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Dual Function of MG53 in Membrane Repair and Insulin SignalingTao Tan³, Young-Gyu Ko^{1,2¶} and Jianjie Ma^{3¶}

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Abstract

MG53 is a member of the TRIM-family protein that acts as a key component of the cell membrane repair machinery. MG53 is also an E3-ligase that ubiquitinates insulin receptor substrate-1 and controls insulin signaling in skeletal muscle cells. Since its discovery in 2009, research efforts have been devoted to translate this basic discovery into clinical applications in human degenerative and metabolic diseases. This review article highlights the dual function of MG53 in cell membrane repair and insulin signaling, the mechanism that underlies the control of MG53 function, and the therapeutic value of targeting MG53 function in regenerative medicine.

1. Introduction

Skeletal muscle comprises about 40% of the human body mass whose main function is to control locomotion. Associated with muscle contraction-relaxation is injury to the sarcolemma membrane that necessitates an active repair mechanism to maintain integrity of the muscle fiber. Skeletal muscle injury-repair and regeneration is a multi-cellular process that involves repair of acute injury to the sarcolemma, mobilization of satellite cells to replace the lost-muscle fibers, and control of fibrotic remodeling for maintenance of muscle function. In muscular dystrophy, compromised sarcolemma integrity or membrane repair triggers the cascade of muscle degeneration that incurs progressive, severe morbidity and ultimately mortality. Developing therapeutic approaches to improve sarcolemma integrity while facilitating regeneration of injured muscle fibers remain a major challenge in muscle physiology research.

In addition to functioning as a contractile machine, skeletal muscle plays an important role in regulating glucose homeostasis, accounting for ~80% of insulin-stimulated glucose disposal¹. Development of insulin resistance is a main underlying cause for metabolic disorders such as obesity and type 2 diabetes. Although metabolic disorders primarily induce defects in nutrient regulation, their burden on the human body extends to other physiological systems²⁻⁴. A less explored complication of metabolic syndrome is its impact on tissue repair. For example, patients with metabolic diseases often are more susceptible to tissue injury and slow to recover after injury^{5,6}.

Repair of injury to the plasma membrane is an important aspect of normal cellular physiology^{3,6-8}, and disruption of this process can result in pathophysiology in a number of different tissues^{3,6,9-11}. MG53 is a tripartite motif protein (TRIM72) that presides over the mechanisms of plasma membrane repair^{12,13}. It facilitates the nucleation of intracellular vesicles to sites of membrane injury to form a repair patch. Genetic ablation of MG53 causes compromised cell membrane repair that can lead to progressive skeletal myopathy and a decreased regenerative capacity of cardiomyocytes^{14,15}. Research efforts have been devoted to translate this basic discovery into clinical applications towards treatment of muscular dystrophy^{16,17}, acute lung injury¹⁸, myocardial infarction^{15,19-21}, and acute kidney injury²².

Aside from a role in cell membrane repair, MG53 also participates in modulation of insulin signaling. MG53 contains the conserved RING motif with intrinsic E3-ligase enzymatic activity to induce ubiquitination and down-regulation of insulin receptor substrate 1 (IRS-1), which negatively impact insulin signal strength in skeletal muscle²³⁻²⁶. Therapeutic interventions that target direct interaction between MG53 and IRS-1 without disruption of membrane repair function for MG53 may be a novel approach for the treatment of metabolic disorders that are associated with insulin resistance.

In this review, we highlight the dual function of MG53 in cell membrane repair and insulin signaling, the mechanism that underlies the transcriptional control of MG53, and the therapeutic value of targeting MG53-mediated cell membrane repair in regenerative medicine.

2. Immuno-proteomic identification of MG53 protein from skeletal muscle

Collaborative study between Drs. Hiroshi Takeshima and Jianjie Ma utilized an immuno-proteomic approach for investigation of proteins involved in myogenesis, Ca signaling and maintenance of membrane integrity in striated muscle²⁷⁻³⁰. The immuno-proteomic approach involves a combination of monoclonal antibody immunohistochemistry, cDNA library screening and gene knockout techniques, leading to the discovery of a group of novel proteins termed mitsugumins (MG) that play important roles in muscle physiology, regenerative medicine, and cardiovascular diseases. For example, MG29 is a synaptophysin-related membrane protein that is essential for the maturation and development of the transverse-tubule membrane structure in skeletal muscle³¹⁻³⁴. MG29 may act as a molecular marker of aging that can shield skeletal muscle against aging-related decreases in Ca homeostatic capacity^{28-32,35,36}. We have identified TRIC, a sarco/endoplasmic reticulum (SR/ER)-resident membrane protein that form a homo-trimeric molecular complex to create a cation selective channel to regulate the efficient Ca release process during excitation-contraction coupling in muscle cells^{37,38}. Another important molecule isolated from our immuno-proteomic library is junctophilin (JP), which possesses the property of physically linking the transverse-tubule and SR membrane, allowing the formation of triad junctions that provide the structural framework for excitation-contraction coupling in skeletal and cardiac muscle³⁹. Further studies have linked alteration of JP and polymorphisms of JP in cardiovascular diseases⁴⁰.

In 2005, Dr. Takeshima cloned the MG53 gene using the immuno-proteomic library. Sequence alignment showed that MG53 belongs to the TRIM-family protein, and the primary amino acid sequence of MG53 is highly conserved across the animal species. Biochemical and RNA-hybridization studies revealed predominant expression of MG53 in mouse skeletal and cardiac muscles. Interestingly, genetic ablation of MG53 gene did not appear to affect the behavior and reproduction of the animals under normal physiological conditions. Subsequent studies in Dr. Ma's laboratory identified a distinct phenotype with the *mg53*^{-/-} mice. When the animals were subjected to down-hill running exercise, the absence of MG53 led to excessive injury to the skeletal and cardiac muscles due to defective membrane repair capacity. Toward understanding the biological function of MG53, Cai et al performed expression of the MG53 cDNA in heterologous cells and observed that overexpression of MG53 increased trafficking of intracellular vesicle to the plasma membrane leading to the appearance of extensive filapodia-like structure⁴¹. These observations led to the hypothesis that MG53-containing vesicles may participate in formation of a repair patch following acute injury to the plasma membrane. What followed next is a series of studies that culminated in the realization of MG53 as an essential component of the cell membrane repair machinery^{12,13}.

Parallel to the immuno-proteomic approach, independent studies from Dr. Young-Gyu Ko's laboratory also identified MG53 from C2C12 myotubes using comparative two-dimensional electrophoresis of detergent-resistant lipid rafts^{23,42}. Lipid rafts or caveolae

enrich a myriad of receptors and signaling molecules and function as a harbor for cell signal transduction. Insulin receptor β (IR β), insulin-like growth factor receptor (IGFR), insulin receptor substrate-1 (IRS-1), phosphatidylinositol-3-kinase (PI-3-K) and Akt are all enriched in the lipid rafts^{43,44}. Studies have shown that caveolae disappear and insulin-elicited phosphorylation of IR β is decreased in the skeletal muscle of cav-3 or cavin-1-disrupted mice, leading to insulin resistance⁴⁵⁻⁴⁷. These data indicate that caveolae are critical membrane compartments for insulin signaling. Thus, proteomic analysis of the lipid rafts is a powerful tool for identifying novel signaling molecules⁴⁸. Cavin-1, gC1qR, raftlin and ezrin have been found from different mammalian cell lines by comparative lipid raft proteomics^{23,49-52}.

