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MG53 is dispensable for T-tubule maturation but critical for maintaining T-tubule integrity following cardiac stress

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ABSTRACT

The cardiac transverse (T)-tubule membrane system is the safeguard for cardiac function and undergoes dramatic remodeling in response to cardiac stress. However, the mechanism by which cardiomyocytes repair damaged T-tubule network remains unclear. In the present study, we tested the hypothesis that MG53, a muscle-specific membrane repair protein, antagonizes T-tubule damage to protect against maladaptive remodeling and thereby loss of excitation-contraction coupling and cardiac function. Using MG53-knockout (MG53-KO) mice, we first established that deficiency of MG53 had no impact on maturation of the T-tubule network in developing hearts. Additionally, MG53 ablation did not influence T-tubule integrity in unstressed adult hearts as late as 10 months of age. Following left ventricular pressure overload-induced cardiac stress, MG53 protein levels were increased by approximately three-fold in wild-type mice, indicating that pathological stress induces a significant upregulation of MG53. MG53-deficient mice had worsened T-tubule disruption and pronounced dysregulation of Ca^{2+} handling properties, including decreased Ca^{2+} transient amplitude and prolonged time to peak and decay. Moreover, MG53 deficiency exacerbated cardiac hypertrophy and dysfunction and decreased survival following cardiac stress. Our data suggest MG53 is not required for T-tubule development and maintenance in normal physiology. However, MG53 is essential to preserve T-tubule integrity and thereby Ca^{2+} handling properties and cardiac function under pathological cardiac stress.

1. Introduction

Repair of damage to the plasma membrane is an active and dynamic process that includes detection of acute membrane injury, recruitment of intracellular vesicles to the injury site, and vesicle fusion to enable formation of a membrane patch to reseal the damaged membrane. MG53, also known as tripartite motif 72 (TRIM72), a muscle-specific member of the tripartite motif/RING B-box Coiled Coil (TRIM/RBCC) family proteins, is a critical component of the sarcolemma repair machinery in striated muscle [1–5]. In response to acute membrane damage, MG53 facilitates vesicle translocation to the injury sites to reseal the damaged membrane within a matter of seconds [5,6]. Mice deficient in MG53 have defective membrane repair function after acute muscle injury and develop progressive myopathy with age [1].

While the majority of studies have analyzed the role of MG53 in striated muscle, genetic ablation of MG53 prohibits emergency sarcolemmal resealing after infrared laser–induced acute membrane damage in intact hearts and increases myocardial vulnerability to ischemia/reperfusion injury [2,3]. In line with a potential role for MG53 in cardiac function, gene delivery of MG53 enhances membrane repair, ameliorates pathology, and improves cardiac functions in hamsters with congestive heart failure [7]. However, the mechanisms by which

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MG53 provides cardiac protection following injury still remain elusive.

It is well-established that cardiac stress is associated with massive deterioration of the cardiomyocyte transverse (T)-tubule membrane system. The organized T-tubule system provides a structural basis for rapid electric excitation, initiation and synchronous triggering of SR Ca^{2+} release, and thus coordinated contraction of each contractile unit throughout the entire myocyte [8,9]. Compelling evidence from our group and others demonstrates that disruption of the T-tubule network is mechanistically involved in excitation-contraction (E-C) coupling dysfunction and the development and progression of heart failure [9–18].

While T-tubule damage is considered to be caused by pathological remodeling, it may also result from ineffective membrane repair in response to injury. Since others have reported that MG53 deletion abolishes membrane resealing after injury and thereby exaggerates ischemia/reperfusion-induced cardiac injury [2,3], our objective in this study was to examine the contribution of MG53 to maintenance of myocyte ultrastructure and cardiac function in developing hearts and following cardiac stress. We hypothesized that the MG53 is involved in T-tubule biogenesis and maintenance. Using MG53-deficient mice, we found that MG53 is dispensable for T-tubule development and cardiac function as late as 10 months of age at baseline. However, in response to LV pressure overload-induced hypertrophy and heart failure, MG53 is essential to maintain T-tubule integrity and thereby preserve Ca^{2+} handling properties and gross cardiac function.

2. Materials and methods

2.1. Animals and MG53 gene knockout mice

Animal experiments were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (publication no. 85–23, revised 1996) and were approved by the Institutional Animal Care and Use Committee at the University of Iowa. MG53 knockout (MG53-KO) mice were generated, as described previously [1]. MG53-KO mice were backcrossed with C57BL/6J mice for 10 generations to generate MG53-KO mice on a C57BL/6J background. Conventional PCR was used for genotyping. Wild-type littermates were used as control mice.

