



Editorial

Mitsugumin-53: Potential biomarker and therapeutic for myocardial ischemic injury?



1. Introduction

Ischemia-reperfusion injury underlies many pathological conditions, including ischemic heart disease, which is a major cause of death worldwide [1]. Myocardial ischemia-reperfusion injury results from an interruption in the blood supply to a region of the heart and is most often the result of acute coronary occlusion. Significant injury also occurs upon reperfusion of the ischemic region, as additional myocardial damage results from the formation of reactive oxygen species [2], which can trigger cell death by activating pro-apoptotic and/or necrotic signaling pathways in cardiomyocytes [3]. The issue of cell death is of paramount importance in the heart since cardiomyocytes are terminally differentiated, and aside from cardiac progenitor cells, the myocardium is very limited with regard to endogenous regenerative capabilities [4]. Cardioprotective interventions hold great promise for lessening the detrimental effects of myocardial ischemia-reperfusion injury [5] and have been shown to reduce contractile dysfunction, arrhythmogenesis and cell death in animal models [6–9]. In 1986, Murry and Reimer originally described the powerful cardioprotective phenomena known as ischemic preconditioning (IPC), whereby brief exposure to periods of ischemia and reperfusion serves to protect the heart against a more prolonged ischemic insult [6]. However, IPC must be performed prior to the onset of ischemia, thereby limiting clinical applicability. Since the initial discovery of IPC, additional protective mechanisms with greater clinical applicability have been described, including ischemic postconditioning [10], and pharmacologic agents such as adenosine [11], cyclosporine [12], and nitric oxide [13]. Mechanistically, there is much that remains to be defined, but these interventions are thought to converge on similar cytoprotective signaling pathways that may include the reperfusion injury salvage kinase (RISK) pathway, which is comprised primarily of phosphoinositide 3-kinase/Akt, ERK1/2, and glycogen synthase kinase 3 beta [14]. Although research devoted to the elucidation of cardioprotective signaling pathways has been ongoing for nearly 40 years, few experimental discoveries have translated successfully into effective therapeutics, and currently, the only established intervention that consistently reduces infarct size in humans is early coronary artery reperfusion, which still has the potential to cause additional myocardial injury as mentioned above. The recent study from Liu *et al.* [15] brings a fresh perspective on potential therapeutic approaches for the treatment of ischemic heart disease with the use of recombinant protein, namely, the membrane repair protein mitsugumin-53.

2. Role of mitsugumin-53 in cardioprotection

Mitsugumin-53, also known as tripartite motif-containing protein 72, is a member of the large tripartite motif-containing family of proteins and was recently characterized by Jianjie Ma's group as a critical membrane repair protein in heart and skeletal muscle [16,17]. Membrane rupture is a main contributor to cardiomyocyte cell death following ischemia-reperfusion injury, and as such, membrane repair is an essential process for preserving cardiomyocyte viability [18]. Following membrane damage, the oxidizing milieu of the extracellular environment leads to the dimerization and activation of mitsugumin-53, which subsequently translocates to the site of injury [16,17]. Mitsugumin-53 functions in membrane repair by recruiting essential repair components, including dysferlin, and serving as a scaffold to facilitate membrane repair [19]. Ischemia-reperfusion injury has been shown to decrease mitsugumin-53 levels in the heart, likely via oxidation-induced degradation [20], and this correlates directly with enhanced cellular injury [21]. In addition, the genetic ablation of mitsugumin-53 exacerbates myocardial ischemia-reperfusion injury, while mitsugumin-53 overexpression has been shown to be protective in cellular models of injury [21]. Mitsugumin-53 also plays an essential role in IPC and postconditioning, in part, by facilitating the activation of various components of the RISK signaling pathway [21,22]. Interestingly, IPC has been shown to maintain mitsugumin-53 levels following ischemia-reperfusion injury, thereby reducing cell death. The maintenance of mitsugumin-53 levels occurs, in part, via IPC-induced S-nitrosylation, which stabilizes mitsugumin-53 by preventing oxidation-induced degradation [20]. Mitsugumin-53 also plays a role in cellular metabolism by functioning as an E3 ubiquitin ligase and targeting such proteins as the insulin receptor and insulin receptor substrate 1 [23].

The study from Liu *et al.* [15] provides a comprehensive examination of recombinant mitsugumin-53 (rhMG53) as a potential therapeutic for myocardial ischemia-reperfusion injury using multiple animal models. In a Langendorff-perfused mouse heart model of ischemia-reperfusion injury, the authors demonstrated a rhMG53-dependent reduction in creatine kinase release and infarct size when administered in the perfusate prior to ischemia or 2 min post-reperfusion. Similarly, using *in vivo* mouse and rat heart models of coronary artery occlusion, the authors demonstrated a dose-dependent reduction in creatine kinase release and infarct size upon the respective intraperitoneal (10 min post-reperfusion) or intravenous tail vein (2 min post-reperfusion) administration of rhMG53. Recognizing the need to further validate the protective effects of rhMG53 in a large-animal model, the authors

