Published in final edited form as:

J Mol Cell Cardiol. 2014 July ; 72: 292–295. doi:10.1016/j.yjmcc.2014.04.004.

S-nitrosylation of TRIM72 mends the broken heart: A molecular modifier-mediated cardioprotection

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1. Introduction

Cardiovascular diseases (CVDs) are a leading cause of death globally, casting substantial economic and societal burdens. This devastating situation imposes a pressing need to identify novel cardioprotective mechanisms that will reinforce and innovate the current medical interventions for CVDs [1–3]. Coronary artery disease (CAD) and heart attack are major causes of ischemic cardiomyopathy. In many cases, ischemic infarction occurs, triggering a series of catastrophic structural changes in the ventricles, resulting in ventricular

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Disclosure statement

The authors do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence (bias) our work.

remodeling. As a consequence, the contractility or pumping capacity of the heart will decline progressively, leading to heart failure [4]. An intuitive tactic to resuscitate ischemic heart from hypoxia is reoxygenation or reperfusion. However, this approach exacerbates cardiac injury by elevating ROS production, which elicits multiple destructive impacts on mitochondria. As a result, pro-apoptotic proteins are released, provoking irreversible cell death and ultimately myocardial infarction [5,6]. Accordingly, investigative efforts addressing approaches to repair and restore injured myocardial function are of great clinical significance. In this regard, the study by Kohr et al. in this issue of JMCC, sheds new light on cellular mechanism promoting cardioprotection and minimizing cardiac injury.

Over the years, mounting efforts have been invested to curtail ischemic reperfusion injury. In 1986, Murry et al. have first reported the beneficial effects of ischemic preconditioning (IPC), in which IPC-treated canine heart demonstrated a prominent attenuation of infarction during hypoxia [7]. However, IPC is administered prior to the onset of ischemic events. Accordingly, Vinten-Johansen et al. introduced the concept of postconditioning (PostC), which is applied at the onset of reperfusion [8]. Enthrallingly, the cardioprotective effects of PostC were comparable with those of IPC, in terms of attenuation of ROS-mediated apoptosis, reduction of infarct size/microvascular injury, as well as improvement of blood endothelial function and hemodynamics [8,9]. However, both IPC and PostC are surgically invasive, involving repetitive balloon inflation and deflation, which can damage the endothelial lining and trigger atherosclerotic lesion, thereby predispose patients with embolism risk [10–12]. To circumvent these shortfalls, adjunctive pharmacological agents (e.g., cyclosporine, glycoprotein IIb/IIIa inhibitors) have been developed and demonstrated a remarkable potency to confine the ischemic risk zone and thereby protect the heart from chronically adverse left ventricular remodeling [13–16].

2. The regulatory role of protein post-translational modifiers in cardioprotection

The leading molecular character of the investigation by Kohr et al. is tripartite motif-containing protein-72, abbreviated as TRIM72 (Q1XH17 from UniProtKB) [17]. TRIM72 is a protein of 477 amino acids containing 16 cysteine residues, including the C144. Cysteine is a unique amino acid residue, in which the sulfhydryl groups of two cysteine residues can form a disulfide bridge and generate a cysteine dimer namely cystine. Such dimerization is pivotal in stabilizing the protein conformation, and therefore governing the protein function. In the presence of aggressive oxidants such as hydrogen peroxide, the sulfhydryl group will be converted to sulfinic acid and sulfonic acid, which abolish the formation of disulfide bond, and thereby affect the protein function. Apart from oxidation, cysteine is also modulated by other post-translational modifications (PTMs), including S-glutathionylation [18], S-nitrosylation [19], sulfenylation [20], sulfinylation [21], S-ubiquitinylation [22], S-palmitoylation [23], 4-hydroxy-2-nonenal [24], and S-guanylation [25]. In spite of well-characterized chemical nature of cysteine residue, the functional consequences of these PTMs are much less understood, which has limited our ability to translate its application in the clinical arena.

Over the past three decades, the delineation of underlying regulatory mechanisms amid IPC and PostC has been a major focus in the realm of cardiovascular research [26–28]. IPC initiates a spontaneous release of multiple receptor ligands, including adenosine, bradykinin, endogenous opioids, and other growth factors. Upon ligand-receptor binding, a network of pro-survival kinase signaling cascades is triggered, including phosphatidylinositol-3-OH kinase (PI3K)/Akt, p42/p44 extracellular signal-regulated kinase (ERK1/2), and JAK/STAT pathways [29–31], via which a diverse array of mediators are phosphorylated in a hierarchical and concerted fashion. Apart from the pivotal role in signaling cascades, phosphorylation is functionally versatile in nature. In the context of PTM, the attachment of phosphate group at the serine, threonine, and/or tyrosine residues can modulate the protein/receptor activity. In the orchestration of mitochondrial homeostasis by protein kinases, PKC ϵ phosphorylates and activates mitochondrial K⁺-channels, which subsequently augments the K⁺ ions influx and stabilizes the membrane potential to salvage mitochondria from swelling/rupture [32]. Similarly, PKA phosphorylates complex IV of the electron transport chain, which in turn promotes ATP production via mitochondrial respiration and attenuates ROS production [33]. Analogous to phosphorylation, O-GlcNAcylation (O-GlcNAc) has recently been characterized as a potent modulator, which can positively and negatively regulate cardiovascular function [34]. Majority of the work has hitherto been focused on the positive regulatory role of O-GlcNAc in cardioprotection, via the protection of cardiomyocytes from calcium overload [35], apoptosis [36], calpain proteolysis [36], oxidative stress [37], and inflammation [38]. Emerging evidences of PTM changes during ischemic conditioning suggest that PTMs, as a regulatory paradigm that directly alters the protein function and stability, offer an alternative option for cardioprotection. The findings by Kohr et al. provide another novel PTM regulatory mechanism in preserving cardiac function during ischemic injury.

