Published in final edited form as:

Future Cardiol. 2008 ; 4(1): 65–75. doi:10.2217/14796678.4.1.65.

Appetite for destruction: E3 ubiquitin-ligase protection in cardiac disease

Monte S Willis, MD, PhD,

University of North Carolina, Department of Pathology & Laboratory Medicine, Carolina Cardiovascular Biology Center, 2340B Medical Biomolecular Research Building, Chapel Hill, NC 27599–7525, USA, Tel: +1 919 843 1938; Fax: +1 919 843 4585; monte_willis@med.unc.edu

Jonathan C Schisler, PhD, and

University of North Carolina, Division of Cardiology & Carolina Cardiovascular Biology Center, 8200 Medical Biomolecular Research Building Chapel Hill, NC 27599–7126, USA, Tel: +1 919 843 2757; Fax: +1 919 843 4585; schisler@med.unc.edu

Cam Patterson, MD[†]

University of North Carolina, Division of Cardiology & Carolina Cardiovascular Biology Center, 8200 Medical Biomolecular Research Building Chapel Hill, NC 27599–7126, USA, Tel: +1 919 843 6477; Fax: +1 919 843 4585; cpatters@med.unc.edu

Abstract

Over the course of 3 billion heartbeats in an average human lifetime, the heart must maintain constant protein quality control, including the coordinated and regulated degradation of proteins via the ubiquitin-proteasome system (UPS). Recent data highlight the specificity by which the UPS functions in the context of cardiac hypertrophy, ischemic heart disease and cardiomyopathies. Although curbing the appetite of the proteasome through the use of inhibitors in animal models of cardiac disease has proven effective experimentally, recent studies report proteasome inhibition as being cardiotoxic in some patients. Therefore, focusing on specific regulatory components of the proteasome, such as members of the E3 ubiquitin-ligase family of proteins, may hold promise for targeted therapeutics of cardiac disease. This review focuses on the UPS, its specific role in cardiac disease and opportunities for novel therapies.

Keywords

atrogin-1; bortezomib; cardiac; CHIP; hypertrophy; ischemia; MDM2; MuRF1; reperfusion; VelcadeTM

Cardiac proteins are in a dynamic state of flux, where a balance of continual degradation and synthesis occurs in a highly regulated and specific manner. This balance is pivotal to cellular function both at steady state and in response to stress. Most long- and short-lived proteins are degraded by the ubiquitin-proteasome system (UPS), representing over 80% of all intracellular

[†]Author for correspondence University of North Carolina, Division of Cardiology & Carolina Cardiovascular Biology Center, 8200 Medical Biomolecular Research Building, Chapel Hill, NC 27599-7126, USA, Tel.: +1 919 843 6477; Fax: +1 919 843 4585; E-mail: cpatters@med.unc.edu.

Financial & competing interests disclosure: The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

proteins [1]. These proteins not only include structural proteins turned over owing to wear and tear, but also regulatory proteins involved in signaling pathways that mediate common cardiac pathologies. The specificity of the UPS lies mainly in the E3 ubiquitin ligases, which recognize proteins and target them for degradation by the 26S proteasome. Therapeutically, experimental evidence suggests that nonspecific inhibition of the 26S proteasome may protect against ischemic heart disease [2–7]. However, there are concerns because of cardiotoxicity identified in cancer patients treated with the US FDA-approved proteasome inhibitor, bortezomib (Valcade™). In this review, we will focus on the very recent discovery of multiple E3 ubiquitin ligases that modulate cardiac disease experimentally, and represent more specific therapeutic targets than is currently being proposed using proteasome inhibition to ultimately improve outcomes of cardiac disease.

Ubiquitin-proteasome system: the specificity is in the E3 ubiquitin ligases

The UPS utilizes a series of enzymes to construct covalently linked polyubiquitin chains on lysine residues of targeted protein substrates, which then ‘mark’ these proteins for degradation (see Figure 1A for detailed description). Eukaryotic cells express a single ubiquitin-activating enzyme (E1) that activates free ubiquitin for subsequent transfer to one of approximately 50 ubiquitin-conjugating enzymes (E2). Ubiquitin-protein ligases (E3) directly interact with both E2 and the substrate proteins, mediating the transfer of the ubiquitin molecule to the targeted protein. The substrate specificity of the ubiquitination process occurs at the level of the E3 ubiquitin ligases, which bind directly to the protein that is targeted for degradation. For protein substrates to be degraded by the 26S proteasome, the ubiquitination process is repeated, adding additional ubiquitin molecules via a thioester bond to lysine-48 on a preceding ubiquitin molecule (Figure 1B). The resulting polyubiquitinated proteins are then recognized by the 26S proteasome. The 26S proteasome is a multi-megadalton oligomeric complex comprised of two functional units. The 19S regulatory cap (which binds to the polyubiquitinated chains) denatures the substrate protein and passes the protein on to the second component of the 26S proteasome, the 20S barrel, which contains the proteolytic core (Figure 1A). The net result is the conversion of a polyubiquitinated protein to free ubiquitin and peptides that are subsequently used for either protein synthesis or catabolism. Currently, it is estimated that 500-600 E3 ubiquitin ligases reside in the human genome, allowing for a wide range of possible protein substrates that can be targeted for degradation by the UPS [8,9]. Recently, cardiac E3 ubiquitin ligases have been reported to regulate specific cellular processes involved in common heart diseases such as heart failure and ischemic heart disease. The E3 ubiquitin ligase carboxyl terminus of Hsp70-interacting protein (CHIP), atrogin-1 and muscle ring finger (MuRF) proteins, represent distinct molecular regulators by which the heart controls its structure, signaling processes and function. The protective effects of these proteins, outlined below, highlight how therapeutics that increase their activity may be beneficial in halting the progression of heart failure.

