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Article Addendum

Mitsugumin 53 (MG53) facilitates vesicle trafficking in striated muscle to contribute to cell membrane repair

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Repair of the plasma membrane following damage is an important aspect of normal cellular physiology, and disruption of this process is observed in many pathologic states. In a recent series of publications, we resolved that Mitsugumin 53 (MG53) is a novel, muscle-specific member of the tripartite motif/RING B-box Coiled Coil (TRIM/RBCC) family of proteins (TRIM72) that contributes to vesicle trafficking in the course of normal cellular physiology. MG53 can bind phosphatidylserine (PS) with some specificity, and interacts with caveolin-3 (Cav-3) as part of its function in vesicle trafficking. As part of the response to membrane damage in muscle cells, MG53 acts in an oxidation-dependent manner to facilitate vesicle translocation to the sites of membrane injury where these vesicles are involved in patching the membrane. Here we discuss these findings and examine the implications of this work in the field of membrane repair. Further discussion is provided about potential therapeutic applications for MG53.

Repair of acute damage to the plasma membrane is an important aspect of normal cellular physiology,[1,2] and disruption of this process can result in pathophysiology in a number of different tissues.[3-5] Several important previous studies have established the framework of the cell membrane repair response.[6,7] It is known that this process requires the translocation of intracellular vesicles[8] to the injury site though the action of kinesin motor proteins.[9] These vesicles then fuse with the plasma membrane in a Ca^{2+} dependent manner, similar to the mechanism underlie neurotransmitter release from neurons.[10] Three steps would be necessary to mediate this process; sensing the membrane disruption, facilitating vesicle translocation and vesicle fusion with the membrane injury site. While the underlying process of membrane repair is known, the molecular machinery involved in this response is less well defined. Recent studies have identified some molecular components, particularly those involved in a putative pathway specific to striated muscles. Dysferlin is essential for membrane repair in striated muscle,[4] and is thought to contribute to vesicle fusion at the plasma membrane.[11] However, the factor(s) that sense the disruption of the plasma membrane and control rapid trafficking of vesicles to the injury site were not well described.

In a recent series of publications,[12-14] we resolved that Mitsugumin 53 (MG53) is a novel, muscle-specific member of the tripartite motif/RING B-box Coiled Coil (TRIM/RBCC) family of proteins (TRIM72) that contributes to vesicle trafficking in the course of normal cellular physiology.[12] Thought screening of our unique immunoproteomic monoclonal antibody targeting membrane-associated proteins found in striated muscle,[15] we identified a monoclonal antibody (mAb 5259) that would recognize the subsarcolemmal space and the gap within the triad junction. Immunoaffinity purification with this antibody produced a protein of 53 kDa that we named MG53.

MG53 associates with intracellular vesicles and the inner leaflet of the sarcolemma of striated muscle, likely though binding with phosphatidylserine (PS) that is enriched in these membrane surfaces. Such vesicles can be readily observed trafficking through the cell, fusing with the sarcolemma and entering the extracellular space. Overexpression of MG53 results in excessive fusion of these vesicles with the sarcolemmal membrane leading to the formation of filapodia-like tendrils.[12] These vesicles may be involved in trafficking of normal components of the sarcolemmal membrane as such fusion events result in increased K^+-channel density at the sarcolemmal membrane, while decreased MG53 levels lead to diminished K^+-channel activity.[14] This MG53-mediated exocytosis activity seems to be controlled in native striated muscle by the activity of a muscle-specific protein associated with endocytosis, caveolin-3 (Cav-3). Cav-3 can act in an antagonistic manner

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Cai C, Masumiya H, Weisleder N, Pan Z, Nishi M, Komazaki S, Takeshima H, Ma J. MG53 regulates membrane budding and exocytosis in muscle cells. J Biol Chem 2009; 284:3314–22; PMID: 19029292; DOI:10.1074/jbc.M808866200.