utilized a porcine model of angioplasty-induced myocardial infarction and observed that intravenous administration of rhMG-53 reduced infarct size, decreased troponin I release, and decreased TUNEL positive staining as a marker of apoptosis after 24 h of reperfusion. Similar protection was observed with rhMG53 regardless of whether it was administered prior to the onset of ischemia or at 2 min post-reperfusion. Interestingly, a similar degree of protection was also observed even when the administration of rhMG53 was delayed until 30 min post-reperfusion. The authors also examined the sustained protective effects of rhMG53 administration in the porcine heart model by separately examining post-infarction remodeling. Hearts that were administered rhMG53 showed significantly enhanced fractional shortening and ejection fraction, as well as reduced fibrosis at 4 weeks post-injury. Mechanistically, the recruitment of cytosolic mitsugumin-53 to the site of injury has been well characterized [16,17], but the mechanism underlying the recruitment of rhMG53 has not been fully examined. rhMG53 is thought to concentrate at the site of injury by binding phosphatidylserine [24], and the authors provide additional evidence in support of this hypothesis by demonstrating that rhMG53 preferentially targets infarcted tissue, as compared to adjacent or remote myocardium, and upregulates phospho-AKT and phospho-glycogen synthase kinase 3 beta. Regardless of the specific mechanism of action, rhMG53 does appear to provide substantial cardioprotective effects in the animal models that were examined. In addition to the use of mitsugumin-53 as a potential treatment for myocardial ischemia-reperfusion injury, Liu et al. also examined mitsugumin-53 as a potential biomarker for myocardial injury. Using an *in vivo* murine model, the authors demonstrated a low circulating level of mitsugumin-53 in the blood at baseline that increased dose-dependently with myocardial injury and this increase persisted for up to 4 h. In addition, the authors showed that the increase in mitsugumin-53 levels in the perfusate with myocardial injury mirrors that of creatine kinase in the Langendorff-perfused mouse heart. These results are consistent with the findings of Marshall *et al.*, which also identified mitsugumin-53 as a potential marker for necrotic cellular injury [25].

3. Conclusions and future perspectives

Membrane repair is a vital process for maintaining cardiomyocyte viability following ischemia-reperfusion injury, and the study from Liu *et al.* provides compelling evidence in support of the use of rhMG53 as a potential therapeutic to combat the damaging effects of ischemia-reperfusion-induced membrane rupture [18]. The authors demonstrate powerful cardioprotective effects in mouse, rat, and porcine models using rhMG53 [15], and since the porcine heart is anatomically similar to that of the human, these results provide strong support for the use of rhMG53 as a potential therapeutic for ischemic heart disease. In addition to protective effects observed in the myocardium, the authors have also demonstrated that rhMG53 administration can facilitate the repair of damaged skeletal muscle membranes, particularly in the setting of muscular dystrophy [24]. Additionally, rhMG53-dependent protection has also been observed in other cell types, including rat bone marrow-derived stem cells [26]. The fact that mitsugumin-53 can elicit protective effects in multiple cell types clearly broadens the therapeutic potential beyond that of myocardial injury. rhMG53 is also soluble in aqueous solutions and can be stored as a lyophilized powder at room temperature. The use of mitsugumin-53 as a potential biomarker for myocardial injury is also an intriguing possibility, but additional studies will have to be conducted in order to establish the reliability of mitsugumin-53 as a biomarker compared to more commonly used markers, including troponin I, creatine kinase, and lactate dehydrogenase.

Although the protective effects of rhMG53 have been characterized in the heart and skeletal muscle, mechanistic detail is lacking, and future studies will need to address key issues underlying the protective effects of rhMG53. For example, how does an intracellular protein provide

protective effects when administered in the extracellular space? Intracellular mitsugumin-53 works to facilitate membrane repair by forming a dimer and translocating to sites of injury, but additional studies are necessary in order to establish whether rhMG53 also needs to dimerize in order to provide protective effects. Moreover, does rhMG53 need to gain entry into injured cells in order to provide protection? Since the authors demonstrated preferential targeting of damaged tissue by rhMG53, it is possible that rhMG53 facilitates protection by simply entering damaged cells through the site of membrane rupture. This is supported by the upregulation of protective intracellular signaling pathways (i.e., Akt, glycogen synthase kinase 3 beta). However, it is also possible that the mechanism underlying the protective effects of intracellular mitsugumin-53 is completely different from that of rhMG53. Furthermore, does the E3 ubiquitin ligase function play a role in the cytoprotective effects of mitsugumin-53? While membrane rupture is a major factor contributing to cardiomyocyte cell death following ischemia-reperfusion injury, one could envision that mitsugumin-53 may perhaps upregulate or downregulate essential cytoprotective pathways by altering the stability of target proteins. In addition, although enhanced mitsugumin-53 levels appear to be beneficial in the setting of myocardial ischemia-reperfusion injury, mitsugumin-53 upregulation can potentially trigger insulin resistance, obesity, and hypertension by degrading the insulin receptor and insulin receptor substrate 1 [23]. Therefore, it is of critical importance to also determine the half-life of rhMG53 in the bloodstream and to examine the long term effects of acute rhMG53 administration on insulin signaling and cellular metabolism. Mutations and/or modifications to mitsugumin-53 may also serve to optimize the therapeutic benefit of rhMG53. For instance, the stability of rhMG53 itself may be improved, as we have recently demonstrated by developing a mutant variant of mitsugumin-53 that is resistant to oxidation-induced degradation [20]. In conclusion, rhMG53 appears to be a promising cytoprotective agent that has the potential to serve as a biomarker and as a therapeutic for the treatment of myocardial ischemic injury, and additional studies will aim to refine the use of rhMG53 in the heart and beyond.

Disclosures

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Mark J. Kohr*

Department of Environmental Health Sciences, Bloomberg School of Public Health, Johns Hopkins University, 615 N. Wolfe Street, Room E7616, Baltimore, MD 21205, USA
E-mail address: mkohr1@jhu.edu

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