E3 ubiquitin ligases in heart failure & cardiac hypertrophy: regulating with specificity

Cardiac hypertrophy occurs in response to physiologic or pathologic stimuli. While physiologic hypertrophy can result from exercise, pathologic hypertrophy is an adaptive physiologic response to events such as ischemia, heart valve defects, genetic abnormalities, adrenergic overactivation and volume or pressure overload. Although the response to both types of stimuli is initially similar, the sequelae differ as pathologic hypertrophy frequently leads to heart failure, whereas physiologic hypertrophy does not. Induction of pathological left ventricular hypertrophy results in a parallel enhancement of both protein synthesis and proteolysis, the latter of which is regulated by the UPS [10,11]. Several interesting studies have begun to elucidate the exact role of the UPS in cardiac hypertrophy. Increases in ubiquitin, E1, E2 and

E3 (specifically *atrogen-1* and *MuRF1*) mRNA levels occur following the induction of cardiac hypertrophy [12]. Moreover, *atrogen-1* and *MuRF1* have been found to be transcriptionally upregulated in an *in vivo* model of chronic heart failure [13]. Whether or not this increase in *atrogen-1* and *MuRF1* expression is beneficial or detrimental in the defense against pathological hypertrophy remains to be determined. However, as will be discussed in the following sections, there is strong evidence to suggest that these molecules may be key players in inhibiting the development of cardiac hypertrophy.

Cardiac atrogen-1, like all cardiac E3 ligases elucidated to date, has generalized antihypertrophic activity, inhibiting cardiac hypertrophy induced by either pathologic or physiologic stimuli. Atrogen-1 inhibits cardiac hypertrophy induced by pathological stimuli by acting as an E3 ligase to degrade calcineurin [14], which regulates the activity of the transcription factor nuclear factor of activated T cells, which is necessary for the induction of cardiac hypertrophy [15]. As a result of targeting calcineurin for 26S proteasome-dependent degradation, atrogen-1 disrupts a critical pathway involved in adrenergic-stimulated cardiac hypertrophy both *in vitro* and *in vivo* [14,16]. Atrogen-1 also inhibits physiologically-induced cardiac hypertrophy by another mechanism. During the induction of physiologic cardiac atrophy, atrogen-1 targets the transcription factors, Foxo1 and Foxo3, for ubiquitination [17]. Surprisingly, this results in enhanced Forkhead transcriptional activity (instead of ubiquitin-mediated degradation) and subsequent inhibition of Akt-dependent induction of hypertrophy [17]. Further analysis of this process has revealed that atrogen-1 actually mediates the noncanonical addition of ubiquitin molecules via lysine-63-linked chains, instead of the traditional lysine-48 (canonical) linkages (as shown in Figure 1B) [17]. Lysine-63-linked chains have previously been associated with modulating protein function [18,19], however, the atrogen-1-mediated noncanonical ubiquitination of the Forkhead transcription factors reveals a new regulatory mechanism that exists within the heart.

The MuRF proteins are striated muscle-specific proteins that have been implicated in various aspects of contractile regulation and myogenic responses [20]. In addition to the E3 ubiquitin ligase MuRF1, there exist two other MuRF family proteins, MuRF2 and MuRF3. The MuRF family proteins are believed to generally function as mechanical stress sensors, given their localization in the sarcomere and microtubules, as well as their direct and indirect interactions with the giant protein, titin [21–23]. MuRF1 appears to be a key player in the regulation of cardiac hypertrophy, probably through multiple mechanisms. In cardiac muscle *in vitro*, MuRF1 ubiquitinates and degrades the sarcomeric protein, cardiac troponin I, while also inhibiting signaling through protein kinase C ϵ [24,25]. *In vivo*, MuRF 1 regulates the induction of pressure overload-induced cardiac hypertrophy, likely through its direct interaction with the serum response factor (SRF) [26]. By contrast, while *in vitro* studies have similarly linked MuRF2 and SRF activity [27], recent data indicate that MuRF2 does not play a role in the induction of pressure overload-induced cardiac hypertrophy [26]. The extent to which the microtubule-associated MuRF3 plays a role in cardiac hypertrophy has not yet been elucidated.

E3 ubiquitin ligases protect against ischemic heart disease

The common theme to ischemic injury is the induction of cardiomyocyte apoptosis, of which p53 is a key regulator. The transcriptional target genes of p53 include B-cell lymphoma (BCL)-2-associated X protein (BAX), p53-upregulated modulator of apoptosis (PUMA) and apoptotic protease-activating factor (APAF)-1, each of which contribute to apoptosis in distinct and complementary ways (as outlined in Figure 2). The stability of p53 is countered by multiple cardiac E3 ubiquitin ligases, including CHIP, murine double minute (MDM)2 and MDM4, which degrade p53 in a 26S proteasome-mediated manner. The cardioprotective effects of these E3 ligases are believed to be caused, in part, by their interaction with p53 and the subsequent inhibition of cardiomyocyte apoptosis.