2.2. Transaortic banding surgery

Male MG53-KO mice and WT littermates (9–10 weeks) were subjected to pressure overload by transaortic banding (TAB) surgery, as previously described [19]. Briefly, mice were anesthetized with ketamine/xylazine (87.5 mg/kg/12.5 mg/kg, i.p.) and ventilated with a small rodent ventilator. A thoracotomy was created between the second and third intercostal space, and the aortic arch was visualized. Aortic constriction was performed by tying a 7–0 nylon suture ligature against a 27-gauge needle to yield a narrowing 0.4 mm in diameter when the needle was removed and a reproducible TAB of ~ 70%. In sham mice, the aortic arch was visualized but not banded. Mouse survival rate was recorded from 48 h after surgery; mice that died within 48 h of surgery were excluded from the analysis. Left ventricle (LV) function was examined by echocardiography 5 weeks after TAB or sham surgery as previously described [20]. Confocal imaging of T-tubule structure and intracellular Ca^{2+} in subepicardial myocytes from intact hearts was performed on the next day after echocardiography.

2.3. Mouse exercise training protocol

C57BL/6 mice were trained on treadmill at 20° incline every other day for 4 weeks. Each session consisted of 4 sets of 6 min intervals at 80 – 90% of maximum velocity (Vmax), with 4 min of active rest between intervals. The control (sedentary) group was handled like the training group and placed on non-moving treadmill for same time periods.

2.4. Confocal calcium and T-tubule imaging

Cytosolic Ca^{2+} from intact hearts was stained with Rhod-2 AM at 10 μmol/L (AAT BioQuest, CA) in 1.8 mmol/L $[Ca^{2+}]_o$ Tyrode solution *via* Langendorff perfusion at room temperature for 30 min [21]. Calcium transients were acquired using confocal microscope (LSM 510, Carl Zeiss MicroImaging Inc., Germany) with 63× lens in line–scan mode along the transverse axis of myocytes under autonomous beating. After completing Ca^{2+} imaging, hearts were perfused with MM 4–64 (2.5 μmol/L, AAT BioQuest, CA) in $[Ca^{2+}]_o$ free Tyrode solution at room temperature for 30 min to stain T-tubule membranes. The structure of T-tubules was visualized with the same confocal microscope in frame-scan mode. Quantitative analysis of T-tubule integrity was processed with IDL image analysis program as previously reported. The power value (TT_{power}) reflects the strength of the regularity of T-tubule organization [15].

2.5. Protein preparation and western blotting

Heart lysates were prepared as previously described [22]. Briefly, the whole hearts were homogenized and sonicated in lysis buffer (in mmol/L: Tris-HCl 50 [pH 7.4], NaCl 150, NaF 10, Na_3VO_4 1, EGTA 5, EDTA 5, 0.5% Triton X-100, 0.5% Na deoxycholate, 0.1% SDS, and protease inhibitors (#P8340, Sigma-Aldrich)). After standing on ice for 1 h allowing lysis, the homogenates were centrifuged at 13,000*g* 4 °C for 15 min. The supernatants were kept as whole cell proteins. Protein concentration was measured by BCA assay.

Proteins were separated on 4–12% Bis-Tris gels and transferred to PVDF membrane. The membrane was probed with primary antibodies against MG53 (provided by Dr. Jianjie Ma), NCX1 (1:1000, #R3F1, Swant, Switzerland), RyR2 (#MA3-916, Thermo Scientific), SERCA2a (#MA3-919, Thermo Scientific), Junctophilin-2 (JP2, #SC51313, Santa Cruz Biotechnology), Amphiphysin 2 (Bin1, #SC23918, Santa Cruz Biotechnology) and GAPDH (1:10,000, #G8975, Sigma-Aldrich) overnight at 4 °C, followed by incubation with appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000–1:10,000 dilution in PBS solution). The immunoreactions were visualized using an enhanced-chemiluminescent detection kit and the protein bands were quantified with Quantity One 1-D Analysis Software (Bio-Rad, USA).

2.6. Statistical analysis

Data are expressed as mean ± SE. One-way ANOVA followed by Bonferroni procedure was applied to multiple group comparisons. Statistical analyses were carried out using SPSS V15.0 software. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. MG53 protein is not required for maturation of the T-tubule system during cardiac development

We first established the expression pattern of MG53 in whole heart lysates from C57BL/6 mice at postnatal days 1, 3, 5, 7, 9, and 11. MG53 was detected as early as postnatal Day 7, increased substantially at Day 9 and continued to increase at Day 11 (Fig. 1A). We previously reported that some sporadic T-tubules are present at postnatal Day 8, with an obvious T-tubule network apparent at Day 10 [23]. In adult cardiomyocytes, MG53 co-localized with RyR2 in the T-tubule/SR junctional region (Fig. 1B).

Since the pattern of MG53 expression occurred earlier than T-tubule development, we hypothesized that MG53 may participate in T-tubule maturation. Therefore, we examined T-tubule structure in intact hearts from WT and MG53-KO mice using *in situ* confocal imaging. We confirmed that MG53-KO mice have no detectable MG53 protein in heart

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