Dynamin 2 the rescue for centronuclear myopathy

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Centronuclear myopathy is a lethal muscle disease. The most severe form of the disease, X-linked centronuclear myopathy, is due to mutations in the gene encoding myotubularin (MTM1), while mutations in dynamin 2 (DNM2) and amphiphysin 2/BIN1 (AMPH2) cause milder forms of myopathy. MTM1 is a lipid phosphatase, and mutations that disrupt this activity cause severe muscle wasting. In this issue of the JCI, Cowling and colleagues report on their finding of increased DNM2 levels in human and mouse muscle with MTM1 mutations. Partial reduction of Dnm2 in mice harboring Mtm1 mutations remarkably rescued muscle wasting and lethality, and this effect was muscle specific. DNM2 regulates membrane trafficking through vesicular scission, and it is presumed that reducing this activity accounts for improved outcome in X-linked centronuclear myopathy.

Defective membranes in centronuclear myopathies

Centronuclear myopathy (CNM) is an inherited muscle-wasting disease with onset in infancy or childhood. Muscle biopsies from CNM patients characteristically show enlarged and centrally placed nuclei throughout the muscle, and clinically, CNM-associated mutations may result in weakened respiratory musculature, leading to early death without supportive care. Three main forms of CNM are attributable to mutations in myotubularin (MTM1), amphiphysin 2 (AMPH2), and dynamin 2 (DNM2), which all encode membrane-associated proteins that potentially act in muscle at the transverse tubules (T-tubules), membrane invaginations specialized for calcium handling (Figure 1 and ref. 1). The most common and severe form of CNM is X-linked CNM (XLCNM), referred to as myotubular myopathy. XLCNM is clinically evident at birth, with patients presenting with hypotonia and respiratory compromise. Skeletal muscles biopsies from XLCNM patients show abnormal T-tubules, including defective triads, structures critical for excitation-contraction coupling. XLCNM is the result of mutations in MTM1, which encodes a ubiquitously expressed phosphoinositide 3-phosphatase that functions to dephosphorylate PI3P and phosphatidylinositol-3,4-bisphosphate, two phospholipids essential for membrane sorting and signaling (2). MTM1 is required for normal endocytosis, receptor degradation, and early endosome maturation (1).

Mice harboring a deletion of Mtm1 (Mtm1–/y mice) recapitulate human disease and display many characteristic XLCNM-associated features, including muscle weakness, central nuclei, and muscle atrophy (3). Mtm1–/y mice exhibit muscle atrophy, become weak at 3 to 4 weeks of age, and have a greatly reduced life expectancy of only 6 to 12 weeks (3). The role of MTM1 in human muscle has also been effectively modeled in zebrafish using morpholinos to reduce mtm1 expression (MTM MO) (4). MTM MO fish, like XLCNM patients and Mtm1–/y mice, develop weakness and muscle atrophy. MTM MO fish display accumulation of PI3P, especially surrounding muscle nuclei, and T-tubule defects. The phenotypes observed in MTM MO fish provide further support that MTM1 is critical for the normal biogenesis and maintenance of membrane structures within muscle (4). Because of its broad role as a lipid phosphatase, the indispensable targets of MTM1’s enzymatic action are not fully known.

DNM2 is a ubiquitously expressed GTPase that has been implicated in multiple cellular functions, including endocytosis, membrane scission, and cytoskeletal remodeling (5). Dynamins assemble as rings around membrane tubules where they are thought to actively “pinch off” membranes (5). Complete loss of Dnm2 results in embryonic lethality in mice (6). In humans, CNM is caused by dominant DNM2 mutations (7), and overexpression of a CNM-linked DNM2 mutation (R465W) in mouse muscle results in myopathic features, including centralized nuclei, muscle atrophy, and deformed T-tubules (8). Interestingly, overexpression of normal DNM2 in mouse models also produces some of these same features, consistent with the model that increased DNM2 activity contributes to CNM (8). Increased DNM2 activity may lead to excessive membrane scission and pruning, giving the appearance of excessive membrane accumulation around nuclei or at T-tubules.

Genetic reduction of DNM2 in a CNM model

In this issue of the JCI, Cowling and colleagues described elevated protein levels of DNM2 in Mtm1–/y animals and in muscle biopsies from human XLCNM patients (9). To target the increased levels of DNM2 found in Mtm1–/y animals, mice heterozygous for Dnm2 were crossed with Mtm1–/y mice. The in vivo reduction of Dnm2 corrected some histological abnormalities in muscle, but dramatically extended life expectancy from 6 to 12 weeks to beyond one year. To demonstrate that this effect was muscle intrinsic, Cowling and colleagues generated Mtm1–/y mice in which Dnm2 was specifically reduced in skeletal muscle. Reduction of Dnm2 in skeletal muscle alone after disease onset was sufficient to reduce pathology and...
extend the life span in Mtm1−/− animals; how- ever, skeletal muscle–specific Dnm2 deletion did not extend life span in Mtm1−/− animals to the same extent as heterozygous whole body Dnm2 reduction, suggesting that aspects of the phenotype arise from muscle extrinsic effects or the need to reduce Dnm2 earlier in muscle development.