CHIP & MDM2: E3 ubiquitin ligases & the regulation of apoptosis in ischemia-reperfusion injury

The E3 ligase/cochaperone CHIP, a protein highly expressed in cardiac muscle, is characterized by its ability to bind heat-shock proteins (Hsc70-Hsp70 and Hsp90) and ubiquitinate-specific proteins, targeting them for degradation by the 26S proteasome. CHIP is integral in protecting the heart against ischemia-reperfusion (I/R) injury as evidenced by studies carried out in CHIP^{-/-} mice. When mice lacking CHIP undergo cardiac I/R injury, a 50% greater incidence in infarction is identified compared with controls [28]. Furthermore, during reperfusion, CHIP^{-/-} mice suffer an increased incidence of arrhythmias, suggesting a heightened degree of damage compared with wild-type mice. Enhanced cardiomyocyte apoptosis is also identified in CHIP^{-/-} mice following I/R injury, indicating that CHIP inhibits apoptosis during cardiac stress. Although the exact mechanism behind CHIP's antiapoptotic actions is not fully elucidated, recent studies have identified that CHIP interacts with both the wild-type and mutant forms of p53, suppressing its aggregation and inducing 26S proteasome-dependent degradation of p53 in response to stress [29]. MDM2 is an E3 ubiquitin ligase that regulates the degradation and activity of proteins involved in cell growth and apoptosis, such as p53 and apoptosis repressor with caspase recruitment domain (ARC). MDM2 inhibits p53 transcriptional activity by promoting its ubiquitination and proteasomal degradation [30–32]. In cardiomyocytes, MDM2 promotes survival by reducing p53 levels, thereby inhibiting apoptosis during pathologic stress [33]. Inactivation of MDM2 results in elevated cardiomyocyte p53 levels and an increase in I/R-induced apoptosis and reduced left ventricular function [33]. In addition to its actions on p53, MDM2 also regulates ARC protein turnover in an ubiquitin-proteasome-dependent pathway, a process that has been shown to be a significant inhibitor of cardiomyocyte apoptosis [34]. Recently, MDM4, an MDM2 homolog, was also shown to inhibit p53 in cardiomyocytes. Using a conditional knockout, it was found that decreased cardiac MDM4 levels resulted in the development of a dilated cardiomyopathy [35]. A p53-dependent loss of cardiomyocytes was the underlying cause, demonstrating that MDM4 inhibits p53 activity in fully differentiated cardiomyocytes. While MDM4 may have overlapping function with MDM2, its role in I/R injury has not been tested to date.

MuRF3: regulation of sarcomeric protein turnover in ischemia-reperfusion injury

The MuRF3 is a muscle specific RING-finger protein originally identified for its interaction with microtubules and its role in skeletal myoblast differentiation [36]. Recent work has expanded these findings to the heart, and identified that mice lacking MuRF3 display abnormal sarcomere structure and respond dramatically to myocardial infarction (MI). In response to MI, left ventricular dilation, myocyte degeneration and cardiac rupture occur within days [37]. Interestingly, the first substrates of MuRF3 in this study were found to be the four-and-a-half LIM domain protein (FHL2) and γ -filamin, both of which accumulated in the hearts of mice lacking MuRF3. The role of MuRF3 in the integrity and function after MI suggest that the normal turnover of FHL2 and γ -filamin contributes to protection against acute MI [37]. MuRF3 (in addition to MuRF1) also regulates the turnover of key contractile proteins of the sarcomere, including β /slow myosin heavy chain and MHC IIa [38]. The cooperative nature of MuRF1 and MuRF3 is evident in mice deficient in both proteins who develop a skeletal muscle myopathy and hypertrophic cardiomyopathy [38]. MHC accumulation, myofiber fragmentation and diminished muscle performance are also observed, revealing a key role for MuRF1 and MuRF3 in the UPS-dependent turnover of sarcomeric proteins and a potential mechanism of myosin storage myopathies [38].

Pharmacologic 26S proteasome inhibition

Ischemic heart disease

The evidence that has been presented so far demonstrates that E3 ubiquitin ligases are cardioprotective by multiple mechanisms including:

- Cochaperone activity
- Transcriptional coactivation
- Targeting specific proteins for degradation by the 26S proteasome

However, there is also a body of work that suggests that partial or site-specific inhibition of the 26S proteasome can also be protective against I/R injury, suggesting a complex and sometimes contrary role for the UPS during I/R injury.

Inhibition of the 26S proteasome during experimentally induced cardiac I/R injury results in significantly reduced infarct size, in some cases greater than 50%, as well as improved left ventricular function [2–7]. This cardioprotective effect may be due to the induction of heat-shock proteins and/or the inhibition of nuclear factor (NF)- κ B activity. Inhibiting NF- κ B prevents the induction of apoptosis by inhibiting NF- κ B-induced inflammatory intermediates, such as TNF- α (Figure 3) [4–6]. Partial inhibition of NF- κ B as a therapy for cardiac I/R injury makes sense given that anti-NF- κ B activity is a hallmark of a number of drugs (including aspirin, statin compounds and adenosine) used in the treatment of acute coronary syndromes [39–41]. When given therapeutically to patients, the dose of 26S proteasome inhibitors are adjusted to blunt a maximum of 80% 26S proteasome activity [42]. This allows vital cellular processes (such as those described above for E3 ubiquitin ligases that mediate proteasome-dependent degradation) to proceed while still inhibiting NF- κ B and other, as yet undescribed, protective effects.

The first 26S proteasome inhibitor to be identified, bortezomib, was approved by the FDA in 2005 for the treatment of multiple myeloma resistant to multiple therapies [43]. Bortezomib binds the catalytic site of the 26S proteasome with high affinity and specificity, inhibiting its activity (Figure 1A). While this therapy has improved patient outcomes, the therapy does have side effects, most commonly including neurological symptoms and thrombocytopenia [44]. Recent studies have also identified unexpected increases in a spectrum of cardiac complications ranging from the onset of arrhythmias to heart failure [45]. Over 10% of patients (of a total of 69) experienced cardiotoxicity and had in common age greater than 60 years, at least four cycles of bortezomib therapy, and a cumulative dose of at least 20.8 mg/m² [45]. Additional reports also identified heart failure and asymptomatic arrhythmia following bortezomib therapy [46]. The underlying mechanisms of these cardiotoxic side effects associated with bortezomib are not currently known. However, it is known that inhibition of the 26S proteasome is associated with atherosclerotic plaque instability in humans, and a loss of delayed ischemic preconditioning in animals (discussed in detail in [45]). While these studies do not necessarily preclude the use of 26S proteasome inhibition in the treatment of ischemic heart disease, they do suggest that further studies need to be performed to determine appropriate doses and patient profiles before considering 26S proteasome inhibition as a form of therapy for cardiac disease.