Lee et al. isolated lipid raft proteins from C2C12 myoblasts and myotubes and compare them by two-dimensional electrophoresis for identifying novel signaling molecules involved in C2C12 myogenesis²³. With these approaches, MG53 is identified in the lipid rafts of C2C12 myotubes. Further studies show that MG53 is co-localized with caveolin-3 in C2C12 myotubes and mouse skeletal and cardiac muscles. The molecular interaction of MG53 with lipid raft proteins such as caveolin-3, cavin-1, dysferlin, IRS-1 and IR β has been demonstrated by endogenous immunoprecipitation in mouse skeletal and cardiac muscles, indicating that MG53 is a real lipid raft protein^{14,23,24,41,53}.

MG53 protein harbors N-terminal tripartite motif (TRIM) and C-terminal PRY-SPRY (sp1A and ryanodine receptors), making it a member of the TRIM family^{12,23}. There are more than 70 TRIM proteins with a RING finger motif, a B-box and one or two coiled-coil domains. MG53 is named as TRIM72 (**Figure panel a**)⁵⁴. The RING finger motif has a consensus sequence C-X₂-C-X_[9-39]-C-X_[1-3]-H-X_[2-3]-C-X₂-C-X_[4-48]-C-X₂-C, where X is any amino acid. With its unique “cross-brace” arrangement with 2 zinc ions, the RING finger motif has E3 ligase activity, transferring from E2 enzyme to their specific target proteins. Indeed, RING domain of MG53 has E3 ligase activity for IRS-1 ubiquitination with the help of E2 ligase UBE2H because RING domain-disrupted MG53 mutants (C14A and Δ RING) do not induce IRS-1 ubiquitination and degradation^{24,25}. B-boxes also contain zinc-finger motifs and may be linked to cell membrane repair and wound healing^{55,56}. Coiled-coil domains show typical hyper-secondary structure formed by interwinding multiple α -helices and are known to be required for homo- or hetero-oligomerization of TRIM family proteins and interacting with different binding partners. Indeed, MG53 mutant without coiled-coil domain (Δ CC) loses its ability to form homo-oligomer and interact with IRS-1^{23,57}. PRY-SPRY domain of MG53 might be involved in protein-protein interaction because its structure has a pocket for an unknown binding partner⁵⁸.

3. MG53 function in cell membrane injury-repair

Prior to the finding of MG53, studies from other investigators have identified several molecular components involved in membrane repair, particularly those specific to cardiac and skeletal muscles^{59,60}. Bansal et al. showed that dysferlin plays an important role in

maintenance of sarcolemmal membrane integrity⁹. Several mutations in the dysferlin gene have been linked to human muscular dystrophy^{9,61,62}. It was proposed that dysferlin can function as a fusogen to allow vesicles to form a membrane repair patch⁶³. This was based on immunostaining observations that dysferlin concentrates at the injury sites of isolated muscle fibers and the muscular dystrophy that appears in dysferlin knockout mice⁹. However, since the initial study by Bansal and colleagues, there has been no indication that dysferlin itself can facilitate the rapid translocation of vesicles associated with acute membrane damage. Indeed, *dysferlin*^{-/-} muscle maintains the capacity for vesicle translocation to damage sites on the sarcolemma. This suggests that although dysferlin may participate in the final membrane resealing process, proteins other than dysferlin are likely required for nucleation of intracellular vesicles toward the acute injury sites.

While MG53 is a soluble protein in nature, it can tightly associate with intracellular vesicles and the inner leaflet of the sarcolemma of striated muscle, likely through binding with phosphatidylserine (PS) that is enriched in these membrane surfaces^{12,64}. Under live cell imaging, intracellular vesicles containing MG53 can be observed trafficking through the cell, fusing with the sarcolemma and entering the extracellular space^{12,41}. Cai et al first observed that MG53 containing vesicles can translocate to sites of membrane damage following mechanical or laser-induced injury to the plasma membrane¹². Muscle fibers derived from *mg53*^{-/-} mice display defective membrane repair following such injuries. A role for MG53 in muscle membrane repair was further established through electron microscopy ultrastructural analysis that revealed a lack of vesicle accumulation at sites of injury to the *mg53*^{-/-} muscle fibers. Inspired by these initial observations in 2006, it took us nearly 2 years to resolve the mechanism that underlies the nucleation process for MG53-mediated cell membrane repair¹². MG53 appears as a monomer under reduced conditions, as in the cytoplasm of an intact cell. In response to changes in the redox-status, as it occurs during acute damage of the cell membrane, MG53 forms oligomer complexes under oxidized conditions. Through targeted mutagenesis and functional studies, we determined that MG53 oligomer formation involves a critical cystidine residue (C242). Mutation of C242 into alanine (C242A) disrupted MG53 oligomer formation, caused defective trafficking of MG53-containing vesicles to injury sites and dominant-negatively impacted the wild type MG53 function in muscle membrane repair. These studies support a model where the entry of oxidized milieu triggers activation MG53 to facilitate vesicle translocation to the site of membrane disruption. Subsequent entry of extracellular Ca and activity of other fusogenic factors, such as dysferlin or annexin¹², can facilitate fusion of these vesicles at the membrane injury sites to create a membrane repair patch (**Figure panel b**).

Over the past 7 years, we made significant progress in understanding the molecular mechanisms for MG53-mediated membrane repair in muscle physiology and disease. We showed that leucine zipper motifs in the coiled-coil domain of MG53 constitute an additional mechanism that facilitates oligomerization of MG53 during cell membrane repair

57. Two leucine zipper motifs in the coiled-coil domain of MG53 (LZ1 - L176/L183/L190/V197 and LZ2 - L205/L212/L219/L226) are highly conserved across the different animal species. Chemical cross-linking studies show that LZ1 is critical for MG53 homodimerization, whereas LZ2 is not. In addition to the leucine zipper-mediated intermolecular interaction, redox-dependent cross talk between MG53 appears to be an obligatory step for cell membrane repair, since *in vivo* modification of cysteine residues with alkylating reagents can prevent the movement of MG53 toward the injury sites⁵⁷.

In order for MG53 and intracellular vesicles to translocate to membrane injury sites, motor proteins must be involved. We found that nonmuscle myosin type IIA (NM-IIA) interacts with MG53 to regulate vesicle trafficking during cell membrane repair⁶⁵. In cells that are deficient for NM-IIA expression, MG53 cannot translocate to acute injury sites, whereas rescue of NM-IIA expression in these cells can restore MG53-mediated membrane repair. Compromised cell membrane repair is observed in cells with RNAi-mediated knockdown of NM-IIA expression, or following pharmacological alteration of NM-IIA motor function. Thus, NM-IIA acts as a key cytoskeleton motor protein that facilitates vesicle trafficking during MG53-mediated cell membrane repair.

