

## Clinical Implications of Basic Neuroscience Research

# TRIM Proteins in Therapeutic Membrane Repair of Muscular Dystrophy

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Muscular dystrophy represents a major unmet medical need; only palliative treatments exist for this group of debilitating diseases. Because multiple forms of muscular dystrophy arise from compromised sarcolemmal membrane integrity, a therapeutic approach that can target this loss of membrane function could be applicable to a number of these distinct diseases. One promising therapeutic approach involves the process the cell uses to repair injuries to the plasma membrane. Recent discoveries of genes associated with the membrane repair process provide an opportunity to promote this process as a way to treat muscular dystrophy. One such gene is mitsugumin 53 (MG53), a member of the tripartite motif (TRIM) family of proteins (TRIM72), which is an essential component of the membrane repair pathway in muscle. Recent results indicate that MG53/TRIM72 protein can be directly applied as a therapeutic agent to increase membrane repair capacity of many cell types and treat some aspects of the disease in mouse models of muscular dystrophy. There is great potential for the use of recombinant human MG53 in treating muscular dystrophy and other diseases in which compromised membrane integrity contributes to the disease. Other TRIM family proteins may provide additional targets for therapeutic intervention in similar disease states.

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One fundamental requirement for the maintenance of cellular homeostasis is the presence of an intact plasma membrane bordering the intracellular cytosolic space. Rupture of the plasma membrane allows for free exchange between the extracellular milieu and cytosolic contents; if the membrane permeability barrier is not rapidly reestablished, the cell will die. Although a simple lipid bilayer would reseal disruptions by the thermodynamic alignment of the hydrophilic lipid heads, the native plasma membrane is held in tension by its attachment to the cytoskeleton and the extracellular matrix. Thus, there are limits to how much the plasma membrane can be repaired by thermodynamic resealing, and an active process must be available to patch membrane disruptions. The membrane repair process is a conserved, physiologically relevant cellular response to the disruption of the plasma membrane. In many eukaryotic cell types, disruptions of the plasma membrane trigger an active process whereby intracellular vesicles or organelles rapidly traffic to the injury site on the plasma membrane. These vesicles provide a source of additional lipids that can fuse with one another or directly with the plasma membrane to create a membrane repair patch. This response is dependent on calcium ion  $Ca^{2+}$  and has been compared with the vesicle-mediated neurotransmitter release process.<sup>1</sup>

The membrane repair process was first identified *in vivo* in the gut<sup>2</sup> and skeletal muscles but has since been confirmed to occur in a variety of different tissue types.<sup>3</sup> Although the cellular aspects of membrane repair have been known for some time, only recently have several genes been demonstrated to mediate

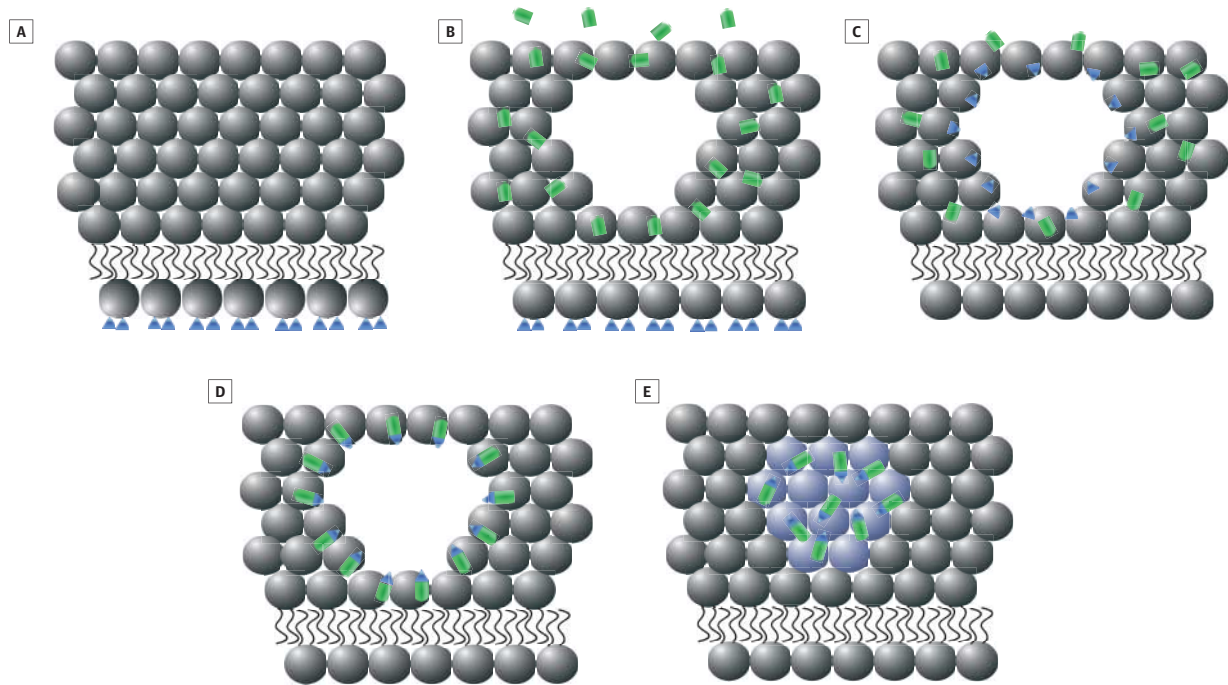
membrane repair. One of the first genes to be directly linked to membrane repair was *dysferlin*<sup>4</sup>; several other genes have now been linked to membrane repair, and there are undoubtedly more to be discovered.