Restenosis injury

The local application of proteasome inhibitors can inhibit experimentally induced vascular restenosis (intimal hyperplasia) [47]. The underlying pathogenesis of this process involves proliferation, inflammation and apoptosis of multiple cells types that are regulated by the UPS. Experimentally, inhibiting the 26S proteasome decreases proliferation, macrophage infiltration

and apoptosis in a balloon model of carotid restenosis in rats [47]. Local application of 26S proteasome inhibitors inhibits experimentally induced intimal hyperplasia through the inhibition of TNF- α -mediated degradation of I κ B α and I κ B β (Figure 3) [47]. More recent studies have identified that endothelial nitric oxide synthase (eNOS) expression by endothelial cells is also regulated by the UPS, such that inhibition of the 26S proteasome results in nearly a threefold increase in eNOS activity and subsequent enhanced endothelial-dependent vasorelaxation [48]. Taken together, these studies demonstrate the potential for site-directed proteasome therapy in vascular injuries associated with revascularization therapies utilized in ischemic cardiac disease. One recent study identified that chronic inhibition of the 26S proteasome can contribute to coronary atherosclerosis, illustrating the necessity of the UPS in maintaining health in the vasculature [49]. These findings further support the concept that general inhibition of the UPS by 26S proteasome inhibitors may result in detrimental side effects in nontargeted tissues.

Role of the ubiquitin-proteasome system in familial hypertrophic cardiomyopathy

The link between UPS dysfunction with several diverse groups of cardiomyopathies has recently been reviewed [50]. We focus here on more recent data involving the role of UPS in familial hypertrophic cardiomyopathy (FHC). FHC is an inherited cardiac disease affecting 0.2% of the population, transmitted as an autosomal dominant trait, and is the leading cause of sudden death in the young, overtly healthy, population [51]. Of the more than 200 mutations identified in 14 different genes, the majority encode sarcomeric proteins [52]. Most of the mutations identified in families with FHC are in the myosin-binding protein-C (cMyBP-C) [53], which is a major constituent of the thick filaments of the sarcomere. cMyBP-C has both structural and regulatory roles, and binds to myosin, titin and actin [54–57]. Recent studies have identified that naturally occurring truncated mutations in cMyBP-C are rapidly degraded by the UPS, and impair its proteolytic capacity [58]. Specifically, these frameshift mutations had markedly lower protein expression, despite mRNA levels matching wild-type cMyBP-C [58], similar to that found in humans [59,60] and transgenic mice [61,62]. Inhibition of the 26S proteasome allows expression of the truncated cMyBP-C mutant protein to wild-type levels, indicating that the ubiquitin proteasome was involved in its degradation. Interestingly, the truncated proteins were found to inhibit the activity of the proteasome, resulting in accumulation of mutated proteins [58]. This study identifies that truncated cMyBP-Cs are both substrates of the UPS, as well as inhibitors of the degradatory process, which is a common finding in protein mutations that accumulate in Alzheimer's disease [63]. The specific E3 ubiquitin ligases that regulate wild-type or mutant cMyBP-C are currently unknown. It would be of great interest to determine what the specific E3 ubiquitin ligases that are responsible for the turnover of cMyBP-C, in order to determine how they might be utilized to alter the severity and progression of FHC.

Conclusion

The UPS has evolved from its initial characterization as simply a means for cells to maintain protein homeostasis. It is now recognized as having a direct role in the regulation of cellular processes intimately involved in common cardiac diseases such as ischemic heart disease and cardiac hypertrophy. As highlighted in this review, E3 ubiquitin ligases, such as atrogin-1, CHIP and MuRF proteins, play a pivotal role in inhibiting molecular pathways underlying ischemic heart disease, cardiac hypertrophy and heart failure. However, despite the potential that E3 ligases have as therapeutic agents, more data need to be gathered in order to understand the extent to which E3 ubiquitin ligases control various cellular processes, both beneficially and detrimentally. Such focus will allow the identification of those proteins that might serve as therapeutic targets in the future.

Future perspective

We are just beginning to delineate the regulation of protein quality control and physiologic and pathological signaling processes by post-translational modifications by the UPS. To date, most of the specificity of the UPS has focused on the substrate specificity of the E3 ubiquitin ligases. However, recent studies have demonstrated that the type of ubiquitin chain (canonical vs non-canonical) added to a substrate depends on the E2 involved [64]. Moreover, these different ubiquitin chains lead to varying amounts of degradation (or protection from degradation) in the presence of 26S proteasome [64]. In the near future, more cardiac E3 ubiquitin ligases will be identified, providing additional mechanisms that can be taken advantage of to modulate cardiac disease. These findings will preclude long-term use of general inhibition of the UPS by 26S proteasome inhibitors, however, utilization of local UPS inhibition may still have a role in cardiac therapy. Moreover, specific therapy targeting E3 ubiquitin ligases and their particular signaling pathways may enhance outcomes in several common cardiac diseases.

Executive summary

The ubiquitin-proteasome system in the heart

- The ubiquitin-proteasome system (UPS) regulates all aspects of cellular processes, including the signaling pathways involved in cardiac disease.
- The E3 ubiquitin ligases, one component of the UPS, confer substrate specificity to this system.
- Carboxyl terminus of Hsp70-interacting protein (CHIP), atrogin-1 and muscle ring finger (MuRF) proteins represent distinct molecular regulators by which the heart controls its structure, signaling processes and function.

