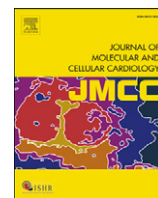


Contents lists available at [ScienceDirect](http://ScienceDirect.com)

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

Ubiquitin-proteasome system and hereditary cardiomyopathies

Saskia Schlossarek^{a,b}, Norbert Frey^{b,c}, Lucie Carrier^{a,b,d,e,*}^a Department of Experimental Pharmacology and Toxicology, Cardiovascular Research Center, University Medical Center Hamburg-Eppendorf, Hamburg, Germany^b DZHK (German Centre for Cardiovascular Research), partner site Hamburg/Kiel/Lübeck, Germany^c Department of Cardiology and Angiology, University of Kiel, Kiel, Germany^d Inserm, U974, Paris F-75013, France^e Université Pierre et Marie Curie- Paris 6, UMR 76, CNRS, UMR 7215, Institut de Myologie, IFR14, Paris F-75013, France

ARTICLE INFO

Article history:

Received 12 July 2013

Received in revised form 13 November 2013

Accepted 17 December 2013

Available online 28 December 2013

Keywords:

Ubiquitin-proteasome system

Hypertrophic cardiomyopathy

Dilated cardiomyopathy

ABSTRACT

Adequate protein turnover is essential for cardiac homeostasis. Different protein quality controls are involved in the maintenance of protein homeostasis, including molecular chaperones and co-chaperones, the autophagy-lysosomal pathway, and the ubiquitin-proteasome system (UPS). In the last decade, a series of evidence has underlined a major function of the UPS in cardiac physiology and disease. Particularly, recent studies have shown that dysfunctional proteasomal function leads to cardiac disorders. Hypertrophic and dilated cardiomyopathies are the two most prevalent inherited cardiomyopathies. Both are primarily transmitted as an autosomal-dominant trait and mainly caused by mutations in genes encoding components of the cardiac sarcomere, including a relevant striated muscle-specific E3 ubiquitin ligase. A growing body of evidence indicates impairment of the UPS in inherited cardiomyopathies as determined by measurement of the level of ubiquitinated proteins, the activities of the proteasome and/or the use of fluorescent UPS reporter substrates. The present review will propose mechanisms of UPS impairment in inherited cardiomyopathies, summarize the potential consequences of UPS impairment, including activation of the unfolded protein response, and underline some therapeutic options available to restore proteasome function and therefore cardiac homeostasis and function. This article is part of a Special Issue entitled "Protein Quality Control, the Ubiquitin Proteasome System, and Autophagy".

© 2013 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/3.0/>).

Contents

1. Introduction	25
2. The ubiquitin-proteasome system	26
3. The ubiquitin-proteasome system in familial cardiomyopathies	26
3.1. The ubiquitin-proteasome system in hypertrophic cardiomyopathy	26
3.2. The ubiquitin-proteasome system in dilated cardiomyopathy	27
3.3. Potential mechanisms leading to UPS impairment in cardiomyopathies	27
3.4. Potential consequences of UPS impairment	29
4. Potential therapeutic UPS interventions	29
5. Conclusion – future directions	29
Disclosures	30
Acknowledgment	30
References	30

1. Introduction

In mammalian cells, most of the proteins are in a dynamic state of flux. The balance of protein synthesis and degradation in each cell is highly regulated and occurs in a specific manner to maintain cellular homeostasis. However, under the circumstances of cardiac remodeling during heart disease this balance can be altered leading to accumulation

* Corresponding author at: Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg Eppendorf, Martinistraße 52, 20246 Hamburg, Germany. Tel.: +49 40 7410 57208; fax: +49 40 7410 55925.

E-mail address: l.carrier@uke.de (L. Carrier).

of potentially toxic proteins. To ensure that these misfolded or aberrant proteins are either repaired or removed, a set of molecular mechanisms works in collaboration or separately as a quality control of the cell. This quality control consists of molecular chaperones and co-chaperones, the autophagy-lysosomal pathway (ALP), and the ubiquitin-proteasome system (UPS). In the past years, the functional significance of the UPS in cardiovascular physiology and disease has become evident. Particularly, UPS alteration is rapidly gaining recognition as a major player in the pathogenesis of several cardiac disorders, including inherited cardiomyopathies.

Cardiomyopathies are myocardial diseases, characterized by abnormal cardiac structure and function, in the absence of other causes that could produce these abnormalities, such as coronary artery disease, hypertension, valvular disease, or congenital heart disease [1,2]. Phenotypically, they are classified into four main forms: hypertrophic, dilated, restrictive, and arrhythmogenic right ventricular cardiomyopathy [3]. The two most prevalent familial forms are hypertrophic (HCM) and dilated (DCM) cardiomyopathies, which are typically associated with mutations in genes encoding proteins of the sarcomere, cytoskeleton, sarcoplasmic reticulum, T-tubules and others [1].

This review will highlight the current knowledge of UPS function in the context of HCM and DCM, discuss potential mechanisms and consequences of UPS dysfunction and propose potential therapeutic interventions.

2. The ubiquitin-proteasome system

The UPS is indispensable for the highly selective degradation of most intracellular cytosolic, nuclear, and myofibrillar proteins. It controls many fundamental biological processes such as cell proliferation, adaptation to stress and cell death, and its major function is to prevent accumulation of damaged, misfolded and mutant proteins [4]. Degradation of proteins by the UPS is an ATP-dependent multistage process that requires first ubiquitination of the target protein prior to its degradation by the 26S proteasome [5,6].

Ubiquitination of the target protein is achieved via an enzymatic cascade involving the concerted action of E1 (ubiquitin-activating), E2 (ubiquitin-conjugating) and E3 (ubiquitin ligase) enzymes. While there are two E1, about 40 E2 and more than 600 E3 enzymes have been described in mammals [6,7]. The process of ubiquitination occurs with spatial, temporal and substrate specificity, which is dictated by the E3 ubiquitin ligases. The E3 ubiquitin ligases have been broadly classified into 2 main categories based on structural similarities: the RING (really interesting new gene) finger domain-containing proteins including the RING-related E3s such as the U-box proteins, and the HECT (homologous to E6-AP carboxy-terminus) domain-containing proteins [7,8]. In addition, hybrids of RING-finger and HECT E3 ubiquitin ligases exist. Recent reviews, including one in the current issue, gave an update on the known striated muscle-specific E3 ubiquitin ligases ([9,10], Willis et al., in press). The last identified cardiac-specific E3 ubiquitin ligase is the F-box protein Fbxl22, which promotes degradation of α -actinin and filamin C [11].

The eukaryotic 26S proteasome is a large, multicatalytic protein complex composed of two subcomplexes: the 20S core particle capped by either one or two 19S regulatory particles (for a detailed description, see [12]). The function of the 19S regulatory particle is to recognize, deubiquitinate, and unfold target proteins, and then to translocate them into the 20S core particle, which houses the proteolytic activities within its central chamber. Three distinct proteolytic activities exist, namely the chymotrypsin-like, trypsin-like and caspase-like activities, and each cleaves preferentially after particular amino acid residues. Many structurally diverse inhibitors of these activities have been discovered or developed and were recently summarized [13].

