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TITLE: Hypermetabolism as a Risk Factor for ALS

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ALS, a fatal adult-onset disease, is a neurodegenerative disorder that affects motor neurons in the brain and the spinal cord. What causes ALS remains incompletely understood and no disease modifying therapy is currently available. Recent discovery of an ALS gene called Tat Activating Regulatory DNA Binding Protein (TDP-43) encoding a protein involved in RNA metabolism, provided opportunities to clarify disease pathogenesis and hold promise for development of new therapies. Our recent discovery in mice that lack Tdp-43 showed that these mice excessively burned out their body fat, likely working through a protein called Tbc1d1 which is known to control fat metabolism in skeletal muscle. To test this hypothesis, we generated conditional Tdp-43 knockout mice using a muscle-specific (MLC) driver of Cre recombinase. Loss of Tdp-43 in skeletal muscle led to adult onset weight loss beginning about 3 months of age followed by premature death within a month. Pathological examination of skeletal muscles revealed degeneration of fibers as well as marked regeneration, observations that are consistent with a myopathy occurring in these mice. These new findings suggest an important role for Tdp-43 in skeletal muscle that may contribute to the pathogenesis of ALS and possibly a select group of myopathies.
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Introduction

Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain and spinal cord, is fatal and at present, there is no effective mechanism-based therapy. Our previous work indicated that hypermetabolism related to loss of TDP-43, an essential RNA binding protein implicated in ALS and Frontotemporal dementia (FTD), may contribute to disease pathogenesis (1). Specifically, we generated a conditional Tardbp knockout mouse model to determine the physiological role of TDP-43 in the adult animal. Surprisingly, we found that post-natal deletion of Tardbp in mice caused dramatic loss of body fat (1). We discovered one downstream targets of TDP-43, termed Tbc1d1, a gene primarily expressed in skeletal muscle and known to regulate body fat metabolism (2) and linked to obesity (3), and is down-regulated in the absence of TDP-43 (1). Thus, our results implicate the role of Tardbp-dependent Tbc1d1 in skeletal muscle to regulate body fat metabolism. That some individuals with ALS showed a hypermetabolic state (4) and that there is evidence for defective energy homeostasis in ALS (5) raised the possibility that hypermetabolism may contribute to the pathogenesis of ALS. Although it is not clear how hypermetabolism influences pathogenesis of ALS, it will be of great interest to test directly in mouse models of ALS whether altered fat metabolism participates in motor neuron degeneration using our conditional Tardbp knockout mouse model that exhibit a lean phenotype. Positive outcomes from these studies will offer the possibility that dietary or nutritional interventions to attenuate excessive fat metabolism would be beneficial for patients with ALS. This is particularly important as the nutritional status is thought to be a prognostic factor for survival in ALS (6) and that personalized nutritional management of patients may be a beneficial strategy for symptomatic treatment of ALS (7). Moreover, strategies to up-regulate Tbc1d1 in skeletal muscles may also be an attractive alternative approach towards development of mechanism-based therapies for ALS. Thus, our proposed studies will have direct impact on the development of therapeutics that can be translated into disease-modifying treatments for this devastating disease of the elderly. Because TDP-43 inclusions has been observed in a variety of other neurodegenerative diseases (reviewed in 8), such as Inclusion Body Myopathy associated with Paget's disease of bone and FTD (IBMPFD), it will also be interesting to test whether hypermetabolism may also be a risk factor in these illnesses. If so, our work will have important therapeutic implications beyond ALS.

Body

1. Loss of Tdp-43 in skeletal muscle leads to adult onset weight loss and premature death

To obtain mice lacking Tdp-43 exclusively in skeletal muscle, we crossbred Tdp-43 F/- mice with MLC-Cre; Tdp-43 +/- mice. Mice expressing Cre recombinase driven by regulatory sequences of the Myosin Light Chain 1/3 locus (MLC) demonstrated Cre activity in only skeletal muscle fibers, specifically in fast twitch (Type II) but not in slow twitch (Type I) fibers. Expected Mendelian ratios were observed from these crosses (Table 1), suggesting that Tdp-43 is dispensable in skeletal muscle during embryonic development.