E3 ubiquitin ligases in heart failure & hypertrophy: regulating with specificity

- Atrogin-1 (MAFbx) is antihypertrophic owing to two separate mechanisms. In pathological hypertrophy, atrogin-1 targets calcineurin for proteasomal degradation, effectively inactivating the calcineurin/nuclear factor of activated T cells signaling pathway, which is necessary for the induction of cardiac hypertrophy. In physiologic hypertrophy, atrogin-1 polyubiquitinates Foxo1 and Foxo3, enhancing their transcriptional activity and inhibiting Akt-dependent cardiac hypertrophy.
- MuRF1 inhibits pathologic cardiac hypertrophy in vivo by inhibiting protein kinase C ϵ signaling, degrading cTnI and/or through direct interactions with the transcription factor serum response factor.

E3 ubiquitin ligases protect against ischemic heart disease

- The E3 ligase/cochaperone, CHIP, binds and stabilizes p53, inhibiting stress-induced apoptosis resulting from ischemic heart disease.
- The E3 ligase, murine double minute (MDM)2, promotes ubiquitination and degradation of p53, inhibiting apoptosis during pathologic stress. The related E3 ubiquitin ligase MDM4 also ubiquitinates and degrades p53.
- The E3 ubiquitin ligase MuRF3 is necessary for sarcomeric structure and the regulated turnover of sarcomeric proteins, four-and-a-half LIM domain protein and γ -filamin. Lack of cardiac MuRF3 increases susceptibility to rupture after the induction of myocardial infarction due to sarcomeric structural defects.

Pharmacologic 26S proteasome inhibition & ischemic heart disease

- 26S proteasome inhibitors (such as bortezomib [Velcade™]) reduce infarct size experimentally, likely by the inhibition of nuclear factor- κ B signaling and the induction of heat-shock proteins.
- However, over 10% of patients taking bortezomib for cancer therapy experience cardiotoxicity.
- The nonspecific targeting of 26S proteasome activity may result in increased atherosclerotic plaque instability, loss of ischemic preconditioning and loss of protein quality control mechanisms.
- E3 ubiquitin ligases specific to striated muscle offer a more direct approach to treating cardiac diseases.

26S proteasome inhibition & restenosis injury

- Local application of 26S proteasome inhibitors experimentally reduce intimal hyperplasia.
- However, experimentally, the chronic use of 26S proteasome inhibitors results in enhanced coronary atherosclerosis.
- Site-directed 26S proteasome therapy in vascular injuries associated with revascularisation therapies may be utilized in the future.

Protein quality control in familial hypertrophic cardiomyopathies

- Familial hypertrophic cardiomyopathies (FHCs) affect 0.2% of the population, and are the leading cause of sudden death.
- Common mutations in the sarcomeric protein cardiac myosin-binding protein-C (cMyBP-C) underlie this disease.
- The inability of the UPS to degrade mutant cMyBP-C or the preferential recognition of mutant cMyB-C may underlie the phenotypes identified in FHCs, and may account for associated cardiac dysfunction.

Conclusion

- E3 ubiquitin ligases specifically regulate signaling pathways to inhibit cardiac hypertrophy and ischemic heart disease.
- 26S proteasome inhibitors are cardioprotective experimentally in ischemic heart disease; however, clinical experience with 26S proteasome inhibitors in cancer patients has uncovered unexpected cardiotoxicity.
- Alternative approaches to more specifically intervene in cardiac disease include the targeting of E3 ubiquitin ligases, such as CHIP, atrogin-1 and MuRF proteins.
- Despite the potential in these molecular targets, much more data are needed outlining the extent to which E3 ubiquitin ligases control multiple cellular processes, both beneficially and detrimentally.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature* 2003;426:895–899. [PubMed: 14685250]