Over the last decade, methods for assessment of UPS function have been established. The evaluation of the UPS includes the determination of steady-state levels of ubiquitinated proteins, ubiquitinating and

deubiquitinating enzymes, and proteasomal subunits by Western blot. In addition, measurements of the proteolytic activities using synthetic fluorogenic substrates are often performed, despite the disadvantage that these small substrates can easily enter the 20S core in an ubiquitination-independent manner and therefore do not reflect the highly-regulated entry of substances into the 20S core. To get insights into the dynamic behavior of the UPS in living cells, fluorescent-labeled UPS components, including fluorescent ubiquitin and fluorescent-tagged proteasomal subunits, have been generated [14]. These tools allowed the discovery of novel features of the UPS in diverse cellular processes, including its different locations such as in the inner surface of the nuclear envelope, the endoplasmic reticulum, or its homogenous distribution in cells. Related to these studies, transgenic reporter mouse models expressing fluorescent substrates of the UPS were then created to decipher the role of the UPS in the whole animal [15,16].

3. The ubiquitin-proteasome system in familial cardiomyopathies

Several disorders, including neurodegenerative and cardiovascular diseases exhibit organ failure due, at least in part, to toxic protein accumulation [5,9,17,18]. Most cases of heart failure with hypertrophic, dilated or ischemic cardiomyopathies exhibit accumulation of ubiquitinated proteins [19,20], abnormal protein aggregation such as preamyloid oligomer formation [21,22], and altered proteasomal activities [23–25]. A body of evidence indicates UPS alterations in inherited HCM and DCM and is discussed below.

3.1. The ubiquitin-proteasome system in hypertrophic cardiomyopathy

HCM is the most prevalent cardiac genetic disease (1:500), characterized by left ventricular hypertrophy (LVH), increased interstitial fibrosis, and diastolic dysfunction [26–28]. HCM is considered as a sarcomeropathy, transmitted in an autosomal-dominant fashion with an incomplete penetrance, and caused by more than 1000 individual mutations in (at least) 10 genes encoding proteins of the cardiac contractile unit [1,28–30]. Most of the disease genes exhibit missense mutations that are expected to produce stable full-length mutant proteins. However, in some HCM genes, mutations are mainly frameshift leading to a premature stop codon and C-terminal truncated polypeptides. This is the case for the most frequently mutated gene, *MYBPC3*, encoding cardiac myosin-binding protein C (cMyBP-C; [29]) and for a much less frequently mutated gene, *FHL1*, encoding four-and-a-half LIM domain protein 1 [31].

It has been shown that truncated cMyBP-Cs resulting from human *MYBPC3* mutations are unstable, not well incorporated into the sarcomere and finally degraded by the UPS after gene transfer in rat cardiac myocytes [32,33]. Continuous degradation of mutant cMyBP-C proteins led to UPS impairment as shown by the accumulation of the UPS substrate Ub^{G76V}-DsRed [33]. The muscle-specific E3 ubiquitin ligase involved was then found to be atrogin-1, whereas MuRF1 did contribute to lowering cMyBP-C level at the mRNA level after gene transfer in cardiac myocytes [34]. The expression of a missense E334K *MYBPC3* mutation also resulted in UPS impairment and accumulation of pro-apoptotic markers, ion channels and Ca²⁺ handling proteins after gene transfer in cardiac myocytes [35,36]. The knock-in of the most frequent human *MYBPC3* mutation (c.772G>A; 13% of unrelated HCM patients, likely with a founder effect in Tuscany; [37]) into the mouse genome revealed that its expression is regulated by both the nonsense-mediated mRNA decay (NMD) and UPS [38]. Moreover, in both homozygous *Mybpc3*-targeted knock-in (KI) and knock-out (KO) mice, which developed LVH with systolic and diastolic dysfunction [38–40], the activities of the proteasome were elevated during the first 3 months of age and positively correlated with the degree of LVH [41]. Interestingly, then, the global activity of the proteasome was impaired with aging only in the KI mice (but not in KO), as shown by the accumulation of the UPS substrate Ub^{G76V}-GFP protein in the heart [41]. Similarly, adrenergic stress induced the same extent

of LVH (but with a specific septum involvement) in heterozygous *Mybpc3*-targeted KI and KO mice as in wild-type mice, but induced a marked reduction in proteasome activity only in heterozygous KI mice [42]. Reduced proteasome activities were found in human myocardial tissue of HCM patients, particularly in those carrying *MYBPC3* gene mutations [24]. Whether UPS impairment contributes to the development of HCM in human is unclear but mouse studies strongly support the view that UPS impairment results from the combination of altered cardiac phenotype plus stress in mice exhibiting a *Mybpc3* mutation.

Besides *MYBPC3*, other disease genes need to be underlined, even if mutations were rare and found in isolated cases of HCM. For example, the expression of missense and truncating *FHL1* mutations as well as missense mutations in *ANKRD1*, encoding ankyrin repeat domain 1, were markedly regulated by the UPS after gene transfer in cardiac myocytes or in rat engineered heart tissue (EHT; [31,43]). *ANKRD1* interacts with the sarcomere-specific MuRF1 and MuRF2 [44], suggesting that its degradation could be mediated by MuRF1. Interestingly, mutations in *TRIM63* encoding MuRF1 cause isolated cases of HCM and reduced the UPS-mediated degradation of mTOR-S6K hypertrophic signaling pathway in transgenic mutant mice [45].

3.2. The ubiquitin-proteasome system in dilated cardiomyopathy

DCM is characterized by increased ventricular dimensions, contractile dysfunction and myocardial fibrosis [2]. In 20–50% of cases DCM is familial and inherited primarily in an autosomal-dominant mode [1]. The genetic basis of DCM is far more heterogeneous than that of HCM. More than 50 single genes are associated with DCM, several of which also cause HCM [46]. The DCM genes encode components of the sarcomere, sarcolemma, nuclear envelope, cytoskeleton, mitochondria, and proteins involved in Ca^{2+} handling [1,46]. Most of DCM cases result from sarcomere gene mutations, with the majority (25%) attributed to truncating mutations in *TTN*, encoding titin [47].

As for HCM, UPS impairment might also play a role in human or experimental models of familial DCM. While *MYBPC3* is the paradigm for UPS impairment in HCM, for DCM these are *CRYAB* encoding α -B-crystallin and *DES* encoding desmin. Mutations in *CRYAB* or *DES* resulted in accumulation of mutant proteins and severe DCM in desmin-related (cardio)myopathy (DRM; [48,49]). A mouse model of DRM, obtained by overexpression of the R120G mutant *CRYAB* (*CryAB*^{R120G}) recapitulated the human phenotype [50] and exhibited marked UPS impairment as revealed by GFPdgn-based UPS reporter mice before the development of hypertrophy and heart failure [51]. Similar observations were made in mutant *Des-D7* transgenic mice [52,53]. In both DRM mouse models, UPS impairment started before the cardiac phenotype and seems to concern the delivery of ubiquitinated proteins into the 20S proteasome.

