels of MuRF1 in mouse hearts increase the likelihood of heart failure.[93] Low levels of MuRF1 and Atrogin/MAFbox remote from the site of infarction are speculated to permit hypertrophy in these areas.[94] MuRF1 not only activates antihypertrophic pathways within the myocardium, it also mediates cardiac atrophy in vivo. In fact, it was demonstrated in 2 different models of cardiac atrophy (dexamethasone-induced and regression of hypertrophy after TAC) that MuRF1 KO mice were more resistant than wild-type mice to hypertrophy regression.[92] Finally, using a gain-of-function approach in mice, it was demonstrated that MuRF1 overexpression induces a broad array of metabolic abnormalities, leading to increased susceptibility to heart failure after TAC.[93] Interestingly, it appears that the inhibitory effect of MuRF1 on myocardial hypertrophy depends on the stimulus, as MuRF1 only prevented maladaptive pathological hypertrophy in vivo but not adaptive physiological hypertrophy.[95]

MuRF1 acts as an E3 ligase targeting the cardiac troponin I (the contractile protein) for degradation through a RING finger–dependent mechanism, thereby reducing contractility in vitro.[85] More recently Li et al[96] showed that MuRF1 protects against ischemia-reperfusion injury through its role in proteasome-dependent degradation of phospho-c-Jun. In 2 ex vivo models of ischemia-reperfusion (cultured cardiomyocytes and isolated hearts), MuRF1 conveyed cardioprotection in ischemia-reperfusion injury that was attenuated after JNK inhibition. MuRF1 also inhibits PKCε-dependent signaling in a proteasome-independent manner, inducing an antihypertrophic signaling pathway.[95]

Murine Double Minute 2

Murine double minute 2 (MDM2) is an ubiquitin ligase that mediates the degradation of p53.[19] A recent study by Birks et al[97] demonstrated a pathophysiological link between dysregulation of the UPS and increased p53 levels in MR, specifically in dilated cardiomyopathy. Thus, the hypothesis has been put forward that p53 is elevated in MR as a result of the dysregulation of UPS components that govern its stability, like MDM2. This finding is particularly relevant in view of the multifaceted role of p53. In addition to its well-known role as central regulator of the stress response, p53 is involved in a broad variety of processes that are related to cardiovascular functions including cell growth, angiogenesis, and apoptosis. p53 is part of a group of molecules upregulated in conditions of hemodynamic overload leading to myocardial hypertrophy.[98] p53 levels are elevated in human hearts from patients with chronic heart failure, secondary to dilated cardiomyopathy,[99] and its inhibition restores cardiac function by promoting angiogenesis. A p53 target gene, PUMA, might be a critical component of the apoptotic signaling pathways leading to MR and heart failure.[100] Cell stress results in increased p53

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levels by removing MDM2 from p53, allowing its levels to increase so that it can activate transcription.[101] In the heart, if MDM2 is inactivated, p53 levels increase, resulting in enhanced ischemia-repurfusion injury, apoptosis, and reduction in left ventricular function.[102] Reduced Mdm2 expression sensitized hearts to IR damage, whereas overexpression led to cardioprotection through prevention of p53-induced apoptosis.[102] MDM2 is also a critical regulator of apoptosis repressor with caspase recruitment domain (ARC), which like p53 regulates cell growth and apoptosis.[103] In mice, MDM2 is upregulated by both oxidative stress and TAC-induced dilated cardiomyopathy, which correlate with reduced ARC levels, suggesting that therapies targeting MDM2 could prevent cardiomyocyte apoptosis. MDM2 also regulates cardiovascular FoxO signaling.[104] Specifically, the Akt-MDM2 pathway acts to regulate endothelial cell FoxO1 levels by ubiquitination and degradation, illustrating a potential mechanism underlying the pathophysiological upregulation of FoxO1 under ischemic conditions.[105] Moreover, MDM2 ubiquination plays an important role in the degradation of β2-adrenergic receptor and β-arrestin, regulating mammalian G protein–coupled receptor function.[106] MDM2 induction potentially represents a cardioprotective response to oxidative stress because it is transactivated in response to H_20_2.[104]