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<th>Tdp-43 F/-</th>
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Table 1: Progeny from crosses between Tdp-43 F/- and MLC-Cre; Tdp-43 +/- mice yielded expected Mendelian frequencies for all genotypes.
Protein expression of Tdp-43 was substantially reduced in skeletal muscle of MLC-Cre;Tdp-43F/− mice (Fig. 1, left panel), but remained unaffected in brain (Fig. 1, right panel) cardiac as well as smooth muscle (data not shown). Levels of Tdp-43 were consistent with that of control mice in a variety of other tissues sampled. Both MLC-Cre;Tdp-43F/− mice and control mice showed comparable growth and development into early adulthood. However, from about 3 months of age, mice lacking Tdp-43 in skeletal muscles decline in weight and show dramatic reduction in body size within a month (Fig. 2). These mice reach end stage within 6 weeks after noticeable onset of weight loss. End stage in MLC-Cre;Tdp-43F/− mice is characterized by an emaciated appearance, a pronounced hunch, inactivity and labored breathing. Kaplan-Meier analysis of a small cohort of mice indicates an average survival of 4 months for MLC-Cre;Tdp-43F/− mice while control littermates survived the span of the experiment (Fig. 3).
These mice were also monitored for muscle strength using the hanging wire test. Mice were suspended from a wire grid and their latency to fall was recorded with a maximum of 60 seconds. The test was carried out every 2 weeks beginning at about 6 weeks of age. There was no appreciable difference in the hang time of Tdp-43 knockout and control mice even at 4 months (data not shown), although this could be confounded by the marked decrease in weights of MLC-Cre; Tdp-43<sup>F/-</sup> mice as they progressed towards end stage. We also noted that Tdp-43 knockout mice were less active and did not move around as much on the wire grid.

2. Mice lacking Tdp-43 in skeletal muscle develops a myopathy characterized by degeneration and regeneration of muscle fibers

Gross dissection of mice lacking Tdp-43 in skeletal muscle at end stage show reduced muscle tone, suggestive of muscle atrophy. These mice had very little body fat compared to control littermates (data not shown). At end stage, knockout mice also had small thymuses, with other major organs appearing grossly normal (data not shown).

Pathological analyses of MLC-Cre; Tdp-43<sup>F/-</sup> mice at end stage show a striking phenotype that is consistent with myopathy of the skeletal muscles (Fig. 4); with little evidence of histological abnormalities in the other organs, including but not limited to the heart, blood vessels and gastrointestinal tract. Muscle samples taken from the cheek, tongue, quadriceps, diaphragm and sternum of Tdp-43 knockout mice showed hypercellularity and large variability in fiber size (data not shown). There was muscle degeneration as evidenced by fibers with loss of striation and hyper-eosinophilic rounded fibers (Fig. 4C, D), as well as increased numbers of small atrophic myofibers (Fig. 4D). Importantly, there was also marked regeneration of muscle, as indicated by the large numbers of atypical, enlarged and pale staining (H&E) nuclei in basophilic fibers (Fig. 4E, F). The large number of central nuclei and internal nuclei in rows without basophilia (Fig. 4F) suggests that loss of Tdp-43 may have triggered aberrant developmental programs that may have arrested prematurely. Several foci of perivascular inflammatory cells were also observed in the subcutaneous fat layer of the leg in MLC-Cre; Tdp-43<sup>F/-</sup> mice (data not shown). In contrast to end-stage mice, preliminary analyses of younger MLC-Cre; Tdp-43<sup>F/-</sup> mice showed that the myopathy preceded the decline in weight. Additional
studies are currently ongoing to determine the molecular pathways underlie the myopathy occurring in these mice lacking Tdp-43 in skeletal muscle.

3. No apparent effects in mutant dynactin mice crossbred to mutant Tbc1d1 mice

To address the question as to whether hypermetabolism exacerbates motor neuron disease by assessing the effect of mutant Tbc1d1 on the onset and survival of mutant dynactin mice, we performed a pilot study with a cohort of mice carrying both mutant dynactin and mutant Tbc1d1. However, we did not observe any affect in terms of disease onset or survival of compound mutant mice (mice carrying both mutant dynactin and mutant Tbc1d1) as compared to the mutant dynactin mice. For this reason, we will not further pursue this line of investigation and focus attention on our unexpected findings of a striking myopathy observed in MLC-Cre;Tdp-43F/- mice which we hypothesize may have important implications towards our understanding of muscle pathology occurring in human myopathy disease, such as IBMPFD.