We also found that PTRF (polymerase I and transcript release factor) can function as a docking protein for MG53 during membrane repair by binding exposed cholesterol at the injury site⁵³. Cells lacking endogenous PTRF exhibit defective trafficking of MG53 to injury sites. Mutations in PTRF associated with human muscular dystrophy result in abnormal subcellular distribution of PTRF and disrupt membrane repair function of MG53. Furthermore, mutagenesis studies identified zinc-binding to the RING and B-box motifs of MG53 contributes to muscle membrane repair and wound healing^{55,56}. AAV-mediated gene delivery showed that enhanced expression of MG53 in the δ -Sarcoglycan-deficient hamsters improves membrane repair and ameliorates muscular dystrophy and heart failure in this animal model¹⁶.

On the basis of these studies, we propose a model for MG53 function during cell membrane repair. Under resting conditions with intact cell membrane structure, MG53 exists as a dimeric structure that involves leucine zipper-mediated intermolecular interaction. The leucine zipper-mediated MG53 protein interaction also allows for accessibility of active sulfhydryl groups (e.g., Cys242), which are critical for formation of a larger oligomeric complex during the nucleation process of cell membrane repair. Acute injury of the plasma membrane creates transient changes in the redox-state near the injury site, where disulfide formation through Cys242 can provide the structural base for formation of an oligomeric structure that is essential for cell membrane repair.

Increased cellular oxidative stress is a common phenotype with many chronic human diseases, such as heart failure, muscle atrophy, diabetes, and neurodegeneration^{66,67}. Overproduction of reactive oxidative species could potentially affect the oxidation state of MG53 and reduce its membrane repair capacity. Thus, targeting the intracellular redox state using antioxidant approaches could have beneficial effects in tissue repair and

regeneration. Our identification of the differential function of the two leucine zipper domains in MG53 could provide an attractive target for molecular intervention to modulate endogenous cell membrane repair function in skeletal and cardiac muscle⁵⁷. This could prove to be useful for treatment of human diseases associated with reduced regenerative capacity and/or altered cardiovascular function.

4. Transcriptional regulation MG53 in skeletal and cardiac muscle

MG53 mRNA and protein are predominantly expressed in mouse skeletal and cardiac muscle tissues^{12,23}. The MG53 mRNA and protein levels are gradually increased during the myogenesis of C2C12 cells, MyoD-overexpressing mouse embryonic fibroblasts, human primary skeletal muscles and mouse satellite cells^{23,25}. All these findings indicate that MG53 transcription is regulated by MyoD which is a master transcription factor for skeletal myogenesis.

With promoter luciferase reporter and chromatin immunoprecipitation (ChIP) assay, MG53 promoter is found to harbor two functional E-boxes²³. The MG53 transcription during C2C12 myogenesis is activated by an IGF-activated PI3K-Akt pathway because MG53 promoter activity is dramatically decreased by PI3K inhibitor (LY294002) or Akt knockdown but not by mTOR, GSK3 β or MAPK inhibitor (rapamycin, LiCl or PD98059) and increased by constitutively active Akt. Indeed, IGF-activated PI3K-Akt pathway has known to be required for the formation and activation of myogenic transcriptional complex because active Akt induces direct phosphorylation of p300 and association of MyoD, MEF2, p300 and P/CAF acetyltransferases^{68,69}.

Myocyte enhancer factor 2 (MEF2) is another myogenic transcription factor which is critical for muscle type remodeling from fast-twitch glycolytic fibers to slow-twitch oxidative fibers^{70,71}. For example, lacZ transgenic mice with MEF2-binding sites in its promoter show that only oxidative fibers have strong β -galactosidase activity⁷². MEF2C- or MEF2D disruption reduces the number of oxidative fibers in mouse soleus⁷³. Among different skeletal muscles, MG53 protein level is the highest in soleus which is mainly composed of slow-twitch oxidative fibers and low in gastrocnemius which is mainly composed of fast-twitch glycolytic fibers^{23,42}. These data indicate that MG53 transcription might be dependent on MEF2 transcriptional activity. Indeed, MG53 promoter is found to contain a functional MEF2-binding site as determined by promoter luciferase reporter assay, chromatin immunoprecipitation (ChIP) assay and electrophoretic mobility shift assay (EMSA)⁴². Chung et al. also demonstrate that as like oxidative fiber-specific genes such MHC-I, and IIA, troponin-I (slow) and myoglobin, MG53 is synergistically transcribed by the combination of MyoD and MEF2 or Mgn and MEF2 during C2C12 myogenesis⁴².

5. MG53 secretion and therapeutic application in treatment of muscle injury

During skeletal muscle contraction and relaxation, injury to the sarcolemmal membrane occurs under physiological conditions. Compromised membrane repair capacity

has been linked to a number of different disease states including muscular dystrophy⁷⁴. In vitro studies and studies using MG53 knockout mice have provided compelling evidence that MG53 is an essential component required for repairing membrane damage in cardiac^{15,19,75} and skeletal muscle^{12,14,16}. Although native MG53 protein is principally restricted to skeletal and cardiac muscle tissues, beneficial effects that protect against cellular injuries are present in nonmuscle cells with overexpression of MG53¹⁷.

In addition to the intracellular action of MG53, injury to the cell membrane exposes a signal that can be detected by MG53, allowing recombinant MG53 protein to repair membrane damage when provided in the extracellular space. Recombinant human MG53 (rhMG53) protein purified from *Escherichia coli* fermentation provided dose-dependent protection against chemical, mechanical, or ultraviolet-induced damage to both muscle and nonmuscle cells. Delivery of exogenous rhMG53 can ameliorate the impact of eccentric contraction in dystrophic mice and cardiotoxin injury in skeletal muscle in normal mice¹⁷. Systemic injection of rhMG53 through multiple routes decreased muscle pathology in the *mdx* dystrophic model.

We conducted additional in vivo studies with rhMG53 and found that intravenous administration of rhMG53 was effective in protection against myocardial infarction²⁰, acute lung injury¹⁸, and acute kidney injury²² in rodent and large animal models of these diseases. These data support the concept of targeting cell membrane repair in regenerative medicine, and present MG53 as a potential biological reagent for restoration of membrane integrity in a broad range of human diseases.

While MG53 is an intracellular protein, a small portion of native MG53 is detected in the circulating blood, which can be elevated following injurious exercise training¹⁷. Similar observation was made when we challenged mice with myocardial infarction injury through ligation of anterior coronary artery⁷⁶. Since native MG53 is present in blood circulation at levels directly correlating with injury or secretory activity of the muscle and heart^{17,20,76}, systemic administration of rhMG53 would unlikely induce an immune response and potentially be a safe biologic reagent. We conducted pharmacokinetic and toxicological evaluations of rhMG53 in murine and canine models, and found that repetitive intravenous administration of rhMG53 did not produce adverse effects in these animals^{17,22}.