Masumiya H, Asaumi Y, Nishi M, Minamisawa S, Adachi-Akahane S, Yoshida M, Kangawa K, Ito K, Kagaya Y, Yanagisawa T, Yamazaki T, Ma J, Takeshima H. Mitsugumin 53-mediated maintenance of K^+ currents in cardiac myocytes. Channels 2009; 3:6-11; PMID: 19202355;

to reduce the activity of MG53 in trafficking of vesicles to the sarcolemmal membrane.[12]

Membrane resealing is considered to be an emergency response to significant disruptions of the plasma membrane integrity, where protein with a related function in normal cellular physiology are repurposed to facilitate membrane resealing.[16] As MG53 functions in trafficking of intracellular vesicles in the course of normal cellular physiology, MG53 is positioned to be involved in the membrane resealing process. Further study revealed that MG53 containing vesicles can translocate to sites of membrane damage following mechanical or laser-induced injury to the plasma membrane.[13] Muscle fibers from MG53 knockout mice display defective membrane resealing following such injuries. Such evidence suggests that MG53 can function in facilitating vesicle translocation during the second step during membrane repair. This role for MG53 was confirmed by electron microscopy that indicated a lack of vesicle translocation to site of injury in MG53 knockout muscle. As would be expected, MG53 mice do develop a mild myopathy that can be exacerbated by eccentric exercise known to induce muscle membrane damage.

After confirming that MG53 was essential for vesicle translocation to the sites of membrane injury, we examined if MG53 might participate in sensing disruption of the plasma membrane. MG53 appears as a monomer under reduced conditions, as in the cytoplasm of an intact cell. However, when in an oxidative environment, as is found extracellularly, MG53 forms oligomer complexes. A targeted mutagenesis approach determined that MG53 oligomer formation requires a specific conserved cystidine residue (C242). Mutant MG53 (C242A) cannot form oligomers, displays defective trafficking to injury sites and can disrupt wild type MG53 function in a dominant negative fashion. Thus, we propose a model where the entry of the oxidized intracellular environment activates MG53 to facilitate vesicle translocation to the site of membrane disruption. Fusion of the these vesicles at the plasma membrane would require the presence of Ca^{2+} from the extracellular space and the activity of fusigenic factors, such as dysferlin, to create a membrane repair patch.

Testing the veracity of this model is an important step in the further study of MG53 in vesicle trafficking and membrane repair. While we found that translocation of vesicles to the plasma membrane could occur in the absence of extracellular Ca^{2+}, the addition of extracellular Ca^{2+} could increase the quantity of MG53 containing vesicles that appeared at injury sites. As MG53 does not contain a consensus Ca^{2+}-binding domain, there may be accessory factors that interact with MG53 to provide this Ca^{2+}-dependent activity. One would expect several accessory factors to contribute to the function of MG53 in both vesicle trafficking and repair of the plasma membrane. For example, it remains to be determined how Cav-3 interacts with MG53 to control intracellular vesicle trafficking and membrane remodeling in muscle cells. Establishing if MG53 can directly affect dysferlin function is also a potential future series of studies.

Another outstanding question raised by our studies involves the application of these findings outside of muscle cells. While Ca^{2+} is known to be required for membrane resealing in a number of different cell types, the role of oxidation in membrane repair has not yet been examined. If entry of the oxidative environment is important in membrane resealing in many cell types, then modulation of the extracellular oxidative state could have therapeutic implications in a number of tissues where compromised membrane repair may be involved, including skeletal and cardiac muscles.[17,18] Additionally, the involvement of a ubiquitous binding target like PS in MG53 function could indicate that MG53 may be able to function in cell types other than muscle. If it can function in non-muscle cell types, MG53 could have significant therapeutic implications. Along these lines, we expect that other TRIM family members may function in a similar fashion as MG53 in other cell types, and discovery of such proteins should constitute an important course of investigation in the future.

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