## Molecular Basis for Membrane Repair

Recent findings established that mitsugumin 53 (MG53) is an essential component of the membrane repair mechanism in striated muscles. This protein is a muscle-enriched member of the tripartite motif (TRIM) family of E3 ubiquitin ligase proteins and is also known as TRIM72. It was initially isolated from a skeletal muscle immunoproteomic library in the laboratory of Hiroshi Takeshima, PhD. The structure of the protein consists of a canonical TRIM domain at the N-terminus and a dual-specificity kinase *splA* (SPRY) domain at the C-terminus, a common configuration of several proteins in the TRIM family.

Initial studies in the laboratory of Jianjie Ma, PhD (Robert Wood Johnson Medical School, Piscataway, New Jersey), resolved that MG53/TRIM72 was an important mediator of the cell membrane repair process that nucleates assembly of the membrane repair patch at the membrane injury site.<sup>5</sup> The loss of MG53/TRIM72 function resulted in muscle fibers that would not properly reseal after membrane damage. A myopathy phenotype could be observed in *mg53*<sup>-/-</sup> mice, establishing that MG53/TRIM72 was essential for normal muscle membrane repair.

Figure. Recombinant Human MG53 (rhMG53) Associates With Phosphatidylserine (PS) During Therapeutic Membrane Repair



A, In a healthy cell, PS (blue) is generally sequestered on the inner leaflet of the plasma membrane. B, rhMG53 (green) does not seem to associate with the intact plasma membrane, but disruption of the membrane enables specific interaction with the injury site. C, After disruption of the plasma membrane, PS flows from the inner leaflet onto the cell surface. D, The appearance of PS on

the membrane surface provides a target for rhMG53 binding to the injury site. E, Association of rhMG53 with the injury site enables more efficient membrane resealing to patch the disruption in the plasma membrane, enabling survival of the cell.

## Action of rhMG53 in Membrane Repair

Other recent studies showed that genetic overexpression of MG53/TRIM72 through viral gene delivery could have therapeutic benefit in muscular dystrophy and related cardiomyopathy.<sup>6</sup> Despite the success of this approach, the challenges associated with viral gene delivery make it attractive to find alternative approaches for treating human diseases that involve compromised membrane resealing.

During biochemical studies with the recombinant human MG53 (rhMG53) protein, a surprising finding revealed that the isolated protein could increase the plasma membrane repair capacity of cells when applied to the extracellular space.<sup>7</sup> Fluorescently tagged rhMG53 protein provided outside the cell can specifically localize to membrane disruptions; this interaction allows for more efficient resealing of disruptions in both isolated muscle cells and nonmuscle cell types. The extent of membrane damage produced by physical, chemical, or electrical permeabilization was measured in multiple assays, including quantification of intracellular enzyme leak into the extracellular space or the entry of indicator dye into the cell. In all tested cases, rhMG53 decreased the amount of membrane damage in cultured cells. Functional rhMG53 can be produced in a variety of different host species, including bacteria (*Escherichia coli*), insect cells via baculovirus methods, and Chinese hamster ovary cells. This isolated protein can be used to confirm the protective effects

of rhMG53 with in vivo damage assays in which intramuscular or intravenous injection of rhMG53 is used to prevent cardiotoxin-mediated myocyte death.<sup>7</sup>

These results indicate that rhMG53 can reduce the extent of membrane injury in vitro and in vivo, but the mechanisms that allow it to function when applied outside the cell must still be studied. The available data indicate that neither expression of MG53/TRIM72 nor expression of dysferlin is required for the rhMG53 to increase the membrane repair capacity of a cell. This suggests that externally applied rhMG53 acts by a unique mechanism when compared with endogenously expressed MG53/TRIM72. Based on previous findings that MG53/TRIM72 specifically binds phosphatidylserine (PS),<sup>7</sup> it is possible that this activity contributes to rhMG53 action outside the cell (Figure). In the plasma membrane of a healthy cell, PS is compartmentalized to the inner leaflet. Injury of the cell or induction of apoptosis exposes PS to the external space. Binding to exposed PS when the membrane is disturbed is one way in which externally applied rhMG53 could identify injury sites on the plasma membrane. This is a likely mechanism for the targeting of rhMG53 to injured cells, but the molecular effects that occur at the injury site to increase membrane resealing are not fully established. Although future studies will be necessary to fully resolve this mechanism, studies in the meantime could directly test whether rhMG53 has the capacity to treat disease states in which compromised membrane integrity contributes to disease progression, such as muscular dystrophy.