Other genes associated with DCM are also subject to UPS-mediated regulation. This is the case for *LMNA* encoding lamins A/C, which are proteins of the nuclear envelope. *LMNA* mutations cause DCM with conduction and/or rhythm defects [54]. Heterozygous *Lmna* ^{Δ K32/+} mice developed DCM and heart failure, and finally died between 35 and 70 weeks of age [55]. DCM was triggered by lamin haploinsufficiency, due to rapid degradation of Δ K32-lamin mutant by the UPS, followed by UPS impairment, leading to accumulation of toxic Δ K32-lamin [55]. A missense mutation in *NKX2.5*, associated with congenital heart disease and adult-onset DCM, resulted in UPS impairment after gene transfer in COS cells [56]. In a recent unbiased approach that aimed at identifying modifying pathways in mouse models of DCM carrying mutant muscle LIM protein, calstarcin-1 or δ -sarcoglycan, alterations of gene expression of UPS components emerged as the most significant predictor of impaired contractile function [57]. In human DCM and end-stage heart failure, marked accumulation of ubiquitinated proteins is a common feature, whereas contradictory findings were obtained for proteasomal activities [19,23–25]. Whether UPS impairment contributes to the development

of DCM in human is not resolved yet, but mouse studies support this view.

3.3. Potential mechanisms leading to UPS impairment in cardiomyopathies

The mechanisms by which gene mutations lead to UPS impairment are not fully elucidated. In the absence of external stress, the expression of the mutation is regulated at several levels by quality control mechanisms in order to reduce as much as possible the amount of misfolded or aberrant poison polypeptides, which could induce damage in cardiac myocytes. Missense mutations are expected to produce stable full-length mutant mRNAs and proteins. Misfolded mutant proteins are recognized by chaperones (such as Hsp70, Hsp90) and co-chaperones (such as CHIP, Bag1, Bag3) that will make the decisions about refolding or degrading them by the UPS and/or the ALP [6]. Therefore, in some cases, the expression of missense mutations may be tightly regulated by the protein quality control systems, resulting in low level of full-length mutant proteins. In the specific case of frameshift or nonsense mutations, an additional quality control takes place at the mRNA level, which is the NMD (Fig. 1 [58]). Low levels (or absence) of mutant proteins and the assumed 50% of wild-type proteins, as expected for autosomal-dominant disease such as cardiomyopathies, result in protein haploinsufficiency, which leads to the cardiac phenotype. In most cases, expression of the wild-type allele partially compensates for protein deficiency. For example, >70% of wild-type cMyBP-C proteins were detected in septal myectomy of HCM patients with *MYBPC3* mutations, even for patients with missense mutations [59–61]. Similarly, heterozygous *Mybpc3*-targeted KO mice, which are considered as pure models of haploinsufficiency, exhibited 75% of cMyBP-C and then developed septal hypertrophy at 10–11 months of age [40], and heterozygous *Mybpc3*-targeted KI mice exhibited 79% of cMyBP-C and developed diastolic dysfunction at 10 weeks of age [38,39]. Finally, heterozygous *Lmna* ^{Δ K32/+} mice exhibited haploinsufficiency before the development of DCM [55].

Several mechanisms could lead separately or in combination to UPS impairment. First, it has been shown that the continuous degradation of mutant proteins by the UPS saturates this system after gene transfer in cardiac myocytes or EHTs [31,33,35,55] and in the *CryAB*^{R120G} DRM mouse model [51,53]. Second, the combination of external stress and overwhelmed UPS could precipitate the system into impairment (Fig. 1). This is the case in some HCM and DCM disease mouse models, in which the UPS continuously degraded mutant proteins in young adult mice carrying *Lmna* or *Mybpc3* mutation and became saturated or impaired only after adrenergic stress or aging [38,41,42,55]. In one of these studies, stress-induced decreased chymotrypsin-like activity was mainly due to reduced level of the β 5-subunit of the proteasome in the cytosol, which could be due to translocation of the proteasome to another cellular compartment [41]. Reversible localization of proteasomal components was observed in other conditions. For example, yeast cells that stop cell cycling relocated the proteasome from the nucleus to the cytosol into storage granules [62]. In neurodegenerative disorders, the proteasome system was also relocated into intracytoplasmic inclusions [63]. Third, misfolded proteins escaping the surveillance of chaperones and UPS tend to form aggregates, which are potentially toxic to the cell. Supporting this, the group of Robbins showed that intracellular amyloidosis was highly prevalent in cardiac myocytes derived from human HCM or DCM hearts [21]. Furthermore, protein aggregation itself impaired proteasome function in cardiac myocytes [64], forming a vicious cycle. Fourth, increased oxidative stress also results in protein aggregation. In the case of aging, this could be due to increased free radicals production by damaged aging mitochondria [65]. Oxidative stress induced protein oxidation and aggregation of oxidized proteins, which bind to the 20S proteasome and irreversibly inhibit its activity [66]. This could cause a vicious cycle and also lead to accumulation of oxidized proteins, which are normally degraded by the proteasome system. Fifth, an altered assembly of the proteasome or a switch in the