3. The cardioprotective role of S-nitrosylation of TRIM72

The nitric oxide (NO)-mediated cardiac protection theory was first introduced by Bolli et al., nitric oxide synthase (NOS) was described as a prominent initiator and mediator in cardioprotective response [39]. A plethora of studies has supported the critical role of NOS as a central hub of the signaling network to substantiate the cardioprotective impacts amid IPC [40–42]. Furthermore, NO and its derivative NO₂⁻ have recently been identified as potent inducers of S-nitrosylation (SNO), a reversible protein PTM which emerges to be an appealing regulatory paradigm critical to the cardioprotection via the orchestration of protein stability, protein binding, as well as the competitive modification of cysteine residue with oxidation [28,43–47]. The report by Kohr et al. in this issue of JMCC elegantly demonstrated a competitive relation between oxidation and SNO modifications on the cysteine 144 residue (C144) of TRIM72, as well as the respective functional consequences of TRIM72 stability alterations, adapting an integrative approach which combines genetically manipulated biological model and mass spectrometry-based proteomic analytical platform [17].

TRIM72, also known as mitsugumin-53 (MG53), is a membrane repair protein, which functions as a key component of the sarcolemmal membrane-repair machinery to maintain cell integrity and thereby its normal cellular function [48,49]. TRIM72 demonstrates a

crucial cardioprotective role in both IPC [50] and PostC [51]. During ischemic reperfusion injury, the augmentation of ROS activates and translocates TRIM72 to the membrane damage site, in which TRIM72 will oligomerize with each other via a disulfide bridge formed at the cysteine 242 (C242). This oligomerization step is critical to reinforce and stabilize the repair complex. In addition to C242, C144 (a study target of Kohr et al.) was found to be an essential residue governing the cardioprotective function by orchestrating the protein stability of TRIM72.

In the experimental setting, Kohr and colleagues overexpressed wild-type TRIM72 (TRIM72_{WT}) or TRIM72 with C144 mutated to serine (TRIM72_{C144S}) in HEK-293 cells, which do not express any endogenous TRIM72. Intriguingly, TRIM72_{C144S}-transfected cells revealed significantly less H₂O₂-induced cell death in comparison with TRIM72_{WT}- or empty vector-transfected cells. This illustrates that C144 is an oxidation-targeting residue, in which the mutation of C144 to serine confers the cells a greater resistance to oxidative stress-induced apoptosis. In parallel, an identical set of cells was treated with a cardioprotective S-nitrosylating agent S-nitrosoglutathione (GSNO). Cells expressing TRIM72_{WT} revealed an increase in SNO level, which was not observed in the TRIM72_{C144S}-expressing counterparts. Furthermore, the nitrosylated TRIM72_{WT}-expressing cells were associated with an attenuation of H₂O₂-induced oxidation. This observation illustrates that C144 is a dual target for both oxidation and SNO modifications. Both PTMs are mutually exclusive events, exerting opposite effects on the stability of TRIM72, in which oxidation of TRIM72 attenuates the stability/abundance of TRIM72; whereas SNO enhances the stability of TRIM72 protein by competing with oxidation of C144. Furthermore, a critical piece of information, which enables us to appreciate the significance of such mutually exclusive oxidation/SNO events, hinges on the respective functional consequences (i.e., the functional role of SNO modification of C144 of TRIM72 in the context of cardioprotection). In this regard, the authors employed a Langendorff-perfused heart model to decipher and examine the cardioprotective outcome of SNO of TRIM72 in the aspect of ischemic reperfusion injury. They illustrated that hearts perfused with GSNO prior to ischemia presented a significantly higher abundance of TRIM72 in comparison with the control hearts. In consistence with the cardioprotective role of TRIM72, the preservation of TRIM72 levels in GSNO-perfused hearts demonstrated a prominent amelioration of apoptosis as reflected by the reduction of infarct size compared with the control counterparts.