2. Bao J, Sato K, Li M, et al. PR-39 and PR-11 peptides inhibit ischemia-reperfusion injury by blocking proteasome-mediated I κ B α degradation. *Am J Physiol Heart Circ Physiol* 2001;281:H2612–H2618. [PubMed: 11709430]
3. Gao Y, Lecker S, Post MJ, et al. Inhibition of ubiquitin-proteasome pathway-mediated I κ B α degradation by a naturally occurring antibacterial peptide. *J Clin Invest* 2000;106:439–448. [PubMed: 10930447]
4. Luss H, Schmitz W, Neumann J. A proteasome inhibitor confers cardioprotection. *Cardiovasc Res* 2002;54:140–151. [PubMed: 12062370]
5. Pye J, Ardeshirpour F, McCain A, et al. Proteasome inhibition ablates activation of NF- κ B in myocardial reperfusion and reduces reperfusion injury. *Am J Physiol Heart Circ Physiol* 2003;284:H919–H926. [PubMed: 12424098]
6. Stangl K, Günther C, Frank T, et al. Inhibition of the ubiquitin-proteasome pathway induces differential heat-shock protein response in cardiomyocytes and renders early cardiac protection. *Biochem Biophys Res Commun* 2002;291:542–549. [PubMed: 11855822]
7. Stansfield WE, Moss NC, Willis MS, Tang R, Selzman CH. Proteasome inhibition attenuates infarct size and preserves cardiac function in a murine model of myocardial ischemia-reperfusion injury. *Ann Thorac Surg* 2007;84:120–125. [PubMed: 17588397]
8. Johnson SA, Hunter T. Kinomics: methods for deciphering the kinome. *Nat Methods* 2005;2:17–25. [PubMed: 15789031]
9. Wilkinson KD, Ventii KH, Friedrich KL, Mullally JE. The ubiquitin signal: assembly, recognition and termination. Symposium on ubiquitin and signaling. *EMBO Rep* 2005;6:815–820. [PubMed: 16113643]
10. Coleman PS, Parmacek MS, Lesch M, Samarel AM. Protein synthesis and degradation during regression of thyroxine-induced cardiac hypertrophy. *J Mol Cell Cardiol* 1989;21:911–925. [PubMed: 2530358]
11. Parmacek MS, Magid NM, Lesch M, Decker RS, Samarel AM. Cardiac protein synthesis and degradation during thyroxine-induced left ventricular hypertrophy. *Am J Physiol* 1986;251:C727–C736. [PubMed: 2946236]
12. Razeghi P, Baskin KK, Sharma S, et al. Atrophy, hypertrophy, and hypoxemia induce transcriptional regulators of the ubiquitin proteasome system in the rat heart. *Biochem Biophys Res Commun* 2006;342:361–364. [PubMed: 16483544] • **Demonstrates, for the first time, how multiple facets of the ubiquitin-proteasome system (UPS) are regulated at the transcriptional level in cardiac disease models, including hypertrophy, hypoxemia and atrophy.**
13. Adams V, Linke A, Wisloff U, et al. Myocardial expression of Murf-1 and MAFbx after induction of chronic heart failure: effect on myocardial contractility. *Cardiovasc Res* 2007;73:120–129. [PubMed: 17145048]
14. Li HH, Kedar V, Zhang C, et al. Atrogin-1/muscle atrophy F-box inhibits calcineurin-dependent cardiac hypertrophy by participating in an SCF ubiquitin ligase complex. *J Clin Invest* 2004;114:1058–1071. [PubMed: 15489953]
15. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 2003;65:45–79. [PubMed: 12524460]
16. Ni YG, Berenji K, Wang N, et al. Foxo transcription factors blunt cardiac hypertrophy by inhibiting calcineurin signaling. *Circulation* 2006;114:1159–1168. [PubMed: 16952979]
17. Li HH, Willis MS, Lockyer P, et al. Atrogin-1 inhibits Akt-dependent cardiac hypertrophy in mice via ubiquitin-dependent coactivation of Forkhead proteins. *J Clin Invest* 2007;117:3211–3223. [PubMed: 17965779] • **Defines a role for the E3 ubiquitin ligase, atrogin-1, in the placement of lysine-63-linked ubiquitin chains in the transcriptional coactivation of Foxo1 and Foxo3a, leading to the disruption of physiologic cardiac hypertrophy.**
18. Deng L, Wang C, Spencer E, et al. Activation of the I κ B kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 2000;103:351–361. [PubMed: 11057907]
19. Spence J, Sadis S, Haas AL, Finley DA. Ubiquitin mutant with specific defects in DNA repair and multiubiquitination. *Mol Cell Biol* 1995;15:1265–1273. [PubMed: 7862120]

20. Hoshijima M. Mechanical stress-strain sensors embedded in cardiac cytoskeleton: Z disk, titin, and associated structures. *Am J Physiol Heart Circ Physiol* 2006;290:H1313–H1325. [PubMed: 16537787]
21. Centner T, Yano J, Kimura E, et al. Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain. *J Mol Biol* 2001;306:717–726. [PubMed: 11243782]
22. Gregorio CC, Perry CN, McElhinny AS. Functional properties of the titin/connectin-associated proteins, the muscle-specific RING finger proteins (MURFs), in striated muscle. *J Muscle Res Cell Motil* 2005;26:389–400. [PubMed: 16477476]
23. Mrosek M, Labeit D, Witt S, et al. Molecular determinants for the recruitment of the ubiquitin-ligase MuRF-1 onto M-line titin. *FASEB J* 2007;21:1383–1392. [PubMed: 17215480]
24. Arya R, Kedar V, Hwang JR, et al. Muscle ring finger protein-1 inhibits PKC ϵ activation and prevents cardiomyocyte hypertrophy. *J Cell Biol* 2004;167:1147–1159. [PubMed: 15596539]
25. Kedar V, McDonough H, Arya R, et al. Muscle-specific RING finger 1 is a *bona fide* ubiquitin ligase that degrades cardiac troponin I. *Proc Natl Acad Sci USA* 2004;101:18135–18140. [PubMed: 15601779]
26. Willis MS, Ike C, Li L, et al. Muscle ring finger 1, but not muscle ring finger 2, regulates cardiac hypertrophy *in vivo*. *Circulation Res* 2007;100:456–459. [PubMed: 17272810]
27. Lange S, Vihola A, Udd B. The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 2005;308:1599–1603. [PubMed: 15802564]
28. Zhang C, Xu Z, He XR, Michael LH, Patterson C. CHIP, a cochaperone/ubiquitin ligase that regulates protein quality control, is required for maximal cardioprotection after myocardial infarction in mice. *Am J Physiol Heart Circ Physiol* 2005;288:H2836–2842. [PubMed: 15665051] • **Describes, for the first time, how the E3 ubiquitin ligase carboxyl terminus of Hsp70-interacting protein provides a critical cardioprotective effect in the setting of ischemia-reperfusion injury.**
29. Tripathi V, Ali A, Bhat R, Pati U. CHIP chaperones wild type p53 tumor suppressor protein. *J Biol Chem* 2007;282:28441–28454. [PubMed: 17666403]
30. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997;387:296–299. [PubMed: 9153395]
31. Kubbutat MH, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. *Nature* 1997;387:299–303. [PubMed: 9153396]
32. Momand J, Zambetti GP, Olson DC, George D, Levine AJ. The Mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* 1992;69:1237–1245. [PubMed: 1535557]
33. Toth A, Nickson P, Qin LL, Erhardt P. Differential regulation of cardiomyocyte survival and hypertrophy by MDM2, an E3 ubiquitin ligase. *J Biol Chem* 2006;281:3679–3689.
34. Foo RS, Chan LK, Kitsis RN, Bennett MR. Ubiquitination and degradation of the anti-apoptotic protein ARC by MDM2. *J Biol Chem* 2007;282:5529–5535.
35. Xiong S, Van Pelt CS, Elizondo-Fraire AC, Fernandez-Garcia B, Lozano G. Loss of Mdm4 results in p53-dependent dilated cardiomyopathy. *Circulation* 2007;115:2925–2930. [PubMed: 17533180]
36. Spencer JA, Eliazar S, Ilaria RL Jr, Richardson JA, Olson EN. Regulation of microtubule dynamics and myogenic differentiation by MURF, a striated muscle RING-finger protein. *J Cell Biol* 2000;150:771–784. [PubMed: 10953002]
37. Fielitz J, van Rooij E, Spencer JA, et al. Loss of muscle-specific RING-finger 3 predisposes the heart to cardiac rupture after myocardial infarction. *Proc Natl Acad Sci USA* 2007;104:4377–4382. [PubMed: 17360532]
38. Fielitz J, Kim MS, Shelton JM, et al. Myosin accumulation and striated muscle myopathy result from the loss of muscle RING finger 1 and 3. *J Clin Invest* 2007;117:2486–2495. [PubMed: 17786241]
39. Holschermann H, Schuster D, Parviz B, Haberbosch W, Tillmanns H, Muth H. Statins prevent NF- κ B transactivation independently of the IKK-pathway in human endothelial cells. *Atherosclerosis* 2006;185:240–245. [PubMed: 16051251]
40. Ross AM, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. A randomized, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *J Am Coll Cardiol* 2005;45:1775–1780. [PubMed: 15936605]