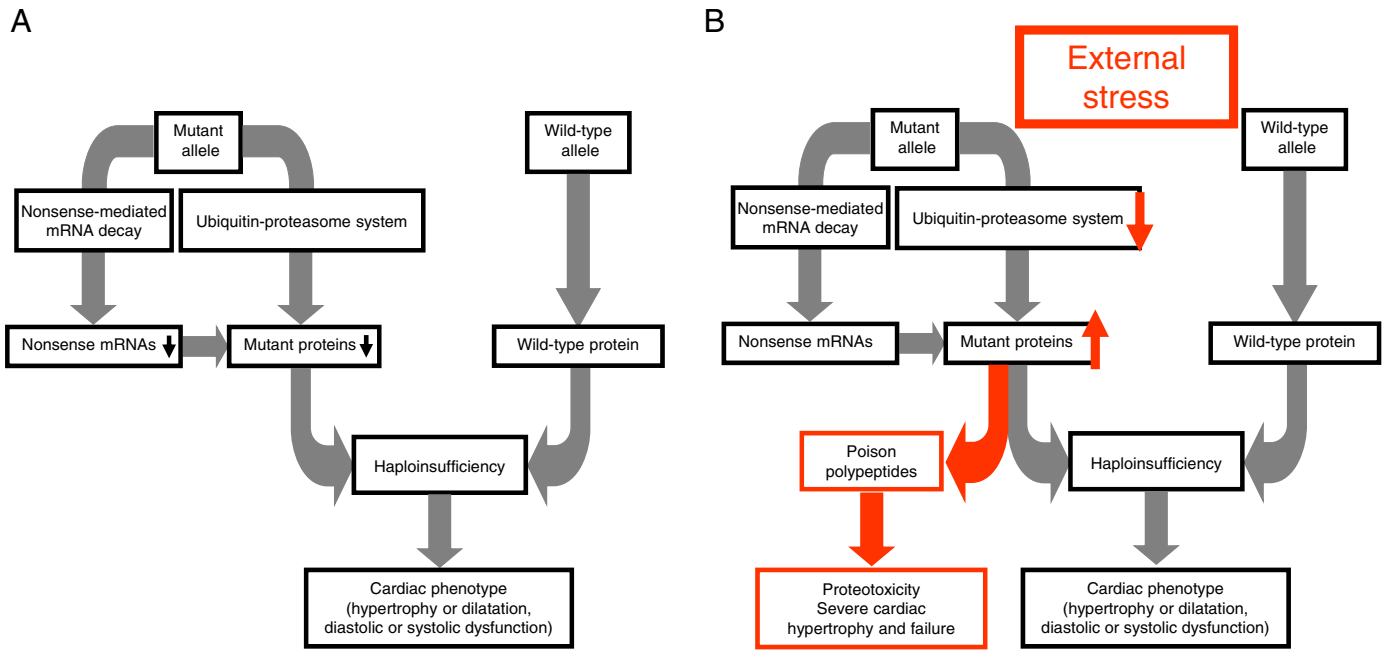


Fig. 1. Potential mechanisms leading to UPS impairment in genetic forms of cardiomyopathies. A) The nonsense-mediated mRNA decay at the mRNA level, and the ubiquitin–proteasome system (UPS) at the protein level regulate the expression of the mutant allele, leading to reduced amount of mutant proteins that could be toxic for cardiac myocytes. Together with the (normal) expression of the wild-type allele, this results in protein haploinsufficiency and the development of the cardiac phenotype (hypertrophy or dilatation, diastolic or systolic dysfunction). B) External stress, e.g. adrenergic stress or aging, could induce impairment of the UPS in cardiomyopathies. Although the exact mechanism is not known, one may suggest that adrenergic stress induces desensitization of the β -adrenoceptors leading to decreased activities of PKA and therefore reduced phosphorylation of proteasome components, known to be associated with decreased proteasome activity. Impaired UPS leads to accumulation of mutant proteins, which could act as poison polypeptides and further impair the phenotype (severe form of cardiac hypertrophy and heart failure). Figure was made from findings obtained in mouse models of hypertrophic and dilated cardiomyopathies [41,42,55].

distribution of proteasomal subpopulations [67] could lead to UPS impairment. A recent study demonstrated an impaired docking of the 19S to the 20S in human end-stage heart failure [68], which could affect the degradation capacity of the proteasome and may explain the diminished proteasomal activity found in human failing hearts [24]. Finally, regulation of the proteasome system involves post-translational

modifications of proteasomal subunits, such as phosphorylation, acetylation or oxidation. For example, in vitro PKA phosphorylation of proteasomal subunits increased proteasomal assembly and activities in the heart [69,70]. Given the evidence of altered β -adrenergic signaling in the diseased heart and particularly in heart failure [71–73], it is possible that the reduced PKA-mediated phosphorylation of the proteasome

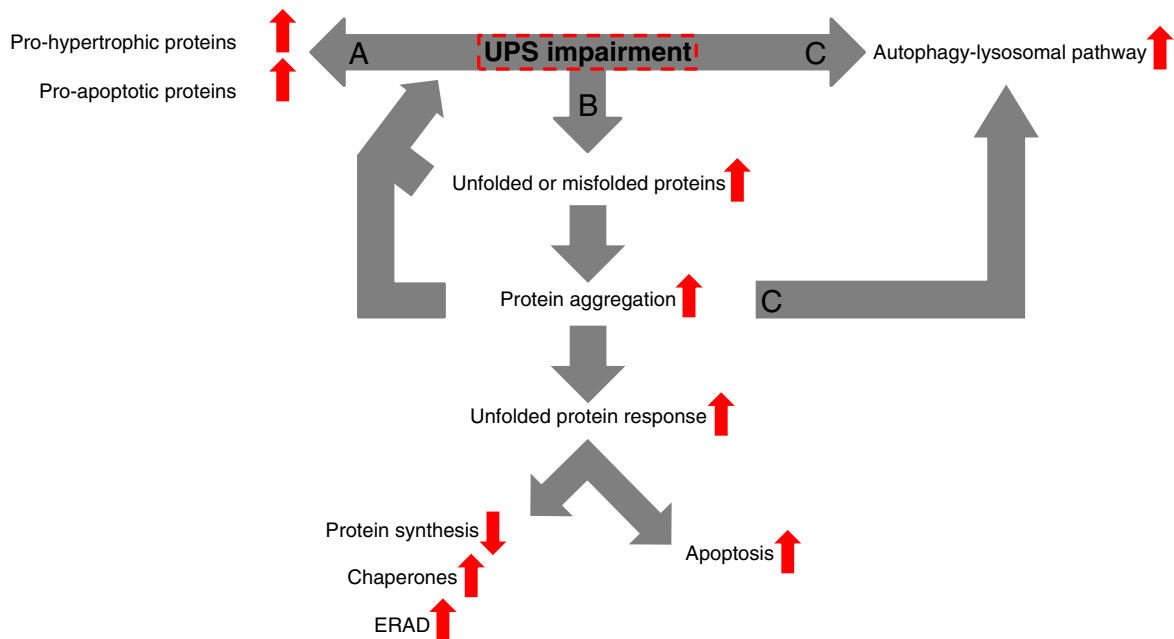


Fig. 2. Potential consequences of UPS impairment in cardiac myocytes. UPS impairment could lead to: A) Accumulation of proteins involved in hypertrophic signaling (e.g. calcineurin) or apoptotic pathway (e.g. p53), which are normally degraded by the UPS. B) Accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), which leads to protein aggregation. This ER stress activates the unfolded protein response, characterized by attenuation of protein synthesis, upregulation of chaperones genes (e.g. GRP78) and ERAD (ER-associated protein degradation). Prolonged stress will lead to apoptosis. Accumulation and/or aggregation of misfolded proteins could itself force UPS impairment. C) Activation of the autophagy-lysosomal pathway directly or indirectly (by protein aggregation) in order to contribute to protein degradation.

components contributes to UPS impairment in cardiomyopathies. Interestingly, reduced PKA-mediated phosphorylation of contractile proteins, such as cardiac troponin I and cMyBP-C was found in HCM [60,61,74] and more generally in human and experimental models of heart failure, such as chronic adrenergic stimulation [75,76].

3.4. Potential consequences of UPS impairment

UPS impairment could have several consequences in cardiac myocytes (Fig. 2). A number of key proteins involved in cardiac hypertrophy and apoptosis pathways are either targets or components of the UPS. For instance, several signaling proteins, such as β -catenin and calcineurin, which mediate cardiac growth (including pathological hypertrophy) are normally degraded by the UPS [77,78]. Similarly, p53 is a target of the E3 ubiquitin ligase MDM2 [79]. Therefore, proteasomal impairment could result in increased levels of pro-hypertrophic and pro-apoptotic factors. It has been shown that UPS impairment activated the calcineurin-NFAT pathway and promoted maladaptive remodeling in cardiac myocytes [80]. Furthermore, reduced proteasomal activities were associated with increased levels of pro-apoptotic p53 in human HCM and failing hearts [24]. Finally, the expression of the HCM p.Glu334Lys MYBPC3 mutation in cardiac myocytes induced UPS impairment, accumulation of pro-apoptotic factors and alteration of Ca^{2+} handling that could result in arrhythmias [35,36].

UPS impairment could also lead to accumulation of unfolded or misfolded proteins and aggregation of proteins (Fig. 2). This might result in ER stress, leading to an adaptive response, which is known as the unfolded protein response (UPR). The UPR promotes attenuation of protein synthesis, transcriptional activation of chaperone genes and activation of ER-associated degradation (ERAD) in order to reduce the load of misfolded proteins [81,82]. If the attempts to resolve the ER stress fail or the UPR is prolonged, UPR-mediated signaling pathways that lead to apoptosis are initiated. An inadequately working UPS (or in this case ERAD) probably stimulates the switch from adaptive to pro-apoptotic response. This hypothesis is supported by the demonstration that proteasome inhibition induced ER-initiated cardiac myocyte death via CHOP-dependent pathways [83]. Of note, accumulation and/or aggregation of misfolded proteins could itself force UPS impairment, forming thereby a detrimental feedback loop.

