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*Review Article*

# Role of ubiquitin-proteasome system (UPS) in left ventricular hypertrophy (LVH)

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**Abstract:** Cardiac hypertrophy is a key compensatory mechanism acting in response to pressure or volume overload, involving some alterations in signaling transduction pathways and transcription factors-regulation. These changes result in enhanced proteins' synthesis leading to Left Ventricular Hypertrophy (LVH). It is known that the main function of Ubiquitin-Proteasome System (UPS) is to prevent accumulation of damaged, misfolded and mutant proteins by proteolysis. But emerging evidences suggest that UPS also attends to the cells' growth, favoring proteins' synthesis, subsequently evolving in LVH. The role of the proteasome in to favor cellular hypertrophy consists in upregulation of the catalytic proteasome subunit, with prevalence of proteins-synthesis on proteins degradation. It is also evident that UPS inhibition may prevent cells' growth opposing to the hypertrophy. In fact in several experimental models, UPS inhibition demonstrated to be able to prevent or reverse cardiac hypertrophy induced by abdominal aortic banding (AAB). That can happen with several proteasome inhibitors acting by multifactorial mechanisms. These evidences induce to hypothesize that, in the future, in patients with the increased volume overload by systemic hypertension, some proteasome-inhibitors could be used to antagonize or prevent LVH without reducing peripheral high blood pressure levels too.

**Keywords:** Ubiquitin-proteasome system (UPS), proteasome-inhibitors, systemic hypertension, increased overload, left ventricular hypertrophy (LVH)

## Introduction

Left ventricular hypertrophy (LVH) consists in an increase of the left ventricular mass secondary to the increase of myocardial walls', **added** in response to pressure or volume overload. While physiological hypertrophy results from physical exercise, pathological hypertrophy responds to the events, such as volume or pressure overload, induced by some conditions as systemic hypertension (SH), aortic or sub-aortic stenosis, or genetic abnormalities [1]. From functional point of view, pathological LVH is distinguished from physiological LVH because myocardial adaptation is unable to satisfy the increased demand, or able to meet this same at the expense of normal LV size and function. So, physiological LVH is defined "adaptive", pathological LVH is named "maladaptive". One key element of LVH is an adaptation of proteins' turnover (e.g. both protein synthesis and degradation), with increase in the volume of cardiac myocytes for an acceleration of synthesis or a reduced degradation rate of constitutive proteins. The increase in protein synthesis rate in the heart results in an increase of sarcomeric proteins by individual cardiac myocytes in response to some factors. In this review, we only refer on the maladaptive LVH induced by SH induced by several biochemical pathways as angiotensin II, endothelin-1, nor-epinephrine, Rho or Ras proteins. Oxidative stress, heat shock proteins, calcineurin and some kinases also play a critical role [2].

### *Ubiquitin*

The Ubiquitin-Proteasome System (UPS) represents the main non-lysosomal mechanism of intracellular cytosolic and nuclear intracellular protein degradation [3]. UPS plays key roles in protein quality by removing damaged, oxidized and/or misfolded proteins [4, 6]. Two consecutive steps characterize the proteolytic activity of UPS. The first one is the ubiquitination, that consists in an enzymatic post-translational

modification of damaged or misfolded proteins. Subsequently, these are transported to the proteasome for their degradation. Ubiquitin has taken its name from it ubiquitous presence in cells. The ubiquitination process involves three enzymatic steps [7]: activation, conjugation, and ligation. In the first step, ubiquitin is activated by an ubiquitin-activated enzyme ($E_1$) in a reaction that requires ATP hydrolysis. In the second step, the activated ubiquitin is transferred to ubiquitin-conjugating-enzyme ($E_2$). Finally, $E_2$ transfers the ubiquitin moiety from $E_1$ to target protein, which is recognized by the ubiquitin-protein ligase ($E_3$). Once the first ubiquitin is bound to its target, the elongation of the poly-ubiquitin chain happens. This is performed by $E_2$ and $E_3$-polyubiquitin chains and can be stabilized and extended further by the action of $E4_S$. This chain subsequently transfers the client protein to the proteasome for degradation [7]. The proteasome degradation represents the second phase of the UPS proteolytic action [8]. UPS attends to some important neurological and cardiovascular impairment. Our group demonstrated that it may favor the carotid plaques' rupture in diabetic patients. These results are presumably given by enhanced inflammatory activity induced by oxidative stress [9].