Interestingly, when using ischemia/reperfusion (I/R) to injure the skeletal muscle, we found that rat muscles were much more resistant to I/R injury as compared to the mouse muscle⁷⁶. We performed a series of biochemical studies and found that key proteins that participate in repair of membrane injury, e.g., dysferlin, caveolin-3, and MG53, show similar expression levels in mouse and rat muscle⁷⁶. Thus, it is unlikely that difference in the intracellular membrane repair mechanism can account for the different response of I/R induced muscle injury in the 2 species. Of interest, we found that endogenous circulating MG53 in rats is significantly higher than that in mice. This finding might explain why the rats are less sensitive to skeletal muscle I/R injury. These results suggest that circulating MG53 might play a role in muscle injury-regeneration and targeting extracellular MG53

function can have beneficial effects on human diseases associated with defective membrane repair capacity.

6. MG53 as an E3-ligase to modulate insulin signaling

Lee et al. investigate the cellular function of MG53 on myogenesis by using various approaches such as adenoviral overexpression, RNA interference and systemic gene disruption^{23,25}. C2C12 myogenesis is compromised by MG53 overexpression but enhanced by MG53 knockdown. Myogenesis of human satellite cells is also highly enhanced by MG53 knockdown. MG53 disruption enhances the myogenesis in mouse satellite cells and MyoD-overexpressing mouse embryonic fibroblasts^{23,25}. Moreover, the overexpression of RING domain-disrupted MG53 mutants (C14A and Δ RING) enhances C2C12 myogenesis, indicating that MG53-mediated protein ubiquitination is required for the negative feedback regulation of MG53 on skeletal myogenesis²⁵.

It has been well known that IGF-IGFR-PI3K-Akt signalling axis is essential for skeletal myogenesis^{77,78}. For example, the knockdown of IRS-1 prevented but the overexpression of constitutively active Akt enhanced C2C12 myogenesis²³. Lee et al. investigate the molecular mechanism of MG53 on the negative feedback inhibition of myogenesis by analyzing IGF signalling after MG53 overexpression and knockdown in C2C12 cells by using adenoviral overexpression and RNA interference²³. The IGF-elicited activation of IRS-1, PI3K and Akt is decreased in MG53-overexpressing C2C12 myoblasts and increased in MG53-knockdown C2C12 myotubes. The molecular interaction between MG53 and IRS-1 is also demonstrated by two independent research groups^{23,24}. These data demonstrated that IRS-1 is a real target of MG53 for regulating skeletal myogenesis. Because IGF-elicited activation of IGFR and ERK1/2 is not changed by MG53 overexpression and knockdown²³ and IGF-stimulated Ras-Raf-ERK1/2 pathway is diverted from IGFR but not from IR β , IGFR is not the target of MG53.

Because IRS-1 is a convergent signaling molecule for both IGF and insulin, MG53-mediated IRS-1 inactivation also leads to the blockage of insulin signaling. For example, MG53 overexpression prevents the insulin-elicited phosphorylation of IRS-1, Akt and ERK1/2 in myoblasts without affecting insulin-elicited phosphorylation of IR β ²⁵. Because insulin-stimulated Ras-Raf-ERK1/2 and PI3K-Akt pathways are diverted from IRS-1 and MG53 inactivates IRS-1, both pathways are inactivated by MG53. The insulin-elicited phosphorylation of IRS-1, Akt and ERK1/2 is increased in the skeletal muscles such as soleus and gastrocnemius-plantaris of mg53^{-/-} mice^{24,25}. However, insulin-elicited phosphorylation of IR1 β is not changed by MG53 disruption²⁵. These data indicate that MG53 negatively regulates IRS-1 but not IR β in the skeletal muscle.

During the myogenesis of C2C12 cells and MyoD-overexpressing MEFs, MG53 protein level is greatly increased and IR β and IGFR protein level is constant whereas IRS-1 protein level is decreased in spite of the slight increase of IRS-1 mRNA level²⁵. MG53 overexpression decreases IRS-1 protein level in C2C12 myoblasts and MG53 knockdown

increases IRS-1 protein level in C2C12 myotubes without changing IR β and IGFR protein level^{24,25}. Moreover, MG53 disruption increases IRS-1 protein level in mouse skeletal muscles such as soleus and gastrocnemius-plantaris. These data indicate that MG53 mediates the degradation of IRS-1 but not of IR β and IGFR. The MG53-induced IRS-1 degradation is abrogated in the presence of MG132, a proteasome inhibitor, indicating that MG53 induces IRS-1 ubiquitination. Interestingly, the MG53-induced IRS-1 degradation is abolished by the overexpression of C14A and Δ RING in HEK293 cells, showing that C14A and Δ RING work as a dominant negative form of MG53 because both C14A and Δ RING interact and form oligomer with MG53.

Yi et al. demonstrated a molecular interaction between MG53 and UBE2H, an E2 ligase enzyme by endogenous and exogenous immunoprecipitation and UBE2H knockdown abrogates MG53-induced IRS-1 ubiquitination, indicating that MG53 induces IRS-1 ubiquitination with the help of E2 ligase UBE2H²⁵. Based on these studies, we propose a model for MG53-mediated ubiquitination of IRS-1 and its implication in insulin signaling in skeletal muscle. MG53 interacts with IRS-1 in the lipid rafts or caveolae of the sarcolemma and induces IRS-1 ubiquitination with the help of E2 ligase enzyme, UBE2H (**Figure panel c**). The ubiquitinated IRS-1 is degraded by proteasome. In the presence of MG53, insulin signaling is decreased because of the low level of IRS-1 protein, leading to the blockage of PI3K-Akt pathway. Thus, GLUT4-containing intracellular vesicles do not fuse with the plasma membrane, leading to the lack of GLUT4 in the plasma membrane and reduced glucose uptake in the skeletal muscle. Glucose and insulin tolerance test and serum level of insulin, leptin, triacylglycerol, free fatty acids and cholesterol show that the HFD-induced insulin resistance was reduced in MG53-disrupted mice.

Different from the observation by Song et al²⁴, Yi et al²⁵ showed that MG53 protein level is not upregulated in the muscle samples derived from mice and human patients with metabolic disorders, suggesting that MG53 upregulation is not a causative factor for the development of insulin resistance. Based on these observations, we propose that therapeutic interventions that target direct interaction between MG53 and IRS-1 without disruption of membrane repair function for MG53 may be a novel approach for the treatment of metabolic diseases that are associated with insulin resistance or compromised membrane repair capacity.

7. Development of recombinant MG53 protein as therapeutic agent for regenerative medicine

The primary amino acid sequence of MG53 does not contain glycosylation or other post-translational modification motifs, raising the feasibility of using an *E. coli* expression system to produce functional rhMG53 proteins^{17,18,20,22,56,76,79}. The chemistry, manufacture and control (CMC) process for rhMG53 has been established that allows for scale-up production of rhMG53 to support our pre-clinical and future clinical studies. The purification protocol of rhMG53 protein contains three chromatography steps. The

expressed rhMG53 protein is first enriched by an anion-exchange column from the soluble supernatant of *E. coli* lysate under a defined buffer solution. Fractions that contain rhMG53 protein were pulled and subjected to a hydrophobic column, which resulted in purification of rhMG53 to greater than 85% purity. After passing through the anion-exchange column for the second time, a final preparation of rhMG53 with greater than 97% purity was achieved, through elution by a slightly different buffer composition. The purified rhMG53 protein can be stored as lyophilized powder, and the lyophilized rhMG53 protein be functionally reconstituted into saline solution, which allows for convenient application as an injectable reagent.