Figure 4: Pathological analysis of myopathy in MLC-Cre;Tdp-43F/- mice. H &E staining of skeletal muscle from MLC-Cre;Tdp-43F/- mice (C-F) and age-matched littermate control (A-B). Note degenerating fiber evidenced by loss of striation and hyper-eosinophilic rounded fibers in panel D (arrow) and regenerating fiber characterized by central nuclei and internal nuclei in rows in panel F (arrowheads).

3. No apparent effects in mutant dynactin mice crossbred to mutant Tbc1d1 mice
Key Research Accomplishments

- To address the role of Tdp-43 in skeletal muscle, we generated a Tdp-43 mouse model that lack Tdp-43 in specifically in skeletal muscle.

- Diminished amount of Tdp-43 in skeletal muscle led to adult onset weight loss beginning at ~3 months of age.

- Diminished amount of Tdp-43 in skeletal muscle caused premature death at ~4 months of age.

- Pathological analysis of skeletal muscle in mice lacking Tdp-43 in skeletal muscle revealed a myopathy characterized by degeneration and atrophy of muscle fibers.

- These results indicate that Tdp-43 plays an important physiological role in skeletal muscle and implicate the participation of Tdp-43 in the pathogenesis of human myopathies with TDP-43 proteinopathy, such as IBMPFD.

Reportable Outcomes

Abstract and presentation of poster by Ms. Sophie Lin, (Graduate Student) at the annual Pathology Young Investigator Day at Johns Hopkins in May, 2012 (See Appendices)

Conclusion

Our investigation into the role of Tdp-43 in skeletal muscle has provided unexpected results that will have profound implications for human myopathies, including IBMPFD. We believe we have created a mouse model of myopathy related to Tdp-43 proteinopathy that will be instrumental in unraveling the pathogenic mechanisms underlying diseases such as IBM or IBMPFD in the future.

References


ABSTRACT

TAR DNA Binding Protein 43 (TDP-43), an essential RNA binding protein, is thought to play an important role in the modulation of gene expression through regulation of transcription, splicing, and stabilization of mRNA. TDP-43 immunoreactive inclusions have been reported in many neurodegenerative diseases, and misregulation of TDP-43 may contribute to disease pathogenesis either through toxic gain-of-function or loss-of-function mechanisms. Discerning between the two has important ramifications for the design of therapeutic strategies, and to achieve this end, our lab generated a conditional Tdp-43 knockout mouse model using the Cre-loxP system. Constitutive knockouts of Tdp-43 are embryonic lethal and postnatal deletion of Tdp-43 resulted in high fatty acid consumption and marked fat loss, presumably through the disruption of glucose metabolism in skeletal muscles. To test this hypothesis, we generated conditional knockouts of Tdp-43 using a muscle specific (MLC) driver of Cre recombinase. Loss of Tdp-43 in skeletal muscle led to adult onset weight loss beginning about 3 months of age and premature death within a month. Pathological examination of skeletal muscles revealed degeneration of fibers characterized by the presence of vacuoles and inclusions, as well as marked regeneration, observations that are consistent with a myopathy occurring in these mice. Moreover, we observed significant reduction in levels of Tbc1d1 and altered splicing of sortilin in skeletal muscle of these knockout mice. We hypothesize that loss of Tdp-43 in skeletal muscle leads to disruption of glucose metabolism and preferential utilization of lipid fuel. We propose that this metabolic adaptation occurs as a result of impaired translocation of glucose transporter GLUT4 to the cell surface in a pathway that is regulated by GTPase activating protein Tbc1d1 and Sortilin. Taken together, these findings suggest an important role for Tdp-43 in skeletal muscle, and implicate defective energy metabolism in the progression of ALS and possibly a select group of myopathies.