*Proteasome*

Proteasome is an eukaryotic structure present both in the cytoplasm and in the nucleus. It is composed of two complexes of proteins: the proteolytic core, or 20S proteasome, **added** containing 28 subunits [10], and one or two regulatory complexes (known as the 19S regulatory complexes), also called proteasome activator [11]. The association of the proteolytic core with the regulatory complex results in a macromolecular structure that has become known as the proteasome 26S [12]. The proteolytic process by proteasome happens in two distinct steps: denaturation (or unrolled mechanism) happening in the regulatory complex (19S) and degradation fulfilled oneself in the proteolytic core (20S). These proteolytic actions in the proteasome occur for the interventions of some enzymes, also called proteases. The clinical importance of the proteasome is rapidly expanding. A proteasome dysfunction can involve in skeletal muscles' loss, common in a number of conditions (as cancer, AIDS, sepsis, renal failure), and in some neurodegenerative disease, as Huntington', stroke, Parkinson, amyotrophic lateral sclerosis' and Alzheimer's diseases [13]. Another potential clinical application of proteasome inhibition is the regression of cancer growth occurred by bortezomib, the first proteasome inhibitor approved for clinical use [14, 15]. But, UPS also acts in regulating proteins' turnover, and its role in maintaining cardiac protein quality control recently is emerging. In this connection, Depre et al. evidenced that proteasome is responsible for both proteolysis and protein synthesis [16]. The role of the proteasome in increasing cardiac tissue has been recently reviewed, even though the process remains largely unknown too. Proteasome protein subunit expression increased when the synthesis of *de novo* proteins exceeds protein degradation, such as found in cardiac cells **added** during chronic pressure overload. Specifically, the raise in protein synthesis induces an upregulation of the catalytic proteasome subunit. An increase in proteasome subunit protein expression was well documented in several models of cardiac hypertrophy [17]. That happens because proteasome promotes the degradation of inhibitors of hypertrophy, such as the inducible cyclic AMP early repressor (ICER) [18]. Another potential mechanism is related to the ribosome assembly (protein synthesis). The third potential mechanism consists in modifying the activity of the elongation factor which participates to protein degradation. In turn, the elongation factor 1A (eF1A) binds amino-acyl tRNA to the A site of the ribosomes [19]. In addition, an experimental study performed on the yeast demonstrated that cellular stress promotes a translocation of the proteasome from the cytosol to the nuclear periphery, where it participates to cell survival by overexpression of the chaperone H11 kinase/Hsp22. This response was accompanied by an increase in proteasome protein expression, an increase in chymotryptic activities, and a decrease in phosphorylation of the α7 protein [20].

*Proteasome inhibitors*

Whereas the activation of cardiac proteasome during pressure overload promotes ventricular hypertrophy [21], Proteasome inhibition induces the block of the proteolytic actions of proteasomes, affecting cardiac cell growth in both adaptive and maladaptive models of cardiac hypertrophy. The mechanism by which protea-