41. Yoo CG, Lee S, Lee CT, Kim YW, Han SK, Shim YS. Effect of acetylsalicylic acid on endogenous I κ B kinase activity in lung epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2001;280:L3–L9. [PubMed: 11133489]
42. Di Napoli M, Papa F. MLN-519. Millennium/PAION. *Curr Opin Investig Drugs* 2003;4:333–341.
43. Bross PF, Kane R, Farrell AT, et al. Approval summary for bortezomib for injection in the treatment of multiple myeloma. *Clin Cancer Res* 2004;10:3954–3964. [PubMed: 15217925]
44. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 2005;352:2487–2498. [PubMed: 15958804]
45. Enrico O, Gabriele B, Nadia C, et al. Unexpected cardiotoxicity in haematological bortezomib treated patients. *Br J Haematol* 2007;138:396–397. [PubMed: 17561972]•• **Reports an unexpected increase in cardiac complications (ranging from heart failure to arrhythmia onset) in patients being treated for multiple myeloma using the proteasome inhibitor, bortezomib.**
46. Berenson JR, Matous J, Swift RA, Mapes R, Morrison B, Yeh HS. A Phase I/II study of arsenic trioxide/bortezomib/ascorbic acid combination therapy for the treatment of relapsed or refractory multiple myeloma. *Clin Cancer Res* 2007;13:1762–1768. [PubMed: 17363530]
47. Meiners S, Laule M, Rother W, et al. Ubiquitin-proteasome pathway as a new target for the prevention of restenosis. *Circulation* 2002;105:483–489. [PubMed: 11815432]
48. Stangl V, Lorenz M, Meiners S, et al. Long-term up-regulation of eNOS and improvement of endothelial function by inhibition of the ubiquitin-proteasome pathway. *FASEB J* 2004;18:272–279. [PubMed: 14769821]
49. Herrmann J, Saguner AM, Versari D, et al. Chronic proteasome inhibition contributes to coronary atherosclerosis. *Circulation Res* 2007;101(9):865–874. [PubMed: 17823377]
50. Wang X, Robbins J. Heart failure and protein quality control. *Circulation Res* 2006;99:1315–1328. [PubMed: 17158347]• **Excellent recent review giving in-depth insight into the regulation of the proteasome and its role in cardiac protein quality control in the context of disease.**
51. Maron BJ. Triggers for sudden cardiac death in the athlete. *Cardiol Clin* 1996;14:195–210. [PubMed: 8724552]
52. Bonne G, Carrier L, Richard P, Hainque B, Schwartz K. Familial hypertrophic cardiomyopathy: from mutations to functional defects. *Circulation Res* 1998;83:580–593. [PubMed: 9742053]
53. Richard P, Charron P, Carrier L, et al. Hypertrophic cardiomyopathy: distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation* 2003;107:2227–2232. [PubMed: 12707239]
54. Flavigny J, Robert P, Camelin JC, Schwartz K, Carrier L, Berrebi-Bertrand I. Biomolecular interactions between human recombinant β -MyHC and cMyBP-Cs implicated in familial hypertrophic cardiomyopathy. *Cardiovasc Res* 2003;60:388–396. [PubMed: 14613868]
55. Freiburg A, Gautel M. A molecular map of the interactions between titin and myosin-binding protein C: implications for sarcomeric assembly in familial hypertrophic cardiomyopathy. *Eur J Biochem* 1996;235:317–323. [PubMed: 8631348]
56. Gruen M, Gautel M. Mutations in β -myosin S2 that cause familial hypertrophic cardiomyopathy (FHC) abolish the interaction with the regulatory domain of myosin-binding protein-C. *J Mol Biol* 1999;286:933–949. [PubMed: 10024460]
57. Squire JM, Luther PK, Knupp C. Structural evidence for the interaction of C-protein (MyBP-C) with actin and sequence identification of a possible actin-binding domain. *J Mol Biol* 2003;331:713–724. [PubMed: 12899839]
58. Sarikas A, Carrier L, Schenke C, et al. Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. *Cardiovasc Res* 2005;66:33–44. [PubMed: 15769446]•• **Reports, for the first time, that mutations in myosin-binding protein-C underlying familial hypertrophic cardiomyopathy (FHC) are degraded preferentially by the UPS, leading to the impairment of the proteolytic activity of the UPS. This study links protein quality control by the UPS in the pathogenesis of FHC.**
59. Moolman JA, Reith S, Uhl K, et al. A newly created splice donor site in exon 25 of the *MyBP-C* gene is responsible for inherited hypertrophic cardiomyopathy with incomplete disease penetrance. *Circulation* 2000;101:1396–1402. [PubMed: 10736283]