To quantify the effect of rhMG53 in ameliorating membrane damage, we developed an in vitro cell population membrane damage assay. In this assay, we measure the quantity of lactate dehydrogenase (LDH), which leaks from the cell interior into the extracellular solution following injury to the cell membrane^{17,18,56}. We have validated this assay with several different forms of injury, including anoxia/reoxygenation, chemical, and mechanical damage with micro-glass beads. We found that applying rhMG53 to the extracellular environment can reduce the degree of membrane damage in a dose-dependent manner. The boiled rhMG53 protein is inefficient due to denaturation of the protein. Using this assay we showed that rhMG53 is more potent at minimizing membrane injury at lower concentrations than other agents, such as poloxamer 188 (P188)¹⁷.

As part of our translational effort, it is important that we establish the safety and pharmacokinetic (PK) property of rhMG53 in circulation. Preliminary evaluation of rhMG53 in mice pharmacology studies has demonstrated no overt signs of toxicity¹⁷. When MG53 was administered intravenously (i.v.) once daily (6 mg/kg) to mice for two weeks, no changes in body weight, serum levels of inflammatory cytokines (IL-6 and TNF α), serum levels of ALT (alanine aminotransferase), LDH (lactate dehydrogenase), or changes in the histopathology of the liver, kidney, lung, spleen and cardiac muscle were reported¹⁷. Recently, we conducted a pilot study with repetitive i.v. dosing of rhMG53 in a beagle dog model²². Histological analyses did not reveal gross abnormality within major vital organs, including heart, lung, kidney, liver, brain and spleen, indicating that the dogs could tolerate repetitive exposure to rhMG53. ELISA determination of serum levels of rhMG53 showed the PK of MG53 remained unchanged from the beginning (first dose) to the end of i.v. administration (seventh dose), with a half-life of ~ 1.4 h at both time points. Thus, systemic administration of rhMG53 did not appear to produce neutralizing antibodies as peripheral tolerance to MG53 has already occurred in body circulation²². From this study, we can expect that rhMG53 can be a safe biological agent for treatment of tissue injuries.

One advantage to the use of rhMG53 as a protein therapeutic reagent in regenerative medicine is that it can be produced effectively in different host cells, including bacteria and mammalian cells, which can simplify production of the protein for preclinical studies and clinical trials. We also showed that rhMG53 can be applied to animal models using subcutaneous injection¹⁷, thus the protein could potentially be self-administered by patients

without the need for professional hospital visits. From the pharmacokinetic measurement of MG53, we know that rhMG53 has limited half-life in circulation (~1.5 hour in rodents and canine^{17,22}). Thus, a sustainable delivery mechanism will be necessary for prevention of chronic muscle injury.

For further understanding the role of circulating MG53 as a myokine for tissue protection, we recently developed a transgenic mouse model that allows for tailored control of MG53 secretion, providing ways to examine the efficacy for circulating MG53 in ameliorating the pathology of skeletal myopathy in animal models of muscular dystrophy, as well as for evaluating the safety profile for sustained MG53 in affecting other organ functions. We constructed a transgene by adding a tissue plasminogen activator (tPA) leader sequence ahead of the mg53 cDNA (tPA-MG53) to allow for secretion of MG53 into the bloodstream⁷⁹. The tPA-MG53 transgene was cloned behind a muscle-specific promoter for generation of the transgenic mouse model. The tPA-MG53 mice are healthy with enhanced lifespan, and display improved muscle function under stress conditions with lesser degree of fibrotic remodeling compared with the wild type mice. Moreover, the tPA-MG53 mice are resistant to ischemia-reperfusion induced brain injury, suggesting a neuroprotective role for circulating MG53 in the bloodstream⁷⁹.

8. Conclusion

Identification of the dual function of MG53 as a component of cell membrane repair and modulator of insulin signaling opens many avenues of cell biological research and translational applications. Given the key contribution of compromised membrane integrity and repair to the pathophysiology of muscular dystrophy and other human diseases, the application of rhMG53 as a “molecular bandage”⁸⁰ may have significant value for improving the regenerative capacity of the injured cells to prevent or treat the underlying cause of degenerative human diseases. While the expression of native MG53 is primarily restricted to striated muscle cells, one can express the MG53 cDNA in non-muscle cells and recapitulate the expected membrane repair function to boost their regenerative capacity under stress conditions. As an E3-ligase that controls function of IRS-1 and insulin signaling in muscle cells, MG53 can potentially modulate glucose uptake into skeletal muscle and the overall glucose metabolism and homeostasis. Molecular or pharmacological approaches that target MG53 and IRS-1 interaction without disruption of membrane repair function for MG53 may offer effective means for treating metabolic disorders that are associated with insulin resistance.

Song et al²⁴ reported MG53 expression was markedly elevated in animal models of insulin resistance. However, their proposed role for MG53-mediated IRS-1 down-regulation in the manifestation of metabolic disorders lacks biological base. Separate report by Yi et al²⁵ and other investigators⁸¹⁻⁸⁴ presented no evidence for MG53 upregulation in diabetes, and muscle samples derived from human diabetic patients and mice with insulin resistance showed normal expression of MG53. There are three additional homologous proteins in the

IRS family, IRS-2, IRS-3, and IRS-4, all contribute to insulin signal transduction. Studies by Terauchi et al⁸⁵ and Tamemoto et al⁸⁶ showed that an absence of IRS-1 is not sufficient to induce type II diabetes. IRS-3 deficiency also does not alter glycemic regulatory capabilities⁸⁷. Other elegant studies also demonstrate that mice with double ablation of IRS-1 and IRS-2 do not exhibit abnormalities in glucose homeostasis^{87,88}. Interestingly, only through a combination of IRS-1 and IRS-3 deficiency are diabetic phenotypes observed, indicating that IRS-1 and IRS-3 serve overlapping physiological functions in insulin signaling⁸⁷. Thus, MG53-mediated IRS-1 down-regulation cannot induce type II diabetes through insulin resistance as the existence of IRS-3 compensates for IRS-1 absence.

We recently showed that circulating MG53 level actually decreases in animals with metabolic syndrome, and sequestration of MG53 at the mitochondria may serve protective roles for MG53 in metabolic diseases⁸¹. In a recent study, Liu et al⁸⁹ showed that gross cardiac-specific overexpression of MG53 induces diabetic cardiomyopathy via transcriptional activation of PPAR- α . In their mhc-MG53 transgenic model, the protein level for MG53 was increased by more than 5 fold in the heart, could lead to uncontrolled activation for MG53 in the nucleus. Importantly for our studies, in our tPA-MG53 mice, we found no evidence of cardiomyopathy, even at the age of 32 month old []. These results highlight the cardioprotective function for MG53 in the circulation, and also suggest possible different mechanisms of action for MG53 in intracellular vs extracellular activities. Clearly more studies are required to dissect the role for the E3-ligase activity MG53 in regulation of metabolic function, because this is fundamental for our effort to translate the basic findings into clinical applications.

