Editorial Commentary: Dichotomous actions of the E3-ligase Ring TRIMmers in cardiac myocytes☆

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The ubiquitin-proteasome system (UPS) is responsible for maintaining cellular homeostasis and protein quality control. The removal of cellular proteins involves a complex series of enzymatic ubiquitination reactions that is shared among all living cells, including those of the cardiovascular system. Protein degradation is a multi-step process, which involves three enzymatic reactions that begin with the covalent modification of the target protein with ubiquitin in the following steps (i) *Activation* involves an ATP dependent E1 ubiquitin-activating enzyme, (ii) *Conjugation* with an E2 ubiquitin-conjugating enzyme and (iii) *Ligation* by E3 ubiquitin ligase. There are hundreds of different E3 enzymes and, for that reason, E3 ligase serves as the substrate recognition site of the UPS.

The cardiovascular UPS is highly dynamic, and is extremely important for maintaining cardiac tissue homeostasis and protein turnover. However, increasing evidence suggests that dysfunction of the UPS resulting in the accumulation of ubiquitinated proteins plays a major role in the pathogenesis of many cardiovascular disease outcomes. In this regard, the most widely studied example of protein ubiquitination in the heart is in the context of cardiac hypertrophy and heart failure. During cardiac hypertrophy, protein turnover, which includes both protein synthesis and protein clearance, is dramatically increased. The therapeutic potential of either activating or inhibiting the UPS has yielded conflicting outcomes. Notably, increased rates of protein clearance following hypertrophy are required to ensure that abnormally synthesized proteins are efficiently eliminated. However, excessive degradation of defective proteins, which is often accompanied by impaired proteasomal function, may contribute to the development of heart failure. Pharmacological inhibition of the proteasome has also demonstrated contradictory outcomes according to treatment duration. For example, the proteasome inhibitor Bortezomib was shown to reverse cardiac hypertrophy in an acute treatment protocol in rat cardiomyocytes. Bortezomib both reduced cell size and inhibited hypertrophy-mediated induction of mRNA and protein synthesis [1]. However, chronic treatment of mice with Bortezomib induced pathological hypertrophy leading to heart failure and increased mortality [2]. This contradiction between acute and chronic administration of Bortezomib highlights the importance of having a delicate balance between protein synthesis and protein degradation in order to maintain cellular protein homeostasis.

The role of the UPS during ischemia-reperfusion (I-R) injury also yields conflicting findings. On one hand, pharmacological inhibitors of the proteasome significantly reduced myocardial infarct size and inflammation in the myocardium following I-R [3]. On the other hand, I-R injury was shown to be attenuated by proteasome activity concordant with increased levels of ubiquitinated proteins [4]. Enhancement of the proteolytic function of the proteasome in mice resulted in a reduction in myocardial infarct size and post-reperfusion cardiac dysfunction [5].

Another cardiovascular pathology commonly associated with impairment of the UPS is familial cardiomyopathy. Increased levels of ubiquitination that results from diminished UPS response was observed in desmin-related cardiomyopathy. In this disease, mutations in desmin and its protein aggregates cause impairment in the ubiquitin system. Increased levels of E1 and E2 enzymes together with impaired ubiquitination, results in the clinical appearance of heart hypertrophy and failure [6]. Taken together, these studies suggest that proteasome inhibition and increased ubiquitination may provide a benefit under specific conditions, while assuming a harmful role under other conditions. Moreover, the extent of UPS manipulation should also be taken into account since total inhibition or total activation may lead to cardiotoxicity or have secondary effects on other physiological processes.

Notably, the tripartite motif-containing (TRIM) protein, a subclass of the type E3 ligase, the really interesting new gene (RING), ubiquitin ligases are the focus in the current issue of Trends in Cardiovascular Medicine. In this review, Borlepawar et al. [7] provide a comprehensive overview of TRIM proteins, and their therapeutic potential in treating cardiovascular disease. The Muscle Ring Fingers (MuRFs) are among the most studied form of TRIMs. Genetic manipulation of MuRFs provided profound information regarding their role in the cardiac myocytes. For example, cardiac-specific overexpression of MuRF1 resulted in wide spread disruption of many primary metabolic processes, including alterations in creatine kinase (CK) activity and increased susceptibility to heart failure [8]. However, MuRF1 was also shown

to have a protective effect during I-R injury. This was attributed to its roles in controlling production of reactive oxygen species (ROS), regulation of mitochondrial function and apoptosis [9]. Furthermore, MuRF1 also plays a key role in suppressing cardiac myocyte fatty acid oxidation, suggesting ubiquitin ligase is responsible for regulating cardiac metabolism in the failing heart [10].