C Terminus of Hsc70-Interacting Protein

In addition to MDM2, other ubiquitin ligases control p53 levels and activity. Through expression screening, Naito and colleagues discovered that CHIP (C terminus of Hsc70-interacting protein) is an endogenous p53 antagonist in the heart.[107] CHIP acts as cochaperone by binding to damaged proteins in concert with Hsp70 or Hsp90, mediating their refolding or their degradation. CHIP integrates the stress responses at multiple levels and appears to be required for maximal cardioprotection after myocardial infarction.[108] CHIP (–/–) mice experienced larger infarct size, increased incidence of arrhythmias, and decreased survival compared with normal littermates when subjected to in vivo coronary occlusion. Moreover, CHIP suppresses p53 levels by targeting it for UPS-mediated degradation. Cellular stresses, such as hypoxia, downregulated CHIP and led to p53 accumulation, whereas CHIP overexpression in vivo prevented p53 accumulation after myocardial infarction.[107] Such an antip53 approach was associated with prevention of myocardial apoptosis and improved LV remodeling. In addition to p53, CHIP also mediates the degradation of myocardin, a key transcription factor of serum response factor, thereby decreasing smooth muscle cell differentiation,[109] and mediates the degradation of FoxO1 (as does Atrogin/MAFbx). Overexpression of CHIP represses FoxO1-mediated transactivation and its proapoptotic function after tumor necrosis factor-α treatment, whereas CHIP knockdown enhances FoxO1-mediated transactivation and its effect on SMC proliferation and survival.[110] Thus, the inhibition of CHIP may serve as a potential therapeutic target for reducing proliferative arterial diseases.

SCF Complex

SCF ligases are generally implicated in hypertrophy, because cardiac-restricted depletion of 1 subunit of the COP9 signalosome, Csn8, induces cardiac hypertrophy and subsequent congestive heart failure, followed by premature death in mice. The COP9 signalosome complex regulates the CRL ligase activity via removing NEDD8 from NEDD8-conjugated Cullins, thereby compromising SCF activity and leading to cardiomyocyte necrotic death.[111] Csn8 is also required for autophagosome maturation in the heart, and impaired autophagosome maturation leads to cardiomyocyte death.[112]

SKP2 is another member of the F-box protein family. In addition to its well-documented role as an oncogene in cell systems mainly involved in the pathogenesis of lymphomas,[8] recent evidence has shown that SKP2/FBXL1 may regulate both vascular smooth cell[113] and cardiomyocyte proliferation.[114] Specifically, impaired SKP2–dependent p27 degradation is related to loss of cardiomyocyte proliferation, whereas overexpression of SKP2 enhances the proliferation of cells induced by Cyclin D1 expression or cyclin-dependent kinase 4 expression, improving cardiomyocyte proliferation and postischemic performance in vivo.[115] Of note, the regulatory role of SKP2 has also been implicated in the proliferation of cardiac fibroblasts through p38 MAPK.[116]

E3-Ligases Within the Notch Pathway

Notch signaling is involved in determining the fate of multiple cell types, including endothelial cell sprouting during embryonic vascular development,[117] and the UPS regulates the turnover of many Notch-signaling components. Activation of Notch receptors results in proteolytic cleavage of the receptor at 2 distinct sites. Positive regulation of Notch signaling is achieved by the ligand of numb-protein X, LNX, by mediating the ubiquitination and degradation of Numb, a notch antagonist.[118] Additionally, the mind bomb (MIB) family of ubiquitin ligases are essential for Notch signaling by mediating the internalization ubiquitination and degradation of membranous Delta.[109] Conversely, several E3 ligases for Notch have been identified. Fbxw7 targets Notch to the proteasome for degradation in the vasculature,[119] in addition to its well-characterized negative regulation of apoptotic JNK signaling in neurons.[120] Additionally, Itch/AIP4 (mice lacking Itch exhibit severe itching) are capable of targeting Notch for degradation,[121,122] inhibiting its downstream signaling.[123] Itch has also been involved in JNK signaling, specifically targeting c-Jun for its degradation in a ubiquitin-mediated manner.[124] Indeed, several lines of experimental evidence recently reviewed by Portbury et al[125] support the concept that both ubiquitination and SUMOylation regulates JNK signal transduction, and that several proteins with ubiquitin ligase activity are involved in cardiovascular JNK signaling.

Nedd4

A key signaling pathway within the cardiovascular system that is regulated by the UPS is vascular endothelial growth factor (VEGF). Important processes, including angiogenesis, permeability, cell proliferation, and survival, are regulated by the VEGF signaling pathway. VEGF receptor 2 degradation is regulated by Nedd4, a HECT domain–containing E3 ligase, in association with Grb10, an adaptor protein, although the exact molecular mechanism is still unclear. Specifically, Grb10 appears to act as a positive regulator of VEGF receptor 2 signaling and protects VEGF receptor 2 from degradation by interacting

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with Nedd4.[126] In addition to its role in the modulation of VEGF, Nedd4 is also known to play a pivotal role in cardiac excitation-contraction coupling. Specifically, it appears to regulate both the cardiac voltage-gated Na^+ channel,[127] and both KCNQ1 and hERG1 potassium channels in cardiomyocytes.[128,129] Recently, Nedd4-1 has been shown to be required for heart development, because knock-out of Nedd4-1 was associated with embryonic lethality and pronounced heart and vasculature abnormalities.[130]