The TRIM family contains over 70 members in the human genome, however, the biological function of most of these proteins remain unexplored. It is possible that other TRIM family proteins may act in a similar fashion as MG53 in the membrane repair process, or they can interact with MG53 to regulate the overall cell membrane repair response. Searching for the interacting partners of MG53 and understanding their intracellular and extracellular actions in tissue repair and regeneration will require dedicated efforts of interdisciplinary approaches.

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Figure Legend

MG53 function in cell membrane repair and insulin signaling. (a) MG53 contains 477 amino acids with RING (Really Interesting New Gene), B-box, and Coil-coil domain at the amino terminus, plus a SPRY domain at the carboxyl terminus. (b) MG53 participates in the nucleation of transport vesicles to sites of membrane injury. It binds to phosphatidylserine (PS) and undergoes oligomerization when the cell changes from reduced to oxidized environment at the injury site. Entry of extracellular Ca^{2+} enables fusion of vesicles for formation of a repair patch. (c) MG53 contains an E3 Ligase activity that facilitates the ubiquitination of IRS-1. The proteasome-mediated degradation of IRS-1 contributes to regulation of insulin signaling in muscle cells.

References:

- 1 Eriksson, J. G. Exercise and the treatment of type 2 diabetes mellitus. An update. *Sports Med* 27, 381–391 (1999).
- 2 Cornier, M. A. *et al.* The metabolic syndrome. *Endocrine reviews* 29, 777–822, doi:10.1210/er.2008-0024 (2008).
- 3 Cooper, S. T. & McNeil, P. L. Membrane Repair: Mechanisms and Pathophysiology. *Physiological reviews* 95, 1205–1240, doi:10.1152/physrev.00037.2014 (2015).
- 4 Moller, D. E. & Kaufman, K. D. Metabolic syndrome: a clinical and molecular perspective. *Annual review of medicine* 56, 45–62, doi:10.1146/annurev.med.56.082103.104751 (2005).
- 5 Giacco, F. & Brownlee, M. Oxidative stress and diabetic complications. *Circulation research* 107, 1058–1070, doi:10.1161/CIRCRESAHA.110.223545 (2010).
- 6 Cooper, S. T. & Head, S. I. Membrane Injury and Repair in the Muscular Dystrophies. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 21, 653–668, doi:10.1177/1073858414558336 (2015).
- 7 Barbee, K. A. Mechanical cell injury. *Ann N Y Acad Sci* 1066, 67–84, doi:10.66/1/67 [pii] 10.1196/annals.1363.006 (2005).
- 8 Blazek, A. D., Paleo, B. J. & Weisleder, N. Plasma Membrane Repair: A Central Process for Maintaining Cellular Homeostasis. *Physiology (Bethesda)* 30, 438–448, doi:10.1152/physiol.00019.2015 (2015).
- 9 Bansal, D. *et al.* Defective membrane repair in dysferlin-deficient muscular dystrophy. *Nature* 423, 168–172, doi:10.1038/nature01573 nature01573 [pii] (2003).
- 10 Doherty, K. R. & McNally, E. M. Repairing the tears: dysferlin in muscle membrane repair. *Trends Mol Med* 9, 327–330, doi:S1471491403001369 [pii] (2003).
- 11 Bazan, N. G., Marcheselli, V. L. & Cole-Edwards, K. Brain response to injury and neurodegeneration: endogenous neuroprotective signaling. *Ann N Y Acad Sci* 1053, 137–147 (2005).
- 12 Cai, C. *et al.* MG53 nucleates assembly of cell membrane repair machinery. *Nature cell biology* 11, 56–64, doi:10.1038/ncb1812 (2009).
- 13 McNeil, P. Membrane repair redux: redox of MG53. *Nature cell biology* 11, 7–9, doi:10.1038/ncb0109-7 (2009).
- 14 Cai, C. *et al.* Membrane repair defects in muscular dystrophy are linked to altered interaction between MG53, caveolin-3, and dysferlin. *The Journal of biological chemistry* 284, 15894–15902, doi:10.1074/jbc.M109.009589 (2009).
- 15 Cao, C. M. *et al.* MG53 constitutes a primary determinant of cardiac ischemic preconditioning. *Circulation* 121, 2565–2574, doi:10.1161/CIRCULATIONAHA.110.954628 (2010).
- 16 He, B. *et al.* Enhancing muscle membrane repair by gene delivery of MG53 ameliorates muscular dystrophy and heart failure in delta-Sarcoglycan-deficient hamsters. *Molecular therapy : the journal of the American Society of Gene Therapy* 20, 727–735,

doi:10.1038/mt.2012.5 (2012).

- 17 Weisleder, N. *et al.* Recombinant MG53 protein modulates therapeutic cell membrane repair in treatment of muscular dystrophy. *Science translational medicine* 4, 139ra185, doi:10.1126/scitranslmed.3003921 (2012).
- 18 Jia, Y. *et al.* Treatment of acute lung injury by targeting MG53-mediated cell membrane repair. *Nature communications* 5, 4387, doi:10.1038/ncomms5387 (2014).
- 19 Zhang, Y. *et al.* MG53 participates in ischaemic postconditioning through the RISK signalling pathway. *Cardiovascular research* 91, 108–115, doi:10.1093/cvr/cvr029 (2011).
- 20 Liu, J. *et al.* Cardioprotection of recombinant human MG53 protein in a porcine model of ischemia and reperfusion injury. *Journal of molecular and cellular cardiology* 80, 10–19, doi:10.1016/j.yjmcc.2014.12.010 (2015).
- 21 Zhou, X., Chen, M., Wang, S., Yu, L. & Jiang, H. MG53 protein: a promising novel therapeutic target for myocardial ischemia reperfusion injury. *International journal of cardiology* 199, 424–425, doi:10.1016/j.ijcard.2015.07.084 (2015).
- 22 Duann, P. *et al.* MG53-mediated cell membrane repair protects against acute kidney injury. *Science translational medicine* 7, 279ra236, doi:10.1126/scitranslmed.3010755 (2015).
- 23 Lee, C. S. *et al.* TRIM72 negatively regulates myogenesis via targeting insulin receptor substrate-1. *Cell death and differentiation* 17, 1254–1265, doi:10.1038/cdd.2010.1 (2010).
- 24 Song, R. *et al.* Central role of E3 ubiquitin ligase MG53 in insulin resistance and metabolic disorders. *Nature* 494, 375–379, doi:10.1038/nature11834 (2013).
- 25 Yi, J. S. *et al.* MG53-induced IRS-1 ubiquitination negatively regulates skeletal myogenesis and insulin signalling. *Nature communications* 4, 2354, doi:10.1038/ncomms3354 (2013).
- 26 Nguyen, N., Yi, J. S., Park, H., Lee, J. S. & Ko, Y. G. Mitsugumin 53 (MG53) ligase ubiquitinates focal adhesion kinase during skeletal myogenesis. *The Journal of biological chemistry* 289, 3209–3216, doi:10.1074/jbc.M113.525154 (2014).
- 27 Weisleder, N., Takeshima, H. & Ma, J. Immuno-proteomic approach to excitation--contraction coupling in skeletal and cardiac muscle: molecular insights revealed by the mitsugumins. *Cell calcium* 43, 1–8, doi:10.1016/j.ceca.2007.10.006 (2008).
- 28 Weisleder, N. *et al.* Muscle aging is associated with compromised Ca²⁺ spark signaling and segregated intracellular Ca²⁺ release. *J Cell Biol* 174, 639–645, doi:jcb.200604166 [pii] 10.1083/jcb.200604166 (2006).
- 29 Weisleder, N. & Ma, J. Altered Ca²⁺ sparks in aging skeletal and cardiac muscle. *Ageing Res Rev* 7, 177–188, doi:S1568-1637(08)00002-0 [pii] 10.1016/j.arr.2007.12.003 (2008).
- 30 Zhao, X. *et al.* Compromised store-operated Ca²⁺ entry in aged skeletal muscle. *Aging Cell* 7, 561–568, doi:ACE408 [pii] 10.1111/j.1474-9726.2008.00408.x (2008).
- 31 Takeshima, H. *et al.* Mitsugumin29, a novel synaptophysin family member from the triad junction in skeletal muscle. *The Biochemical journal* 331 (Pt 1), 317–322 (1998).
- 32 Pan, Z. *et al.* Dysfunction of store-operated calcium channel in muscle cells lacking mg29.