Another family member of the TRIM proteins is MuRF2, which can heterodimerize with MuRF1. Double knockout of these two MuRFs may lead to development of skeletal and cardiac hypertrophy [8] and is associated with hypertrophic cardiomyopathy [11]. The third key MuRF protein is MuRF3. Knockout MuRF3 mice have higher incidences of cardiac rupture following acute myocardial infarction [12]. Interestingly, simultaneous absence of MuRF1 and MuRF3 triggered skeletal muscle myopathy and hypertrophic cardiomyopathy [13], whereas double knockout of MuRF2 and MuRF3 resulted in protein aggregation and skeletal myopathy. MuRF2 and MuRF3-deficient hearts showed decreased systolic and diastolic function, increased expression of the myosin heavy chain isoform and disturbed calcium handling [14]. This suggests a dual role for MuRF2 and MuRF3 in the maintenance of skeletal muscle and cardiac structure and function. Borlepawar et al. point out that the overlapping functions of all three MuRFs may be due to one mutual substrate shared by MuRFs 1, 2 and 3, as revealed in metabolomics analysis [15]. Taken together, these findings suggest that MuRFs are key E3 ligases in the UPS with protective roles in various cardiovascular diseases, through regulation of metabolic processes.

TRIM8 is another (RING)- type E3 ligase that plays a deleterious role in pressure overload-induced cardiac hypertrophy. Mice and neonatal rat cardiomyocytes overexpressing TRIM8 exhibited exacerbated cardiac hypertrophy following aortic banding. In contrast, this effect was rescued when TRIM8 was deficient in the heart [16]. Unlike TRIM8 and its contribution to cardiac hypertrophy, TRIM32 was found to have a protective role in pressure overload induced hypertrophy. This effect could be explained mechanistically through inhibition of AKT signaling, which in turn had beneficial effects on preventing heart failure. This finding was further supported by assessing TRIM32 levels in individuals with hypertrophic cardiomyopathy, which revealed reduced expression levels [17]. TRIM32 was also found to be a key regulator of cell viability and apoptosis in cardiomyocytes via simultaneous activation of p53 and caspase3/7 and inhibition of X-linked inhibitor of apoptosis [18]. Genetically over expressing TRIM32 was shown to rescue hypertrophic effect, which may suggest TRIM32 as a novel therapeutic target in preventing cardiac hypertrophy and heart failure. However, taking into consideration the effects of TRIM32 on apoptotic cells death, this potential therapeutic approach should be interpreted with caution. Interestingly, increased levels of TRIM24 were associated with hypertrophic and dilated cardiomyopathy in the heart. This may be explained by TRIM24 being a binding protein of the pro-hypertrophic protein Dysbinidin. Interestingly, when TRIM24 binds to Dysbinidin, proteasomal degradation by TRIM32 is rescued and leads to protective effects against hypertrophy [18].

TRIM21 plays an essential role in p62-regulated redox homeostasis by ubiquitinating the p62 protein. Cellular deficiency of TRIM21 enhances antioxidant response and reduced cell death in response to oxidative stress. Mice deficient in TRIM21 have less oxidative damage caused by pressure overload heart injury due to increased accumulation of p62 and reduced ROS production [19]. Lastly, TRIM72 has a fundamental role in metabolic disorders and has been implicated in diabetic cardiomyopathy. Notably, TRIM72 has dual roles in the regulation of heart pathology. On one hand, TRIM72 was found to protect the heart from I-R injury, but on the other hand, TRIM72 was found to have a role in developing skeletal muscle insulin resistance. Moreover, TRIM72 is known to con-

trol clearance of insulin receptors and insulin receptor substrate 1, which leads to dyslipidemia and hypertension [20]. Interestingly, TRIM72 is not expressed in the human heart. However, its roles in controlling heart physiology and pathophysiology led to an intriguing study that tested TRIM72 as a biomarker of myocardial injury. This study suggested that since TRIM72 is absent in the human heart, it cannot hold utility as a clinical biomarker of myocardial injury in humans, but its cardioprotective roles could have potential therapeutic applications by using recombinant TRIM72 techniques [21].

The comprehensive and detailed review by Borlepawar et al. [7] highlights the most studied TRIMs in the cardiovascular pathophysiology. This review demonstrates the dual roles of the UPS and especially TRIMs in cardioprotection, by either inhibiting or activating the expression levels of TRIM members. However, it also raises several questions that should be addressed in future studies, such as: (i) How does systemic versus specific upregulation or downregulation of TRIMs affect other organs and tissues of the body? (ii) What is the interaction between the different TRIMs and how does activation or inhibition of one TRIM affects another TRIM? (iii) What is the switch for each of the TRIMs that leads to either adaptive or maladaptive degradation by the UPS? In conclusion, this review emphasizes a very central mechanism of the cellular homeostasis, which has tremendous clinical potential in the cardiovascular pathophysiology. Further studies that include bioinformatics analyses and screening for possible new candidates and their interactions would be able to shed more light on their cellular mechanisms. However, the thin line that separates the dual effects of the TRIMs should be kept in mind in order to avoid unwanted outcomes and worsening of existing conditions.

Acknowledgment

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