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REVIEW

Post-translational Modifications in Heart Failure: Small Changes, Big Impact

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Heart failure is a complex disease process with various aetiologies and is a significant cause of morbidity and death world-wide. Post-translational modifications (PTMs) alter protein structure and provide functional diversity in terms of physiological functions of the heart. In addition, alterations in protein PTMs have been implicated in human disease pathogenesis. Small ubiquitin-like modifier mediated modification (SUMOylation) pathway was found to play essential roles in cardiac development and function. Abnormal SUMOylation has emerged as a new feature of heart failure pathology. In this review, we will highlight the importance of SUMOylation as a regulatory mechanism of SERCA2a function, and its therapeutic potential for the treatment of heart failure.

Keywords Heart failure • Post-translational modifications • SUMO • SERCA2a

Introduction

Congestive heart failure is a major cause of morbidity and mortality throughout the world [1,2]. Over 23 million people world-wide and more than 5.8 million adults in the United State are living with heart disease. Heart failure accounts for over one million hospitalisations, nearly 300,000 deaths, and approximately 40 billion dollars in Medicare expenses annually with five-year survival being less than 50% in the United States despite advances in pharmacological treatment and device therapy [1,3,4]. Thus, there is an ultimate need for novel therapies to treat heart failure. Over the last decade, many studies have focussed on the elucidation of new molecular mechanisms associated with heart failure to identify novel drug targets. Positive inotropic agents, which stimulate and increase muscle contractility, have become the primary focus of new therapeutic approaches for the treatment of heart failure [5–7].

Post-translational modification with small ubiquitin-like modifier (SUMO) protein, SUMOylation, plays a significant role in the functional regulation of proteins by altering structure, enzymatic activity, stability or degradation, localisation, protein-protein interactions, and diverse signalling cascades [8]. SUMOylation is responsible for the dynamic reaction to environmental stimuli in physiological and pathological states. Altered SUMOylation of specific proteins has been linked to diverse human diseases and disorders [9–11]. In addition, recent studies support an important role of SUMOylation in pathogenic mechanisms involved in heart failure such as oxidative stress and hypertrophic stimuli [12].

In this review, we summarise the role of SUMOylation in the heart. In particular, we focus on the role of SUMO1 in SERCA2a-mediated heart function and discuss the need for targeting SERCA2a SUMOylation as a new therapeutic approach to treat heart failure.

SUMOylation/deSUMOylation Process

Ubiquitin (Ub) and ubiquitin-like proteins (Ubls) can covalently bind to substrates and subsequently regulate their function [13]. SUMO belongs to a large family of ubiquitin-related proteins. There are four SUMO isoforms (SUMO1, SUMO2, SUMO3 and SUMO4) in mammalian cells. SUMO2

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and SUMO3 are almost identical (only three N-terminal residues are different), whereas SUMO1 is approximately 48% identical at the amino acid level to SUMO2/3. SUMO4 shares 85% identity with SUMO2/3, however it is not clear whether SUMO4 can be processed and conjugated to substrates [14–16]. SUMO isoforms are functionally distinct as they modify different substrates [17–19] and they differ in their subcellular localisation patterns and dynamics [17,20]. SUMO reversibly attaches to lysine residues via enzymatic cascade reaction called SUMOylation [21]. Similar to ubiquitin, all SUMO proteins need to be processed by specific cysteine proteases, SENP (Sentrin-specific Protease), into their mature form. The mature SUMO is then adenylated by SUMO E1 activating enzyme (SAE1/SAE2) in an ATP dependent reaction and is subsequently transferred to a SUMO E2 conjugating enzyme (Ubc9) [22,23]. In some cases, Ubc9 can directly recognise substrate proteins and catalyse the transfer of SUMO to the substrates [24–27]. SUMO E3 ligase functions as an adaptor protein that stimulates efficacy of SUMOylation [28–30] by facilitating the transfer of SUMO from Ubc9 to the substrate, a process particularly important for the substrates lacking SUMO recognition sites. The covalently linked SUMO proteins can be removed by SENPs, a process referred to as deSUMOylation [31]. To date, six members of the SENP family have been identified in humans, which have distinct subcellular localisation and different preference for SUMO isoforms. Interestingly, some SENPs have dual functionality in that they both process SUMO to its mature form and also cleave the isopeptide bond between SUMO and its substrate protein [31]. This functional diversity of SENPs suggests that SUMOylation is a dynamic process, which needs to be tightly regulated in the cell. Moreover, unlike ubiquitination, which targets proteins to proteasomal degradation, SUMOylation leads to changes in stability, activity and/or sub-cellular localisation of the modified proteins that modulates a diverse range of cellular processes [8,32–34]. However, it is still unknown how substrate specificity of SUMOylation is achieved by the mechanisms constituted with a limited number of enzymes, such as a single SUMO E1 enzyme, a single SUMO E2 enzyme and few SUMO E3 ligases are known.