Nature cell biology 4, 379–383, doi:10.1038/ncb788 (2002).

- 33 Nagaraj, R. Y. *et al.* Increased susceptibility to fatigue of slow- and fast-twitch muscles from mice lacking the MG29 gene. *Physiological genomics* 4, 43–49 (2000).
- 34 Pan, Z. *et al.* Co-expression of MG29 and ryanodine receptor leads to apoptotic cell death: effect mediated by intracellular Ca²⁺ release. *The Journal of biological chemistry* 279, 19387–19390, doi:10.1074/jbc.C400030200 (2004).
- 35 Brotto, M. A. *et al.* Defective maintenance of intracellular Ca²⁺ homeostasis is linked to increased muscle fatigability in the MG29 null mice. *Cell research* 14, 373–378, doi:10.1038/sj.cr.7290237 (2004).
- 36 Kurebayashi, N. *et al.* Changes in Ca²⁺ handling in adult MG29-deficient skeletal muscle. *Biochemical and biophysical research communications* 310, 1266–1272 (2003).
- 37 Yazawa, M. *et al.* TRIC channels are essential for Ca²⁺ handling in intracellular stores. *Nature* 448, 78–82, doi:nature05928 [pii] 10.1038/nature05928 (2007).
- 38 Zhao, X. *et al.* Ca²⁺ overload and sarcoplasmic reticulum instability in tric-a null skeletal muscle. *The Journal of biological chemistry* 285, 37370–37376, doi:10.1074/jbc.M110.170084 (2010).
- 39 Takeshima, H., Komazaki, S., Nishi, M., Iino, M. & Kangawa, K. Junctophilins: a novel family of junctional membrane complex proteins. *Molecular cell* 6, 11–22 (2000).
- 40 Landstrom, A. P. *et al.* Junctophilin-2 expression silencing causes cardiocyte hypertrophy and abnormal intracellular calcium-handling. *Circulation. Heart failure* 4, 214–223, doi:10.1161/CIRCHEARTFAILURE.110.958694 (2011).
- 41 Cai, C. *et al.* MG53 regulates membrane budding and exocytosis in muscle cells. *The Journal of biological chemistry* 284, 3314–3322, doi:10.1074/jbc.M808866200 (2009).
- 42 Jung, S. Y. & Ko, Y. G. TRIM72, a novel negative feedback regulator of myogenesis, is transcriptionally activated by the synergism of MyoD (or myogenin) and MEF2. *Biochemical and biophysical research communications* 396, 238–245, doi:10.1016/j.bbrc.2010.04.072 (2010).
- 43 Ishikawa, Y., Otsu, K. & Oshikawa, J. Caveolin; different roles for insulin signal? *Cellular signalling* 17, 1175–1182, doi:10.1016/j.cellsig.2005.03.025 (2005).
- 44 Inokuchi, J. Membrane microdomains and insulin resistance. *FEBS letters* 584, 1864–1871, doi:10.1016/j.febslet.2009.10.012 (2010).
- 45 Cohen, A. W. *et al.* Caveolin-1-deficient mice show insulin resistance and defective insulin receptor protein expression in adipose tissue. *American journal of physiology. Cell physiology* 285, C222–235, doi:10.1152/ajpcell.00006.2003 (2003).
- 46 Oshikawa, J. *et al.* Insulin resistance in skeletal muscles of caveolin-3-null mice. *Proceedings of the National Academy of Sciences of the United States of America* 101, 12670–12675, doi:10.1073/pnas.0402053101 (2004).
- 47 Liu, L. *et al.* Deletion of Cavin/PTRF causes global loss of caveolae, dyslipidemia, and glucose intolerance. *Cell metabolism* 8, 310–317, doi:10.1016/j.cmet.2008.07.008 (2008).
- 48 Kim, B. W. *et al.* Lipid raft proteome reveals that oxidative phosphorylation system is

associated with the plasma membrane. *Expert review of proteomics* 7, 849–866, doi:10.1586/epr.10.87 (2010).

- 49 Saeki, K., Miura, Y., Aki, D., Kurosaki, T. & Yoshimura, A. The B cell-specific major raft protein, Raftlin, is necessary for the integrity of lipid raft and BCR signal transduction. *The EMBO journal* 22, 3015–3026, doi:10.1093/emboj/cdg293 (2003).
- 50 Gupta, N. *et al.* Quantitative proteomic analysis of B cell lipid rafts reveals that ezrin regulates antigen receptor-mediated lipid raft dynamics. *Nature immunology* 7, 625–633, doi:10.1038/ni1337 (2006).
- 51 Hill, M. M. *et al.* PTRF-Cavin, a conserved cytoplasmic protein required for caveola formation and function. *Cell* 132, 113–124, doi:10.1016/j.cell.2007.11.042 (2008).
- 52 Kim, K. B. *et al.* Proteome analysis of adipocyte lipid rafts reveals that gC1qR plays essential roles in adipogenesis and insulin signal transduction. *Proteomics* 9, 2373–2382, doi:10.1002/pmic.200800811 (2009).
- 53 Zhu, H. *et al.* Polymerase transcriptase release factor (PTRF) anchors MG53 protein to cell injury site for initiation of membrane repair. *The Journal of biological chemistry* 286, 12820–12824, doi:10.1074/jbc.C111.221440 (2011).
- 54 Hatakeyama, S. TRIM proteins and cancer. *Nature reviews. Cancer* 11, 792–804, doi:10.1038/nrc3139 (2011).
- 55 Cai, C. *et al.* Zinc Binding to MG53 Protein Facilitates Repair of Injury to Cell Membranes. *The Journal of biological chemistry* 290, 13830–13839, doi:10.1074/jbc.M114.620690 (2015).
- 56 Li, H. *et al.* Modulation of wound healing and scar formation by MG53 protein-mediated cell membrane repair. *The Journal of biological chemistry* 290, 24592–24603, doi:10.1074/jbc.M115.680074 (2015).
- 57 Hwang, M., Ko, J. K., Weisleder, N., Takeshima, H. & Ma, J. Redox-dependent oligomerization through a leucine zipper motif is essential for MG53-mediated cell membrane repair. *American journal of physiology. Cell physiology* 301, C106–114, doi:10.1152/ajpcell.00382.2010 (2011).
- 58 Park, E. Y. *et al.* Crystal structure of PRY-SPRY domain of human TRIM72. *Proteins* 78, 790–795, doi:10.1002/prot.22647 (2010).
- 59 Bansal, D. & Campbell, K. P. Dysferlin and the plasma membrane repair in muscular dystrophy. *Trends in cell biology* 14, 206–213 (2004).
- 60 McNeil, P. L. & Kirchhausen, T. An emergency response team for membrane repair. *Nat Rev Mol Cell Biol* 6, 499–505 (2005).
- 61 Weiler, T. *et al.* Identical mutation in patients with limb girdle muscular dystrophy type 2B or Miyoshi myopathy suggests a role for modifier gene(s). *Hum Mol Genet* 8, 871–877 (1999).
- 62 Saito, A. *et al.* Miyoshi myopathy patients with novel 5' splicing donor site mutations showed different dysferlin immunostaining at the sarcolemma. *Acta Neuropathol* 104, 615–620 (2002).
- 63 Glover, L. & Brown, R. H., Jr. Dysferlin in membrane trafficking and patch repair. *Traffic* 8, 785–794 (2007).
- 64 Kim, S., Seo, J., Ko, Y. G., Huh, Y. D. & Park, H. Lipid-binding properties of TRIM72. *BMB reports* 45, 26–31 (2012).