## rhMG53 in the Treatment of Muscular Dystrophy

Disruption of the plasma membrane by physiologic stress is a common occurrence in many tissues in the body. Pathophysiologic levels of stress can overcome the native membrane repair capacity of those tissues, with pathologic effects. Compromised membrane repair capacity has been linked to various disease states in which the reduced membrane repair capacity cannot repair the accumulated damage.

The most clearly relevant human disease is muscular dystrophy, for which mutations in a number of different genes involved in membrane repair have been linked to the development of limb girdle muscular dystrophy (LGMD). The muscular dystrophies are a diverse group of degenerative muscle diseases with variable symptoms that result from mutations in various genes and are classified based on their clinical phenotype. The more common X-linked dystrophinopathies, such as Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy, produce extensive muscle degeneration early in life that lead to immobility, cardiac complications, and, in the case of DMD, death, usually after the second decade of life. Patients with LGMD generally share the presentation of muscle weakness primarily in the limb musculature, with the proximal muscles displaying greater weakness than more distal muscles. The age at onset, extent of muscle wasting, and pathologic cardiac involvement vary based on the particular genetic disruption, with mutations in more than 15 genes leading to LGMD of various types and severity.

Congenital muscular dystrophy includes degenerative muscle diseases with genetically heterogeneous origins and variable symptoms. Patients with congenital muscular dystrophy generally have a common presentation of symptoms near birth or during infancy, including displaying muscle weakness and hypotonia with advancing skeletal muscle disease that can lead to immobilization and respiratory failure. Cardiac and nervous system involvement are sometimes associated with congenital subtypes.

Current treatments for muscular dystrophies center on palliative measures with occupational approaches that allow patients to remain mobile as long as possible. However, none of these treatments have any efficacy with the underlying disease process. Although there are promising possible future therapies for muscular dystrophies, including gene therapy replacement of affected genes and stem cell treatment, these treatments are still years away from application in human patients. Genetic replacement approaches are further complicated by the number of different genes mutated in the various muscular dystrophies. As a result, there is a great unmet medical need for intermediate therapies to reduce the pathologic effects of muscular dystrophies, particularly if they can be applied effectively in different types of muscular dystrophy.

One common element in the muscular dystrophies is that many different types display altered membrane integrity. In DMD, the absence of dystrophin increases the fragility of the sarcolemmal membrane of striated muscle cells. Other muscular dystrophies are directly linked to compromised membrane repair capacity, particularly some forms of LGMD that result from certain mutations in dysferlin or caveolin-3. A potential therapeutic agent would be more vi-

able if it could treat multiple forms of LGMD, perhaps by targeting a conserved pathologic mechanism shared in several of these distinct diseases.

Because rhMG53 can boost membrane repair capacity, it may be able to address multiple types of muscular dystrophy with a single therapeutic approach. As a first step of testing its efficacy in the treatment of disease, rhMG53 was injected into a mouse model of DMD, the *mdx* dystrophic mice that lack expression of dystrophin.<sup>7</sup> In these experiments, rhMG53 had protective effects against increased membrane permeability after an eccentric running exercise in the *mdx* mice. Further studies examined the effects of short-term injection of rhMG53 during the initial wave of myonecrosis that occurs in these mice shortly after they first become ambulatory. This short-term rhMG53 treatment significantly reduced the area of muscle displaying myonecrosis and/or fibrosis in these animals and directly affected the permeability of dystrophic muscle fibers; the infiltration of Evans blue dye into the muscle fibers was reduced with the injection of rhMG53. These short-term trials provide initial evidence that rhMG53 may be an effective treatment for muscular dystrophy arising from fragility of the sarcolemmal membrane. Additional studies will be necessary to establish whether long-term application of rhMG53 can provide prolonged relief from the symptoms of the disease. It would also be useful to expand these studies into models of other muscular dystrophies that involve other genetic lesions but share a common pathologic mechanism, one involving death of myocytes due to breakdown of the sarcolemmal membrane.