SUMOylation and Heart Disease

Discovered in 1996, SUMOylation has been found to be highly relevant in signal transduction, particularly in response to cellular stress and disease [35]. Importantly, a number of studies suggested that SUMOylation also plays a role in human disease pathogenesis [11,36–38]. Indeed, critical regulatory proteins for human disease states, including neurodegenerative diseases and cancer, are substrates of SUMOylation [36]. There is also growing evidence that the SUMOylation pathway is involved in cardiac physiology and pathology. Studies employing transgenic and knockout approaches have suggested the roles of SUMOylation in the heart. Global SUMO1 knockout mice developed congenital heart disease, including atrial and ventricular septal defects [39], and cardiac SUMO1 knockdown mice showed progression of cardiac dysfunction and sudden death [40]. However, SUMO2 knockout mice showed defects in embryonic development without any specific cardiac phenotype [41]. In the absence of SENP2, a deSUMOylating enzyme, SENP2 knockout mice led to embryonic lethality with defects in the embryonic heart [42]. In addition, cardiac specific overexpression of SENP2 or SENP5 in mice led to dilated cardiomyopathy demonstrating the negative effects of excessive de-SUMOylation on cardiac function [43,44]. On the other hand, increased SUMO2/3 conjugation together with elevated expression levels of SENP1 and SENP5 was recently observed in human failing hearts caused by idiopathic cardiomyopathy [44,45]. These studies suggest the importance of SUMOylation/deSUMOylation in normal cardiac development and function.

A more direct link has been proposed between defective SUMOylation and human heart disease based on a target-specific study. For instance, a mutation analysis of Lamin A, which is a critical scaffolding protein important for nuclear structure, revealed that its deficiency causes human familial dilated cardiomyopathy. Importantly, Lamin A is SUMOylated at lysine 201, however, Lamin A E203G and E203K mutants, located in the SUMOylation consensus sites, have been shown to have reduced levels of SUMOylation and are associated with familial dilated cardiomyopathy [46]. Mutation in TRPM4 (E7K), which is a calcium activated non-selective cation channel, has been associated with familial atrial fibrillation as a result of defective SUMOylation [47], which can lead to congestive heart failure and stroke.

Furthermore, several transcriptional factors/co-factors (GATA4, Nkx2.5, Myocardin, SRF1), mitochondrial protein Drp1, cardiac ion channel Kv2.1 and cardiac metabolic proteins (PPAR, PGC1 alpha, AMPK) have been reported as SUMOylation substrates [48], however their roles in pathologic conditions need to be elucidated.

SERCA2a Dysfunction and Gene Therapy in Heart Failure

Calcium cycling is a central mechanism of cardiomyocyte function and the sarcoplasmic reticulum (SR) is the primary calcium storage organelle in muscle cells. In cardiomyocytes, removal of cytosolic calcium is mainly accomplished by the cardiac isoform of the SR calcium ATPase (SERCA2a). SERCA2a actively transports calcium from the cytosol to the SR during myocardial relaxation. Abnormal regulation of SR calcium cycling due to SERCA2a dysfunction is a common feature of human heart failure [49–52]. Indeed, SERCA2a expression and activity are significantly reduced in human heart failure [52,53]. Animal models of heart failure and failing human ventricular myocytes isolated from patients with end-stage heart failure showed that SERCA2a gene transfer could restore calcium uptake into the SR and improve velocity of muscle contraction and relaxation [54–58]. Additional beneficial effects of SERCA2a gene transfer were also observed in diverse animal models such as improvements in cardiac function, restoration of cardiac energetics, reduction of ventricular arrhythmias, and