Whereas the UPS usually degrades the majority of proteins, the ALP is the other proteolytic system which is primarily responsible for degradation of (generally) long-lived or aggregated proteins and cellular organelles [6,84]. The ALP engulfs proteins or organelles into autophagosomes, which subsequently fuse with lysosomes to form auto(phago)lysosomes, in which lysosomal proteases degrade autophagosomal content [85]. Although autophagy is generally considered to be independent of the UPS, growing lines of evidence indicate that the UPS and ALP act as a consortium in the removal of misfolded proteins [84,86,87]. Another potential consequence of UPS inhibition is the ALP activation. Several proteins such as p62, NBR1 and HDAC6 seem to play a major role in the interplay between the UPS and ALP [6,84]. Proteasome inhibition activated autophagy *in vitro* and *in vivo*, likely as a compensatory mechanism to alleviate proteotoxic stress [87–89].

4. Potential therapeutic UPS interventions

Since the UPS plays a role in many fundamental biological processes, targeting this system for therapy is complex. In the last decade, inhibition of the proteasome has come into focus for the treatment of cardiac diseases. The irreversible proteasome inhibitor epoxomicin has been demonstrated to completely prevent the development of LVH in a mouse model of short-term pressure overload induced by transverse aortic constriction (TAC; [90]). Similarly, partial inhibition of the proteasome with low doses of the reversible proteasome inhibitor bortezomib significantly attenuated hypertrophic heart growth in hypertensive

Dahl salt-sensitive rats [91]. Furthermore, administration of epoxomicin two weeks after TAC, i.e. at a stage of pronounced hypertrophy, resulted in regression of hypertrophy and stabilization of cardiac function in mice [92]. Comparably, treatment with the irreversible proteasome inhibitor PS-519 significantly diminished isoprenaline-induced hypertrophy in mice [93]. However, conflicting data exist that argue against a cardioprotective role of proteasome inhibition. Chronic administration of bortezomib induced LVH to a similar extent as induced by TAC and resulted in heart failure and premature death in mice [80]. Chronic treatment with the reversible inhibitor MLN-273, an analogue of bortezomib, led to LVH, diastolic dysfunction and a reduction in cardiac output in pigs [94]. Importantly, while bortezomib is generally well tolerated by patients with multiple myeloma, this therapy was associated with the occurrence of cardiac complications, including cardiac dysfunction or even heart failure in elderly patients or patients with preexisting cardiac problems [95–98]. In summary, while complete and sustained proteasome inhibition, particularly under circumstances in which the UPS is already dysfunctional, is expected to rather worsen than to rescue the phenotype, partial or short-term proteasome inhibition may mediate a protective effect in the heart. An alternative therapeutic approach would consist in specifically targeting E3 ubiquitin ligases to reduce UPS-mediated protein degradation. For example, small molecule inhibitors of MDM2 have been developed to induce cancer cell death by stabilizing p53 protein levels [99]. However, they would be not suitable in the therapy of cardiac diseases due to enhanced cardiac apoptosis. Similarly, it has been shown that the UPS-mediated degradation of the cyclin-dependent kinase inhibitor p27 mediated pathological cardiac hypertrophy [100]. Therefore, stabilization of p27 level by targeting its specific E3 ubiquitin ligase SCF-SKP2 or by preventing its degradation using a specific inhibitor [101] could be beneficial. So far, no molecules targeting specifically the cardiac E3 ubiquitin ligases have been developed.

On the other hand, and in light of reduced proteasomal activities or global reduction of proteasome function that was observed in human and experimental models of cardiomyopathies [24,33,35,36,41,42,51,55,102], a reactivation of the proteasome function is expected to be more appropriate and beneficial. Support for this hypothesis came from a recent study showing that proteasomal enhancement induced by overexpression of proteasome activator 28 alpha (PA28 α) attenuated cardiac hypertrophy, delayed premature death, and protected against acute myocardial ischemia/reperfusion injury in a mouse model of DRM [103]. However, no drugs are currently available to mimic this effect. The first small compound capable of enhancing proteasome-mediated protein degradation via inhibiting the deubiquitinase USP14 was recently reported and used for neurodegenerative disorders associated with proteotoxicity [104]. However, it still remains to be evaluated in cardiac myocytes and in the heart *in vivo*. Another way to enhance proteasome function would be to target protein kinase G (PKG). PKG is activated by the PDE5 inhibitor sildenafil, which raises cGMP level. Sildenafil elicited reverse remodeling and improved LV diastolic function in failing patients and animal models [105–107]. Recently, the group of Wang showed that sildenafil activated the proteasome system [108]. Therefore, stimulation of PKG by sildenafil administration is potentially a novel therapeutic strategy to treat cardiomyopathies associated with UPS impairment.

5. Conclusion – future directions

The UPS regulates several functions involved in cardiac physiology. The recent identification of HCM gene mutations in a ubiquitin E3 ligase and dysregulation of several UPS components in HCM or DCM support the view that the UPS contributes to the pathogenesis of inherited cardiomyopathies. In light of the findings of UPS impairment, we believe that global proteasome inhibition is likely to be harmful, at least as a long-term treatment for inherited cardiomyopathies. On the other hand, global activation of the proteasome is likely to be a more promising approach to pursue. Finally, a comprehensive understanding of the mechanisms of UPS impairment in different models of inherited

cardiomyopathies, including cellular and animal models as well as human failing ventricular samples should result in the discovery of cardiac-specific targets within the complexity of the UPS for therapeutic benefit.

Disclosures

None.

Acknowledgment

This work was supported by the Leducq Foundation (Research grant Nr. 11, CVD 04), the seventh Framework Program of the European Union (Health-F2-2009-241577; BIG-Heart project), the DZHK (German Center for Cardiovascular Research) and the German Ministry of Research and Education (BMBF).