Based on their data, Cowling et al. propose that DNM2 and MTM1 function in an overlapping pathway, but there are no reports of a direct interaction between these two proteins. DNM2 is enriched at the Z band of muscle, adjacent to but not directly overlapping with T-tubules, and DNMT2 is also seen in the membranes surrounding myonuclei. Many DNM2 mutations fall near the pleckstrin homology domain, which may insert directly into membranes to participate in membrane fission. These observations suggest a broader complex may be critical for the restricted action of MTM1 and DNM2 on membrane biogenesis and function. The large size of DNM2 may require alternative approaches to provide adequate resolution to fully define its associated membranes in muscle.

AMPH2, also known as BIN1 (bridging integrator protein), is a Bin–Amphiphysin–Rvs (BAR) domain–containing protein, known to sense membrane curvature and to bend membranes (1). Membrane bending is a key aspect of forming intracellular tubules in all cell types and also in muscle. Recent work has shown that BIN1 binds phosphatidylinositol-4,5-bisphosphate at the T-tubule and is capable of binding both DNM2 and MTM1 (11, 12). The binding of MTM1 to BIN1 enhances tubule formation in vitro, and mutations in MTM1 that cause CNM result in reduced tubule formation in vitro. MTM1-deficient muscle lacks normal BIN1 localization at the T-tubule (12); therefore, MTM1 loss may result in defective membrane biogenesis through upregulation and altered localization of phosphoinositides. The increase in DNM2 observed in XLCNM patients may arise as a consequence of altered lipid signaling.

Currently, there are no drug therapies for CNM patients, and care relies on respiratory support and management. To date, most therapeutic approaches have focused on MTM1 replacement. For example, local restoration of MTM1 with viral delivery improved both the structural and functional deficits of Mtm1 deficiency in mice (13). Alternatively, direct enzyme replacement therapy of MTM1 in mice markedly improved muscle strength after only two weeks of treatment (14). These preclinical approaches for MTM1 replacement are promising, but have yet to be tested in a clinical setting.

The rescue of XLCNM through the genetic reduction of DNM2 is a viable approach for this rare form of muscle disease; however, many genetically diverse forms of inherited myopathies share aspects of the phenotypes seen in CNM. For example, mutations in dysferlin (DYSF) and caveolin 3 (CAV3) also result in some histological abnormalities shared with CNM, including T-tubule defects and muscle weakness (17, 18). Similarly to MTM1, DYSF and CAV3 also regulate endocytosis and colocalize with BIN1 at the T-tubule (19–21). Herein, Cowling and colleagues showed abnormal internalization of CAV3 in Mtm1−/− muscle, which was rescued by Dnm2 reduction. Interestingly, both overexpression of normal DNM2 in mice and DNM2 loss-of-function studies in mice dramatically improve longevity in a mouse model of Mtm1-mediated myopathy. Mutations in AMPH2/BIN1 (red) also cause CNM. These proteins are involved in phosphoinositide (PIP) biogenesis, membrane bending, and membrane fission. Mutations in these genes affect membranes throughout the muscle, including those that surround nuclei and those in the T-tubule.
function mutations in mice and humans results in abnormal DYSF internalization (10, 16). Whether mislocalization of CAV3 and DYSF within the myofiber contributes to CNM disease progression remains to be determined. These data suggest that a common pathway links MTM1, DNM2, BIN1, DYSF, and CAV3 in the biogenesis and maintenance of muscle, specifically at the T-tubule. If DNM2 levels are found to be upregulated in these other forms of myopathy, then targeting DNM2 becomes a common therapeutic strategy for a wider range of muscle disease.

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11. Kojima C, et al. Regulation of Bin1 SH3 domain activity as the world’s fourth-leading cause of death, current medical interven- tions have little to offer beyond symptomatic relief. Meanwhile, the prevalence of COPD is expected to continue to rise as low- and middle-income countries join in the developed world’s tobacco addiction. If we are to avoid this grim projection, we must expand our knowledge of the basic mechanisms behind lung injury and repair before translating these findings into novel therapeutic treatments.

Pulmonary emphysema results from an imbalance between elastases and anti-elastases

It has been more than 50 years since Lau- ret and Eriksson first identified a deficiency in α-1 antitrypsin, the major inhibitor of neutrophil elastase (ELANE), as the cul- prit behind hereditary pulmonary emphy- sema (1). Since the involvement of ELANE in pulmonary emphysema was first report- ed, our understanding of the disease’s

Cigarette smoke is the most common cause of pulmonary emphysema, which results in an irreversible loss of lung structure and function. TH1 and TH17 immune responses have been implicated in emphysema pathogenesis; however, the drivers of emphysema-associated immune dysfunction are not fully understood. In this issue of the JCI, Shan and colleagues found that peroxisome proliferator–activated receptor γ (PPARγ) is downregulated in APCs isolated from the lungs of emphysematous chronic smokers and mice exposed to cigarette smoke. Furthermore, treatment with a PPARγ agonist prevented emphysema development and appeared to reduce emphysema-associated lung volume expansion in mice exposed to cigarette smoke. Further work will need to be done to evaluate the potential of PPARγ agonists to restore lung capacity in emphysematous patients.

Pulmonary emphysema is a major compo- nept of chronic obstructive pulmonary dis- ease (COPD) and involves the loss of alveo- lar units distal to the terminal bronchioles. Even though COPD holds an unenviable position as the world’s fourth-leading cause of death, current medical interven- tions have little to offer beyond symptomatic relief. Meanwhile, the prevalence of

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PPARγ in emphysema: blunts the damage and triggers repair?

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