some inhibition prevents the development of cardiac hypertrophy is multifactorial. Stansfield et al. found that the PS-519, a proteasome-inhibitor that permanently interrupts the chymotryptic activity of the 20S proteasome, prevents LVH and promote its regression by blocking the $I_{\kappa}B$-degradation. It is known that this is able to prevent the nuclear translocation and activation of NF-$_{\kappa}$B, an inflammatory transcription factor involved in cardiac hypertrophy [22]. Previously, several groups have evidenced that inhibition of NF-$_{\kappa}$B may attenuate the development of hypertrophy both in vitro and in vivo [23, 24]. It is known that the first proteasome-inhibitor approved for clinical use is PS-341, also known as Bortezomib (Velcade). It is a dipeptide boronic acid which reversibly inhibits the chymotryptic-like activity and the caspase-like activity of the proteasome. This is functionally similar but structurally different from PS-519 because partially acts inhibiting NF-$_{\kappa}$B [25]. Mainers et al. described that low doses of Velcade induced a significant suppression of cardiac hypertrophy by partially inhibiting the chymotrypsin-like activity in the liver [26]. It must be referred that this proteasome inhibitor is also employed for the regression of multiple myeloma, other hematological malignances, and some solid tumors [27], even if some authors have mentioned a potential cardiotoxicity of bortezomib in patients treated for malignancies [28]. Another proteasome-inhibitor is MG-262. In an experimental model, Tang et al. showed that a dose of this inhibitor is sufficient to reduce proteasomal activity [29]. Interestingly, the study of Tang et al. also showed that an acute dose of MG-262 was sufficient to reduce proteasomal activity and to increase the activity of calcineurin-nuclear factors of activated T cell (NFATs) signaling in cardiac myocytes [29]. But, the cardiac effects can be different depending on the given doses. In fact, a low dose of the proteasome inhibitor may prevent or interrupt LVH, by an interference with the degradation of antihypertrophic factors, whereas a high dose may give additional stress to the heart and promote transition to heart failure, via activation of the calcineurin-NFAT pathway. In animal models, Hedhli et al. showed that the proteasome inhibitor, Epoxomicin, induces a regression of cardiomyocyte hypertrophy and affects collagen degradation at the same time (maintaining their mutual relation), via activation of matrix metalloproteinases (MMPs) [30]. Another specific proteasome inhibitor, MG132, has recently demonstrated to reduce LVH in rats through regulation of ERK1/2 and JNK1 signaling pathway, that contributes to the hypertrophic growth. The cardioprotection of MG132 is at least in part, caused by the interruption of the activation of mitogen-activated protein kinase (MAPK) signaling pathway [31]. Treatment with this inhibitor, reducing LVH, improves cardiac function as determined by increased fractional shortening, attenuated LV diastolic pressure, and decreased lung weight/Body-Weight ratio. In their study, the authors found that MG132 has no effect on blood pressure [31], demonstrating that MG132 may be used as a pharmacological inhibitor of LVH, independently of to reduce high levels of systemic pressure.

## Conclusive remarks and future directions

LVH represents the response to the increased ventricular wall stress and is a compensatory mechanism that allows maintenance of normal cardiac function until a definite value of the increased overload. Several studies demonstrated that LVH is a more important risk factor for cardiovascular morbility and mortality in hypertensive patients. It is known that LVH requires an increase in the cellular accumulation of proteins resulting from the synthesis of *de novo* proteins exceeding protein degradation [32]. UPS is the major non-lysosomal pathway for intracellular proteins'-degradation. Its activation has been described in different models of cells' hypertrophy. Specifically, cardiac cell growth is accompanied by a proteasome subunit dysfunction provoked by several mechanisms. On the contrary, proteasome inhibition results in suppressing protein synthesis that contrasts cells' growth, opposing in this way to the progression of myocyte hypertrophy. It was also evidenced that the treatment of animal models with a specific a proteasome inhibitor suppresses the development of the extracellular matrix (collagen), that supports the myocardial cells. These acquisitions could be used for LVH treatment. With reference to this issue, it is known that the main measures to blunt SH and consequent LVH include several anti-hypertensive drugs. ACE-inhibitors that block the renin-angiotensin axis and β-blockers that attenuate the sympathetic stimulation are included among these drugs. Inhibitors of angiotensin-converting enzyme involve a decrease in after-

load, a decrease in scar tissue, an inhibition of apoptosis, and a decrease in LVH [33]. On the other hand, β-blockers reduce high values of systo-diastolic pressure, and antagonize the LVH development at same time [34]. As far as we previously affirmed, proteasome inhibitors could participate in the anti-hypertrophic effects of ACE-inhibitors and β-blockers, although this has not been explored in the heart. In the future, some of these (such as MG132) could be used as components of anti-hypertensive drugs, exclusively opposing to LVH and decreased left contractile function, without affecting the high blood pressure levels, reducing the major risk factors induced by SH.

## Disclosure of conflict of interest

None.

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