References

- Friedrich FW, Carrier L. Genetics of hypertrophic and dilated cardiomyopathy. *Curr Pharm Biotechnol* 2012;13:2467–76.
- Watkins H, Ashrafian H, Redwood C. Inherited cardiomyopathies. *N Engl J Med* 2011;364:1643–56.
- Richardson P, McKenna W, Bristow M, Maisch B, Mautner B, O'Connell J, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of cardiomyopathies. *Circulation* 1996;93:841–2.
- Zolk O, Schenke C, Sarikas A. The ubiquitin-proteasome system: focus on the heart. *Cardiovasc Res* 2006;70:410–21.
- Mearini G, Schlossarek S, Willis MS, Carrier L. The ubiquitin-proteasome system in cardiac dysfunction. *Biochim Biophys Acta* 2008;1782:749–63.
- Lilienbaum A. Relationship between the proteasomal system and autophagy. *Int J Biochem Mol Biol* 2013;4:1–26.
- Metzger MB, Hristova VA, Weissman AM. HECT and RING finger families of E3 ubiquitin ligases at a glance. *J Cell Sci* 2012;125:531–7.
- Komander D, Rape M. The ubiquitin code. *Annu Rev Biochem* 2012;81:203–29.
- Schlossarek S, Carrier L. The ubiquitin-proteasome system in cardiomyopathies. *Curr Opin Cardiol* 2011;26:190–5.
- Pagan J, Seto T, Pagano M, Cittadini A. Role of the ubiquitin proteasome system in the heart. *Circ Res* 2013;112:1046–58.
- Spaich S, Will RD, Just S, Kuhn C, Frank D, Berger IM, et al. F-box and leucine-rich repeat protein 22 is a cardiac-enriched F-box protein that regulates sarcomeric protein turnover and is essential for maintenance of contractile function in vivo. *Circ Res* 2012;111:1504–16.
- Tomko Jr RJ, Hochstrasser M. Molecular architecture and assembly of the eukaryotic proteasome. *Annu Rev Biochem* 2013;82:415–45.
- Kisselev AF, van der Linden WA, Overkleeft HS. Proteasome inhibitors: an expanding army attacking a unique target. *Chem Biol* 2012;19:99–115.
- Salomons FA, Acs K, Dantuma NP. Illuminating the ubiquitin/proteasome system. *Exp Cell Res* 2010;316:1289–95.
- Lindsten K, Menendez-Benito V, Masucci MG, Dantuma NP. A transgenic mouse model of the ubiquitin/proteasome system. *Nat Biotechnol* 2003;21:897–902.
- Kumarapeli AR, Horak KM, Glasford JW, Li J, Chen Q, Liu J, et al. A novel transgenic mouse model reveals deregulation of the ubiquitin-proteasome system in the heart by doxorubicin. *FASEB J* 2005;19:2051–3.
- Lindsten K, Dantuma NP. Monitoring the ubiquitin/proteasome system in conformational diseases. *Ageing Res Rev* 2003;2:433–49.
- Ciechanover A, Brundin P. The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* 2003;40:427–46.
- Weekes J, Morrison K, Mullen A, Wait R, Barton P, Dunn MJ. Hyperubiquitination of proteins in dilated cardiomyopathy. *Proteomics* 2003;3:208–16.
- Kostin S, Pool L, Elsasser A, Hein S, Drexler HC, Arnon E, et al. Myocytes die by multiple mechanisms in failing human hearts. *Circ Res* 2003;92:715–24.
- Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kaye R, Maloyan A, et al. Desmin-related cardiomyopathy in transgenic mice: a cardiac amyloidosis. *Proc Natl Acad Sci U S A* 2004;101:10132–6.
- Gianni D, Li A, Tesco G, McKay KM, Moore J, Raygor K, et al. Protein aggregates and novel presenilin gene variants in idiopathic dilated cardiomyopathy. *Circulation* 2010;121:1216–26.
- Birks EJ, Latif N, Enesa K, Folkvang T, Luong le A, Sarathchandra P, et al. Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy. *Cardiovasc Res* 2008;79:472–80.
- Predmore JM, Wang P, Davis F, Bartolone S, Westfall MV, Dyke DB, et al. Ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies. *Circulation* 2010;121:997–1004.
- Baumgarten A, Bang C, Tschirner A, Engelmann A, Adams V, von Haehling S, et al. TWIST1 regulates the activity of ubiquitin proteasome system via the miR-199/214 cluster in human end-stage dilated cardiomyopathy. *Int J Cardiol* 2013;168:1447–52.
- Elliott P, Andersson B, Arbustini E, Bilinska Z, Cecchi F, Charron P, et al. Classification of the cardiomyopathies: a position statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2008;29:270–6.
- Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, et al. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Thorac Cardiovasc Surg* 2011;142:e153–203.
- Frey N, Luedde M, Katus HA. Mechanisms of disease: hypertrophic cardiomyopathy. *Nat Rev Cardiol* 2012;9:91–100.
- Schlossarek S, Mearini G, Carrier L. Cardiac myosin-binding protein C in hypertrophic cardiomyopathy: mechanisms and therapeutic opportunities. *J Mol Cell Cardiol* 2011;50:613–20.
- Charron P, Carrier L, Dubourg O, Tesson F, Desnos M, Richard P, et al. Penetrance of familial hypertrophic cardiomyopathy. *Genet Couns* 1997;8:107–14.
- Friedrich FW, Wilding BR, Reischmann S, Crocini C, Lang P, Charron P, et al. Evidence for *FHL1* as a novel disease gene for isolated hypertrophic cardiomyopathy. *Hum Mol Genet* 2012;21:3237–54.
- Flavigny J, Souchet M, Sebillon P, Berrebi-Bertrand I, Hainque B, Mallet A, 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;294:443–56.
- Sarikas A, Carrier L, Schenke C, Doll D, Flavigny J, Lindenberg KS, et al. Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. *Cardiovasc Res* 2005;66:33–44.
- Mearini G, Gedicke C, Schlossarek S, Witt CC, Kramer E, Cao P, et al. Atrogin-1 and MuRF1 regulate cardiac MyBP-C levels via different mechanisms. *Cardiovasc Res* 2010;85:357–66.
- Bahrudin U, Morisaki H, Morisaki T, Ninomiya H, Higaki K, Nanba E, et al. Ubiquitin-proteasome system impairment caused by a missense cardiac myosin-binding protein C mutation and associated with cardiac dysfunction in hypertrophic cardiomyopathy. *J Mol Biol* 2008;384:896–907.
- Bahrudin U, Morikawa K, Takeuchi A, Kurata Y, Miake J, Mizuta E, et al. Impairment of ubiquitin-proteasome system by E334K cMyBP-C modifies channel proteins, leading to electrophysiological dysfunction. *J Mol Biol* 2011;413:857–78.
- Olivetto I, Girolami F, Ackerman MJ, Nistri S, Bos JM, Zachara E, et al. Myofibrillar protein gene mutation screening and outcome of patients with hypertrophic cardiomyopathy. *Mayo Clin Proc* 2008;83:630–8.
- Vignier N, Schlossarek S, Fraysse B, Mearini G, Kramer E, Pointu H, et al. Nonsense-mediated mRNA decay and ubiquitin-proteasome system regulate cardiac myosin-binding protein C mutant levels in cardiomyopathic mice. *Circ Res* 2009;105:239–48.
- Fraysse B, Weinberger F, Bardswell SC, Cuervo F, Vignier N, Geertz B, et al. Increased myofibrillar Ca(2+) sensitivity and diastolic dysfunction as early consequences of *Myhbc3* mutation in heterozygous knock-in mice. *J Mol Cell Cardiol* 2012;52:1299–307.
- Carrier L, Knoell R, Vignier N, Keller DI, Bausero P, Prudhon B, et al. Asymmetric septal hypertrophy in heterozygous cMyBP-C null mice. *Cardiovasc Res* 2004;63:293–304.
- Schlossarek S, Englmann DR, Sultan KR, Sauer M, Eschenhagen T, Carrier L. Defective proteolytic systems in *Myhbc3*-targeted mice with cardiac hypertrophy. *Basic Res Cardiol* 2012;107:1–13.
- Schlossarek S, Schuermann F, Geertz B, Mearini G, Eschenhagen T, Carrier L. Adrenergic stress reveals septal hypertrophy and proteasome impairment in heterozygous *Myhbc3*-targeted knock-in mice. *J Muscle Res Cell Motil* 2012;33:5–15.
- Crocini C, Arimura T, Reischmann S, Eder A, Braren I, Hansen A, et al. Impact of *ANKRD1* mutations associated with hypertrophic cardiomyopathy on contraction parameters of engineered heart tissue. *Basic Res Cardiol* 2013;108:349.
- Witt CC, Witt SH, Lerche S, Labeit D, Back W, Labeit S. Cooperative control of striated muscle mass and metabolism by MuRF1 and MuRF2. *EMBO J* 2008;27:350–60.
- Chen SN, Czernuszewicz G, Tan Y, Lombardi R, Jin J, Willerson JT, et al. Human molecular genetic and functional studies identify *TRIM63*, encoding Muscle RING Finger Protein 1, as a novel gene for human hypertrophic cardiomyopathy. *Circ Res* 2012;111:907–19.
- McNally EM, Golbus JR, Puckelwartz MJ. Genetic mutations and mechanisms in dilated cardiomyopathy. *J Clin Invest* 2013;123:19–26.
- Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, et al. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;366:619–28.
- Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, et al. A missense mutation in the alphaB-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet* 1998;20:92–5.
- Goldfarb LG, Dalakas MC. Tragedy in a heartbeat: malfunctioning desmin causes skeletal and cardiac muscle disease. *J Clin Invest* 2009;119:1806–13.
- Wang X, Osinska H, Klevitsky R, Gerdes AM, Nieman M, Lorenz J, et al. Expression of R120G-alphaB-crystallin causes aberrant desmin and alphaB-crystallin aggregation and cardiomyopathy in mice. *Circ Res* 2001;89:84–91.
- Chen Q, Liu JB, Horak KM, Zheng H, Kumarapeli AR, Li J, et al. Intracellular amyloidosis impairs proteolytic function of proteasomes in cardiomyocytes by compromising substrate uptake. *Circ Res* 2005;97:1018–26.
- Wang X, Osinska H, Dorn II GW, Nieman M, Lorenz JN, Gerdes AM, et al. Mouse model of desmin-related cardiomyopathy. *Circulation* 2001;103:2402–7.
- Liu J, Chen Q, Huang W, Horak KM, Zheng H, Mestril R, et al. Impairment of the ubiquitin-proteasome system in desminopathy mouse hearts. *FASEB J* 2006;20:362–4.
- Cattin ME, Muchir A, Bonne G. 'State-of-the-heart' of cardiac laminopathies. *Curr Opin Cardiol* 2013;28:297–304.
- Cattin ME, Bertrand AT, Schlossarek S, Le Bihan MC, Skov Jensen S, Neuber C, et al. Heterozygous *Lmna*ΔE32 mice develop dilated cardiomyopathy through a