Von Hippel-Lindau

The UPS regulates the degradation of hypoxia-inducible factors (HIFs), transcriptional activators that regulate genes encoding proteins involved in cell survival during hypoxia, including VEGF.[131] Under normoxic conditions, HIF1 is hydroxylated by the prolyl-4-hydroxylase domain proteins (PHDs). Hydroxylation is required for the recognition and degradation of HIF1 by the ubiquitin ligase, von Hippel-Lindau. This degradation ceases under conditions of hypoxia, where hydroxylation of HIF1 no longer occurs. HIF1 is required for the induction of angiogenic factors and vascular growth after pressure overload and is required to prevent the development of cardiac hypertrophy. However, HIF1 activity is repressed by p53 after sustained pressure overload, resulting in hypertrophy and cardiac failure. Thus, the antiangiogenic properties of p53 act through inhibition of HIF1 to play a crucial role in the transition to heart failure.[132]

Siah2

The ubiquitin ligase, Siah2, also controls HIF1α availability through its regulation of the stability of prolyl hydroxylases (PHDs) 1 and 3 under hypoxic conditions.[133] Kim et al[134] recently reported that Siah2 is a key regulator of hypoxia-induced mitochondrial fission and plays a relevant role in ischemic injury through the modulation of Fis/Drp1 complex, in particular contribution by Siah2 to mitochondria function occurs through its regulation of the stability of A-kinase anchoring protein 121 (AKAP121).

Therapeutic Intervention of the UPS System in Heart Disease

Tumor cells are sensitive to proteasome inhibition, making the proteasome an ideal target for the development of anticancer therapies. Indeed, there is ongoing research to identify novel proteasome inhibitors. Bortezomib, a highly selective, reversible inhibitor of the 26S proteasome, is already available for treatment of multiple myeloma and mantle cell lymphoma. Other inhibitors that act on different components of the proteasome are also currently being developed and tested.[135] The evidence summarized in this review suggests that proteasome inhibitors represent an attractive and novel approach for the treatment of cardiac diseases, including hypertrophy, heart failure, or ischemia reperfusion. However, considering that both the inhibition and the enhancement of proteasome function are potentially able to confer cardioprotection, several questions remain to be answered in this relatively new field. As previously mentioned, it appears that the extent of proteasome inhibition determines whether there is cardioprotection or cardiotoxicity. Because the UPS is involved in essential cellular processes, inhibition of enzymes common to the entire pathway, as is the case with the proteasome, may nonspecifically impair many processes and give rise to toxicity, particularly in long-term therapies.[73,74]

Targeting the specific myocardial E3 ligases (discussed within this review) could theoretically represent another UPS-based cardiological therapy. In this regard, small molecules capable of binding and inhibiting specific E3s have been developed, such as specific phosphopeptide derivatives that span the phosphorylation targeting domains in different substrates, which can serve as baits to the respective E3s.[136,137] A better approach may be the development of small molecules that are substrate-specific and bind, preferentially, to specific substrates or to their ancillary proteins rather than to an E3. However, at this time, these approaches are limited by the absence of structural information pertaining to the interface between E3 and substrate. Intensive research is needed to establish how the cardiac proteasome system differs from the proteasome system in other organs, and careful vigilance for extracardiac effects will be critical.

A final intriguing treatment strategy involves the use of cyclin-dependent kinase inhibitors to treat heart disease, exploiting the shared signaling pathways driving both cancer and myocardial hypertrophy. It has recently been demonstrated that the degradation of p27, a cyclin-dependent kinase inhibitor, plays a critical role in cardiac remodeling by mediating the pathological growth of cardiomyocytes.[138] Because it is well known that p27 levels are controlled by the SCF-SKP2 ubiquitin ligase, manipulation of p27 protein levels by enhancing its signaling[139] or inhibiting its degradation using specific inhibitors[140] could be beneficial in the treatment of heart failure.

Conclusions

The UPS regulates fundamental cell functions including mitosis, DNA replication and repair, cell differentiation and transcriptional regulation, and receptor internalization, which all play a role in heart biology. Looking to the future, it is possible to envision the identification of an ever-increasing number of substrates of the UPS and their specific E2/E3 complexes in the heart. From a therapeutic standpoint, it will be critical to modulate the UPS to function within an optimal zone, where the target of cardioprotection is reached, while retaining the critical role of basal UPS-dependent protein quality control. In the near future we can expect the development of novel therapeutic agents capable of optimal modulation of the UPS for cardioprotection. Major challenges remain, but patients with heart diseases are likely to benefit from these efforts.

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