- 65 Lin, P. *et al.* Nonmuscle myosin IIA facilitates vesicle trafficking for MG53-mediated cell membrane repair. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 26, 1875–1883, doi:10.1096/fj.11-188599 (2012).
- 66 Kehrer, J. P. & Klotz, L. O. Free radicals and related reactive species as mediators of tissue injury and disease: implications for Health. *Critical reviews in toxicology* 45, 765–798, doi:10.3109/10408444.2015.1074159 (2015).
- 67 Fischer, R. & Maier, O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. *Oxidative medicine and cellular longevity* 2015, 610813, doi:10.1155/2015/610813 (2015).
- 68 Wilson, E. M. & Rotwein, P. Control of MyoD function during initiation of muscle differentiation by an autocrine signaling pathway activated by insulin-like growth factor-II. *The Journal of biological chemistry* 281, 29962–29971, doi:10.1074/jbc.M605445200 (2006).
- 69 Serra, C. *et al.* Functional interdependence at the chromatin level between the MKK6/p38 and IGF1/PI3K/AKT pathways during muscle differentiation. *Molecular cell* 28, 200–213, doi:10.1016/j.molcel.2007.08.021 (2007).
- 70 Potthoff, M. J. & Olson, E. N. MEF2: a central regulator of diverse developmental programs. *Development* 134, 4131–4140, doi:10.1242/dev.008367 (2007).
- 71 Bassel-Duby, R. & Olson, E. N. Signaling pathways in skeletal muscle remodeling. *Annual review of biochemistry* 75, 19–37, doi:10.1146/annurev.biochem.75.103004.142622 (2006).
- 72 Wu, H. *et al.* MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *The EMBO journal* 19, 1963–1973, doi:10.1093/emboj/19.9.1963 (2000).
- 73 Potthoff, M. J. *et al.* Histone deacetylase degradation and MEF2 activation promote the formation of slow-twitch myofibers. *The Journal of clinical investigation* 117, 2459–2467, doi:10.1172/JCI31960 (2007).
- 74 Han, R. *et al.* Dysferlin-mediated membrane repair protects the heart from stress-induced left ventricular injury. *The Journal of clinical investigation* 117, 1805–1813, doi:10.1172/JCI30848 (2007).
- 75 Wang, X. *et al.* Cardioprotection of ischemia/reperfusion injury by cholesterol-dependent MG53-mediated membrane repair. *Circulation research* 107, 76–83, doi:10.1161/CIRCRESAHA.109.215822 (2010).
- 76 Zhu, H. *et al.* Amelioration of ischemia-reperfusion-induced muscle injury by the recombinant human MG53 protein. *Muscle & nerve* 52, 852–858, doi:10.1002/mus.24619 (2015).
- 77 Rommel, C. *et al.* Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. *Science* 286, 1738–1741 (1999).
- 78 Goodman, C. A., Mayhew, D. L. & Hornberger, T. A. Recent progress toward understanding the molecular mechanisms that regulate skeletal muscle mass. *Cellular signalling* 23, 1896–1906, doi:10.1016/j.cellsig.2011.07.013 (2011).
- 79 Yao, Y. *et al.* MG53 permeates through blood-brain barrier to protect ischemic brain injury. *Oncotarget*, doi:10.18632/oncotarget.7965 (2016).
- 80 Burkin, D. J. & Wuebbles, R. D. A molecular bandage for diseased muscle. *Science translational*

medicine 4, 139fs119, doi:10.1126/scitranslmed.3004082 (2012).

- 81 Ma, H. *et al.* Effect of metabolic syndrome on mitsugumin 53 expression and function. *PloS one* 10, e0124128, doi:10.1371/journal.pone.0124128 (2015).
- 82 Ma, L. L. *et al.* Hypertrophied myocardium is refractory to sevoflurane-induced protection with alteration of reperfusion injury salvage kinase/glycogen synthase kinase 3 β signals. *Shock* 40, 217–221, doi:10.1097/SHK.0b013e3182a0674b (2013).
- 83 Xu, Y. *et al.* Hypercholesterolemic myocardium is vulnerable to ischemia-reperfusion injury and refractory to sevoflurane-induced protection. *PloS one* 8, e76652, doi:10.1371/journal.pone.0076652 (2013).
- 84 Yuan, H. *et al.* Proteomic analysis of skeletal muscle in insulin-resistant mice: response to 6-week aerobic exercise. *PloS one* 8, e53887, doi:10.1371/journal.pone.0053887 (2013).
- 85 Terauchi, Y. *et al.* Development of non-insulin-dependent diabetes mellitus in the double knockout mice with disruption of insulin receptor substrate-1 and beta cell glucokinase genes. Genetic reconstitution of diabetes as a polygenic disease. *The Journal of clinical investigation* 99, 861–866. (1997).
- 86 Tamemoto, H. *et al.* Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372, 182–186, doi:10.1038/372182a0 (1994).
- 87 Laustsen, P. G. *et al.* Lipotrophic diabetes in Irs1(-/-)/Irs3(-/-) double knockout mice. *Genes & development* 16, 3213–3222, doi:10.1101/gad.1034802 (2002).
- 88 Long, Y. C., Cheng, Z., Copps, K. D. & White, M. F. Insulin receptor substrates Irs1 and Irs2 coordinate skeletal muscle growth and metabolism via the Akt and AMPK pathways. *Mol Cell Biol* 31, 430–441, doi:10.1128/MCB.00983-10 MCB.00983-10 [pii] (2011).
- 89 Liu, F. *et al.* Upregulation of MG53 induces diabetic cardiomyopathy through transcriptional activation of peroxisome proliferation-activated receptor α . *Circulation* 131, 795–804, doi:10.1161/CIRCULATIONAHA.114.012285 (2015).