There are certain advantages to using rhMG53 as a protein therapeutic to treat muscular dystrophy. Because native MG53 appears in the blood stream of normal mice and other animals, the immune system is exposed to the protein during normal physiologic function. This suggests that administering additional therapeutic rhMG53 should present a minimal risk of immunologic or toxicologic effects. An additional advantage of using rhMG53 is that it can be produced effectively in different hosts, including bacteria and mammalian cells, which can simplify production of the protein for preclinical studies and clinical trials in muscular dystrophy. rhMG53 can also be effectively applied by subcutaneous injection in animal models,<sup>7</sup> so it could potentially be self-administered by patients without the need for additional clinic visits.

Of course, there are still significant unanswered questions regarding the development of rhMG53 as a treatment for muscular dystrophy. One challenge that remains is to establish whether the pharmacokinetics of unmodified rhMG53 are sufficient to be effective in patients with muscular dystrophy. One would expect that rhMG53 would have to be provided when dystrophic muscle fibers are initially injured, and thus treatment would be required throughout a patient's life. Should the pharmacokinetics prove insufficient, there are well established protein engineering approaches to improve delivery of protein drugs for a variety of human diseases. One additional concern could arise from the role of natively expressed MG53/TRIM72 in myogenesis.<sup>8</sup> Because genetic modification of MG53/TRIM72 can alter myogenesis in cell-based model systems, any possible effects of rhMG53 on myogenic signaling must be examined to determine whether these could affect its therapeutic use.

## Future Directions for Therapeutic Membrane Repair

Given the key contribution of compromised sarcolemmal membrane integrity and repair to the progression of various muscular dystrophies, these diseases represent a good first target for the treatment of human diseases by the modulation of membrane repair capacity. However, there are many other disease states in various tissue types wherein compromised membrane repair capacity contributes to disease progression, such as neurodegeneration and acute lung injury. There are even more diseases in which the ultimate pathologic end point includes breakdown of the plasma membrane, death of individual cells, and progression into eventual organ failure, including heart failure and muscular dystrophy. Application of rhMG53 as a “molecular bandage” to injured cells may increase the membrane integrity of these cells and allow them to recover from damage that would normally result in cell death.<sup>9</sup> Although not all cells would be able to recover, current results in studies with the *mdx* mouse model and cell damage assays indicate that enough cells can be spared to improve the structure and function of a target tissue.<sup>7</sup> Boosting membrane repair to prevent cell death could target disease states in which traumatic injury leads to a sudden wave of cell death or chronic diseases in which continual cell death contributes to prolonged decreases in tissue function.

Because rhMG53 proved effective in increasing membrane repair in different nonmuscle cell types,<sup>7</sup> one future application of rhMG53 may be in treating both acute and chronic diseases outside the striated muscles. The brain is one tissue type with limited regenerative capacity that could benefit from treatments to increase membrane repair capacity. If rhMG53 can effectively diminish neuronal cell death after injury, it would have multiple potential applications in the neuronal system. Traumatic brain injuries involve mechanical damage to the cell membrane that could be diminished by modulating the membrane repair capacity of these cells. Breakdown of the cell membrane and death of the

cell contributes to chronic neurodegenerative diseases, such as Alzheimer disease, and previous efforts to increase membrane repair show protective effects at the level of the individual neuron. Application of rhMG53 in such diseases would require delivery of the protein to the brain, as well as establishment of the time window required for effective treatment.

Because rhMG53 appears to be effective in nonmuscle cell types, it is also interesting to note that although the expression of native MG53/TRIM72 is primarily restricted to striated muscle cells, it is possible to express the complementary DNA in numerous nonmuscle cell types and fully recapitulate the expected membrane repair function. Mechanical or chemical disruption of the plasma membrane results in translocation of exogenous green fluorescent protein-tagged MG53/TRIM72 to the membrane injury sites in nearly all cell types that have been tested.<sup>7</sup> This response is dependent on the expression of nonmuscle myosin type IIA, but it seems independent of the expression of known interaction partners of MG53, including dysferlin and caveolin-3.<sup>10</sup>

One possible explanation for the ability of MG53/TRIM72 to recapitulate activity in nonnative cell types is that it interacts with components of basal repair machinery present in many different cell types. In this scenario, MG53/TRIM72 would have evolved as a protective mechanism to compensate for the increased damage to the sarcolemmal membrane that occurs in striated muscle because of the contractile nature of the tissue. Another possibility is that other TRIM family proteins may serve a function similar to that of MG53/TRIM72 in other cell types. The TRIM family is a large gene family with more than 70 members in the human genome, but very few of these proteins have been extensively studied. Many of them have a domain structure similar to that of MG53/TRIM72, with a C-terminal SPRY domain. In other tissue types, there may be additional TRIM family proteins that act similarly to MG53/TRIM72 in the membrane repair process. If so, this would open several different therapeutic possibilities for modulating membrane repair across a wide variety of tissues and disease states, possibly establishing new biomarkers for the detection of various pathologic conditions.

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