4. Summary and future perspectives

In summary, the work presented by Kohr et al. [17] provides a renewed perspective regarding the regulatory paradigm of SNO in cardioprotection. They harnessed and synergized the power of genetic manipulation as well as targeted quantitative proteomics technology, unveiling a unique feature of C144 of TRIM72 as a dual target of both oxidation and SNO modifications. Both forms of PTMs were shown to be mutually exclusive, yet they exerted dichotomous functional consequences in cardioprotection. Specifically, oxidation of TRIM72 triggered cell death by augmenting proteasome-mediated degradation of TRIM72; whereas SNO of TRIM72 ameliorated the infarct size via preserving TRIM72 expression and consequently, retaining its membrane-repairing capability against oxidative stress.

The novelty of the study by Kohr et al. is highlighted in the new regulatory scheme it offers; whereby a single PTM change on an individual protein target may induce “a phenotype-switching effect”. Over the past decades, our ability to define molecular modifications has been transformed from a protein specific manner to amino acid residue targeted fashion. Mass spectrometry has revolutionized our capacity to understand the chemical properties of molecular identifications and their functional roles in cellular processes. Analytical chemistry tools have established themselves as powerful approaches to identify and quantify macromolecules of the biological system in a precise and robust manner [52–55]. These new technological advancements have enabled us to exponentially expand the current cardiovascular investigations in multiple dimensions, including depth and scope. Insights gained from SNO targeting TRIM72 will be helpful for future studies to understand other cardioprotective-relevant proteins, in particular, mitochondrial proteins, which represent approximately 35% of cellular targets of SNO [19]. Intriguingly, multiple mitochondrial proteins have been shown to be S-nitrosylated, including glutaredoxins [56], aldehyde dehydrogenase [57], caspase [58], and electron transport chain complex I [59]. The outcome of SNO-mediated PTM may play essential roles in governing mitochondrial processes, including Ca^{2+} handling and energy metabolism [46,60,61]. To this end, mass spectrometry-based analytical platform will aid the identification of novel SNO-targeted mitochondrial proteins. Future scientific exploration in this direction will shed light on additional targets of SNO as well as their roles in the context of mitochondrial biology in cardioprotection.

Recent progress in biomedical science has been enriched with an integrated view on the temporal and spatial organizations of cellular molecules; this perspective has led our comprehension of cellular function on a global level as well as offered mechanistic insight into the dynamic regulations of molecular pathways. In parallel to the scenario presented here on SNO and oxidation as antagonist regulators, a number of other PTMs have been reported as competitive modifiers, including phosphorylation and O-GlcNAc. Both types of PTMs have contributed significantly to the regulation of cardioprotective signaling [62]. With the implementation of quantitative mass spectrometry-based analytical tool, investigations have been able to measure and to delineate the contextual relationships underlying molecular modifications amid the pathogenesis of cardiac diseases [63,64]. Interestingly, both phosphorylation and O-GlcNAc may target the same amino acid residues (serine and threonine), exerting a ying–yang occupation of the amino acids. In the modality of NIH/3T3 fibroblasts, the dynamic changes of phosphorylation site in response to elevated O-GlcNAc have been characterized, supporting the competitive relationship between phosphorylation and O-GlcNAc [65]. This molecular modification is reminiscent to what has been observed here between oxidation and SNO as demonstrated by Kohr et al. [17].

It has been well documented that SNO is a reversible PTM [19], whereas oxidation of cysteine residue is likely to be a permanent endpoint. Furthermore, oxidation of TRIM72 renders it vulnerable for degradation. Therefore, replacing SNO PTM by oxidation would generate a unique and one-directional impact. Moreover, it requires the SNO PTM occupancy to prevent oxidation to take place. It remains to be established whether or not such intriguing phenomenon of competitive PTMs on the same amino acid exists beyond the SNO and oxidation PTMs in the context of cardioprotection. It is conceivable that a subpopulation of the oxidized TRIM72 may remain, however, the majority of the TRIM72

proteins would exist either in the form of TRIM72_{C144S} or in the form of unmodified TRIM72_{C144}. The temporal dynamics of the SNO modifications as well as the subcellular localization of the modified vs. unmodified isoforms of TRIM72 would be important to determine the phenotypic outcome following ischemic injury.

The capability to catalog such spatiotemporal pattern of PTMs of the cardiac cell proteome in an amino acid site-specific fashion will enable us to construct a global PTM map and PTM sequence code, contributing to our mechanistic understanding of PTM regulation on cardiac function. Moreover, the spatio-temporal organization of the cardioprotective proteins (e.g., TRIM72) also affords selectivity and specificity for the design of drug targets; therefore, providing unique benefit for future applications in clinical therapeutics. In conclusion, three decades of investigations in conditioning-induced cardioprotection have advanced our understanding of cardiovascular biology during ischemic injury. Challenges and opportunities remain in translating this knowledge from bench to the clinical arena. Functional information and outcomes consequent to molecular modifications of cardioprotective proteins will shed light on the design of drug targets as well as therapeutics strategies in cardioprotection.

Acknowledgments

The work was made possible by the support from the National Institutes of Health (NIH-R37-HL-063901), as well as an endowment from Theodore C. Laubisch at UCLA to Dr. Peipei Ping.

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