- combined pathomechanism of haploinsufficiency and peptide toxicity. *Hum Mol Genet* 2013;22:3152–64.
- [56] Costa MW, Guo G, Wolstein O, Vale M, Castro ML, Wang L, et al. Functional characterization of a novel mutation in NKX2-5 associated with congenital heart disease and adult-onset cardiomyopathy. *Circ Cardiovasc Genet* 2013;6:238–47.
- [57] Ivandic BT, Mastitsky SE, Schonsiegel F, Bekeredjian R, Eils R, Frey N, et al. Whole-genome analysis of gene expression associates the ubiquitin-proteasome system with the cardiomyopathy phenotype in disease-sensitized congenic mouse strains. *Cardiovasc Res* 2012;94:87–95.
- [58] Carrier L, Schlossarek S, Willis MS, Eschenhagen T. The ubiquitin-proteasome system and nonsense-mediated mRNA decay in hypertrophic cardiomyopathy. *Cardiovasc Res* 2010;85:330–8.
- [59] Marston S, Copeland O, Gehmlich K, Schlossarek S, Carrier L. How do MYBPC3 mutations cause hypertrophic cardiomyopathy? *J Muscle Res Cell Motil* 2012;33:75–80.
- [60] van Dijk SJ, Dooijes D, Dos Remedios C, Michels M, Lamers JM, Winegrad S, et al. Cardiac myosin-binding protein C mutations and hypertrophic cardiomyopathy. Haploinsufficiency, deranged phosphorylation, and cardiomyocyte dysfunction. *Circulation* 2009;119:1473–83.
- [61] van Dijk SJ, Paalberends ER, Najafi A, Michels M, Sadayappan S, Carrier L, et al. Contractile dysfunction irrespective of the mutant protein in human hypertrophic cardiomyopathy with normal systolic function. *Circ Heart Fail* 2012;5:36–46.
- [62] Laporte D, Salin B, Daignan-Fornier B, Sagot I. Reversible cytoplasmic localization of the proteasome in quiescent yeast cells. *J Cell Biol* 2008;181:737–45.
- [63] Kopito RR. Aggresomes, inclusion bodies and protein aggregation. *Trends Cell Biol* 2000;10:524–30.
- [64] Liu J, Tang M, Mestrlil R, Wang X. Aberrant protein aggregation is essential for a mutant desmin to impair the proteolytic function of the ubiquitin-proteasome system in cardiomyocytes. *J Mol Cell Cardiol* 2006;40:451–4.
- [65] Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med* 2000;29:222–30.
- [66] Davies KJ. Degradation of oxidized proteins by the 20S proteasome. *Biochimie* 2001;83:301–10.
- [67] Drews O, Tsukamoto O, Liem D, Streicher J, Wang Y, Ping P. Differential regulation of proteasome function in isoproterenol-induced cardiac hypertrophy. *Circ Res* 2010;107:1094–101.
- [68] Day SM, Divald A, Wang P, Davis F, Bartolone S, Jones R, et al. Impaired assembly and post-translational regulation of 26S proteasome in human end-stage heart failure. *Circ Heart Fail* 2013;6:544–9.
- [69] Lu H, Zong C, Wang Y, Young GW, Deng N, Souda P, et al. Revealing the dynamics of the 20S proteasome phosphoproteome: a combined CID and electron transfer dissociation approach. *Mol Cell Proteomics* 2008;7:2073–89.
- [70] Asai M, Tsukamoto O, Minamoto T, Asanuma H, Fujita M, Asano Y, et al. PKA rapidly enhances proteasome assembly and activity in *in vivo* canine hearts. *J Mol Cell Cardiol* 2009;46:452–62.
- [71] Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? *Circ Res* 2003;93:896–906.
- [72] Eschenhagen T. Beta-adrenergic signaling in heart failure—adapt or die. *Nat Med* 2008;14:485–7.
- [73] Movsesian MA, Bristow MR. Alterations in cAMP-mediated signaling and their role in the pathophysiology of dilated cardiomyopathy. *Curr Top Dev Biol* 2005;68:25–48.
- [74] Sequeira V, Wijnter PJ, Nijenkamp LL, Kuster DW, Najafi A, Witjas-Paalberends ER, et al. Perturbed length-dependent activation in human hypertrophic cardiomyopathy with missense sarcomeric gene mutations. *Circ Res* 2013;112:1491–505.
- [75] El-Armouche A, Pohlmann L, Schlossarek S, Starbatty J, Yeh YH, Nattel S, et al. Decreased phosphorylation levels of cardiac myosin-binding protein-C in human and experimental heart failure. *J Mol Cell Cardiol* 2007;43:223–9.
- [76] Kuster DW, Bawazeer AC, Zaremba R, Goebel M, Boontje NM, van der Velden J. Cardiac myosin binding protein C phosphorylation in cardiac disease. *J Muscle Res Cell Motil* 2012;33:43–52.
- [77] Nastasi T, Bongiovanni A, Campos Y, Mann L, Toy JN, Bostrom J, et al. Ozz-E3, a muscle-specific ubiquitin ligase, regulates beta-catenin degradation during myogenesis. *Dev Cell* 2004;6:269–82.
- [78] Li HH, Kedar V, Zhang C, McDonough H, Arya R, Wang DZ, 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;114:1058–71.
- [79] Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. *Nature* 1997;387:296–9.
- [80] Tang M, Li J, Huang W, Su H, Liang Q, Tian Z, et al. Proteasome functional insufficiency activates the calcineurin-NFAT pathway in cardiomyocytes and promotes maladaptive remodelling of stressed mouse hearts. *Cardiovasc Res* 2010;88:424–33.
- [81] Groenendyk J, Sreenivasaiah PK, Kim do H, Agellon LB, Michalak M. Biology of endoplasmic reticulum stress in the heart. *Circ Res* 2010;107:1185–97.