60. Rottbauer W, Gautel M, Zehelein J, et al. Novel splice donor site mutation in the cardiac myosin-binding protein-C gene in familial hypertrophic cardiomyopathy. Characterization of cardiac transcript and protein. *J Clin Invest* 1997;100:475–482. [PubMed: 9218526]
61. Flavigny J, Souchet M, Sébillon P, et al. COOH-terminal truncated cardiac myosin-binding protein C mutants resulting from familial hypertrophic cardiomyopathy mutations exhibit altered expression and/or incorporation in fetal rat cardiomyocytes. *J Mol Biol* 1999;294:443–456. [PubMed: 10610770]
62. Yang Q, Sanbe A, Osinska H, Hewett TE, Klevitsky R, Robbins J. *In vivo* modeling of myosin binding protein C familial hypertrophic cardiomyopathy. *Circulation Res* 1999;85:841–847. [PubMed: 10532952]
63. Lindsten K, de Vrij FM, Verhoef LG, et al. Mutant ubiquitin found in neurodegenerative disorders is a ubiquitin fusion degradation substrate that blocks proteasomal degradation. *J Cell Biol* 2002;157:417–427. [PubMed: 11980917]
64. Kim HT, Kim KP, Lledias F, et al. Certain pairs of ubiquitin-conjugating enzymes (E2s) and ubiquitin-protein ligases (E3s) synthesize nondegradable forked ubiquitin chains containing all possible isopeptide linkages. *J Biol Chem* 2007;282:17375–17386. [PubMed: 17426036]
65. Hall G, Hasday JD, Rogers TB. Regulating the regulator: NF- κ B signaling in heart. *J Mol Cell Cardiol* 2006;41:580–591. [PubMed: 16949095]
66. Kopp EB, Ghosh S. NF- κ B and rel proteins in innate immunity. *Adv Immunol* 1995;58:1–27. [PubMed: 7741027]

Figure 1. Overview of the ubiquitin-proteasome system

The degradation of proteins via the ubiquitin-proteasome system is dependent on the sequential addition of ubiquitin molecules on targeted protein substrates. **(A)** Free ubiquitin is activated by the E1 enzyme and transferred to an E2 enzyme. E3 ubiquitin ligases mediate the transfer of the ubiquitin molecule from the E2 to the substrate protein, a process that is repeated, resulting in polyubiquitin chain formation on the protein substrate. Polyubiquitin chains are subsequently recognized by the 26S proteasome, resulting in cleavage of the protein substrate into small peptides and the release of ubiquitin molecules. **(B)** Homologous polyubiquitin chains formed via linkages at lysine-48 of the presiding ubiquitin molecule result in a chain conformation that is recognized by the 26S proteasome. Conversely, polyubiquitin chains coupled via lysine-63 linkages yield chain conformations that are not recognized by the 26S proteasome; rather, these linkages alter the function of the protein substrate. Lys: Lysine; Ub: Ubiquitin.

Figure 2. Mechanisms of p53-mediated apoptosis

The transcription factor p53 regulates the induction of apoptosis by regulating BAX, PUMA and APAF-1 expression. The permeability of the mitochondria and subsequent release of cytochrome C is regulated by a balance between antiapoptotic (i.e., BCL-2) and proapoptotic (i.e., BAX) factors. The increase of the proapoptotic BAX and its effector molecule, PUMA, in response to p53, interacts with and overcomes the antiapoptotic BCL-2, resulting in the release of mitochondrial cytochrome C. Cytochrome C, together with ATP and APAF-1, acts to activate caspase 9 in the activation of apoptosis through the intrinsic pathway. APAF: Apoptotic protease-activating factor; BAX: BCL-2-associated X protein; BCL-2: B-cell lymphoma-2 protein; CHIP: Carboxyl terminus of Hsp70-interacting protein; MDM: Murine double minute; PUMA: p53-upregulated modulator of apoptosis.

Figure 3. Ubiquitin-proteasome system-mediated resulation of the nuclear factor- κ B activation pathway

Activation of the multimeric IKK mediates the phosphorylation of I κ B. The modified I κ B is then recognized by the SCF- β TrCP E3 ubiquitin ligase and targeted for degradation via the ubiquitin-proteasome system, liberating the p50/p65 nuclear factor (NF)- κ B dimer. This results in the exposure of a nuclear localization signal on p50/p65, previously obscured by I κ B, promoting the nuclear translocation of p50/p65 and the subsequent activation of the NF- κ B transcriptional program. Multiple pathways pertinent to cardiac hypertrophy and failure are activated by NF- κ B-mediated transcription, such as cell adhesion, apoptosis, inflammation and immune-response signaling molecules. AGT: Angiotensinogen; CBF: Complement factor B; IAP: Inhibitor of apoptosis protein; ICAM: Intercellular adhesion molecule; I κ B: Inhibitor of κ B; IKK: I κ B kinase complex; P: Phosphate; SAA: Serum amyloid A; SCF- β TrCP: Skip-Cullin-F box β -transducin repeat-containing protein; Ub: Ubiquitin; VCAM: Vascular cell adhesion molecule.

Figure adapted from [65,66].