- [82] Jager R, Bertrand MJ, Gorman AM, Vandenabeele P, Samali A. The unfolded protein response at the crossroads of cellular life and death during endoplasmic reticulum stress. *Biol Cell* 2012;104:259–70.
- [83] Fu HY, Minamoto T, Tsukamoto O, Sawada T, Asai M, Kato H, et al. Overexpression of endoplasmic reticulum-resident chaperone attenuates cardiomyocyte death induced by proteasome inhibition. *Cardiovasc Res* 2008;79:600–10.
- [84] Zheng Q, Wang X. Autophagy and the ubiquitin-proteasome system in cardiac dysfunction. *Panminerva Med* 2010;52:9–25.
- [85] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell* 2008;132:27–42.
- [86] Ding WX, Yin XM. Sorting, recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome. *Autophagy* 2008;4:141–50.
- [87] Lamark T, Johansen T. Autophagy: links with the proteasome. *Curr Opin Cell Biol* 2010;22:192–8.
- [88] Zheng Q, Su H, Ranek MJ, Wang X. Autophagy and p62 in cardiac proteinopathy. *Circ Res* 2011;109:296–308.
- [89] Zheng Q, Su H, Tian Z, Wang X. Proteasome malfunction activates macroautophagy in the heart. *Am J Cardiovasc Dis* 2011;1:214–26.
- [90] Depre C, Wang Q, Yan L, Hedhli N, Peter P, Chen L, et al. Activation of the cardiac proteasome during pressure overload promotes ventricular hypertrophy. *Circulation* 2006;114:1821–8.
- [91] Meiners S, Dreger H, Fechner M, Bieler S, Rother W, Gunther C, et al. Suppression of cardiomyocyte hypertrophy by inhibition of the ubiquitin-proteasome system. *Hypertension* 2008;51:302–8.
- [92] Hedhli N, Lizano P, Hong C, Fritzy LF, Dhar SK, Liu H, et al. Proteasome inhibition decreases cardiac remodeling after initiation of pressure overload. *Am J Physiol Heart Circ Physiol* 2008;295:H1385–93.
- [93] Stansfield WE, Tang RH, Moss NC, Baldwin AS, Willis MS, Selzman CH. Proteasome inhibition promotes regression of left ventricular hypertrophy. *Am J Physiol Heart Circ Physiol* 2008;294:H645–50.
- [94] Herrmann J, Wohlert C, Saguner AM, Flores A, Nesbitt LL, Chade A, et al. Primary proteasome inhibition results in cardiac dysfunction. *Eur J Heart Fail* 2013;15:614–23.
- [95] Enrico O, Gabriele B, Nadić A, Sara G, Daniele V, Giulia C, et al. Unexpected cardiotoxicity in haematological bortezomib treated patients. *Br J Haematol* 2007;138:396–7.
- [96] Hachaneffoglu A, Tarkun P, Gonullu E. Acute severe cardiac failure in a myeloma patient due to proteasome inhibitor bortezomib. *Int J Hematol* 2008;88:219–22.
- [97] Bockorny M, Chakravarty S, Schulman P, Bockorny B, Bona R. Severe heart failure after bortezomib treatment in a patient with multiple myeloma: a case report and review of the literature. *Acta Haematol* 2012;128:244–7.
- [98] Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *N Engl J Med* 2005;352:2487–98.
- [99] Weissman AM, Yang Y, Kitagaki J, Sasiela CA, Beutler JA, O'Keefe BR. Inhibiting Hdm2 and ubiquitin-activating enzyme: targeting the ubiquitin conjugating system in cancer. *Ernst Schering Found Symp Proc*; 2008. p. 171–90.
- [100] Hauck L, Harms C, An J, Rohne J, Gertz K, Dietz R, et al. Protein kinase CK2 links extracellular growth factor signaling with the control of p27(Kip1) stability in the heart. *Nat Med* 2008;14:315–24.
- [101] Wu L, Grigoryan AV, Li Y, Hao B, Pagano M, Cardozo TJ. Specific small molecule inhibitors of Skp2-mediated p27 degradation. *Chem Biol* 2012;19:1515–24.
- [102] Tsukamoto O, Minamoto T, Okada K, Shintani Y, Takashima S, Kato H, et al. Depression of proteasome activities during the progression of cardiac dysfunction in pressure-overloaded heart of mice. *Biochem Biophys Res Commun* 2006;340:1125–33.
- [103] Li J, Horak KM, Su H, Sanbe A, Robbins J, Wang X. Enhancement of proteasomal function protects against cardiac proteinopathy and ischemia/reperfusion injury in mice. *J Clin Invest* 2011;121:3689–700.
- [104] Lee BH, Lee MJ, Park S, Oh DC, Elsasser S, Chen PC, et al. Enhancement of proteasome activity by a small-molecule inhibitor of USP14. *Nature* 2010;467:179–84.
- [105] Takimoto E, Champion HC, Li M, Belardi D, Ren S, Rodriguez ER, et al. Chronic inhibition of cyclic GMP phosphodiesterase 5A prevents and reverses cardiac hypertrophy. *Nat Med* 2005;11:214–22.
- [106] Guazzi M, Vicenzi M, Arena R, Guazzi MD. PDE5 inhibition with sildenafil improves left ventricular diastolic function, cardiac geometry, and clinical status in patients with stable systolic heart failure: results of a 1-year, prospective, randomized, placebo-controlled study. *Circ Heart Fail* 2011;4:8–17.
- [107] Bishu K, Hamdani N, Mohammed SF, Kruger M, Ohtani T, Ogut O, et al. Sildenafil and B-type natriuretic peptide acutely phosphorylate titin and improve diastolic distensibility *in vivo*. *Circulation* 2011;124:2882–91.
- [108] Ranek MJ, Terpstra EJ, Li J, Kass DA, Wang X. Protein kinase g positively regulates proteasome-mediated degradation of misfolded proteins. *Circulation* 2013;128:365–76.