Wild-type human TDP-43 expression causes TDP-43 phosphorylation, motor deficits, early mortality, and mitochondrial aggregation in transgenic mice

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ABSTRACT

Transactivation response DNA-binding protein 43 (TDP-43) is a principal component of ubiquitinated inclusions in frontotemporal lobar degeneration with ubiquitin-positive inclusions and amyotrophic lateral sclerosis (ALS). While TDP-43 has been ascribed a number of roles in normal biology, including mRNA splicing and transcription regulation, elucidating disease mechanisms associated with this protein is hindered by our incomplete understanding of the normal functions of this protein and the lack of models to dissect such functions. We have generated **transgenic (TDP-43$_{PrP}$) mice** expressing full-length human TDP-43 (hTDP-43) driven by the mouse prion promoter to provide a tool with which to analyze the role of wild-type hTDP-43 in the brain and spinal cord. Moderate overexpression of hTDP-43 resulted in **TDP-43 truncation, increased cytoplasmic and nuclear ubiquitin levels, as well as intranuclear and cytoplasmic aggregates which were immunopositive for phosphorylated TDP-43.** Of note, **abnormal juxtanuclear aggregates of mitochondria** were observed, accompanied by enhanced levels of Fis1 and phosphorylated DLP1, key components of the mitochondrial fission machinery. Conversely, a marked reduction in mitofusin 1 expression, which plays an essential role in mitochondrial fusion, was observed in TDP-43$_{PrP}$ mice. TDP-43$_{PrP}$ mice also showed **reactive gliosis, axonal and myelin degeneration, gait abnormalities and early lethality.** This TDP-43 transgenic line provides a valuable tool for identifying potential roles of wild-type TDP- 43 within the central nervous system and for studying TDP-43-associated neurotoxicity.

RESULTS

Reduced Brain and Body Weight and Abnormal Escape Response in TDP-43$_{PrP}$ Mice

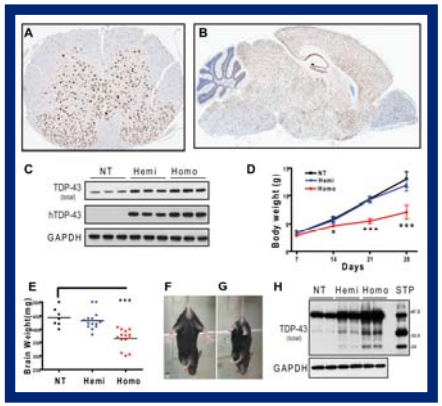

Figure 1. TDP-43$_{PrP}$ mice expressing hTDP-43 in the brain and spinal cord display reduced brain and body weight and abnormal escape response. (A-B) hTDP-43 distributed throughout the gray matter of the spinal cord (A) and brain (B) in homozygous TDP-43$_{PrP}$ mice. (C) Western blots of brain lysates using antibodies that detect either total TDP-43 or human TDP-43 only. (D) Compared to NT and hemizygous mice, homozygous TDP-43$_{PrP}$ mice had significant deficits in body weight. By 28 days, the average body weight of homozygous TDP-43$_{PrP}$ mice was approximately half that of controls. (E) At 1 month, brain weight of homozygous TDP-43$_{PrP}$ mice was significantly lower than that of age-matched NT and hemizygous mice. (F-G) Upon tail elevation, homozygous mice (G) held their hindlimbs close to their body and failed to show proper escape extension while NT mice (F) showed normal escape response by splaying their hindlimbs. (H) Immunoblot of spinal cord lysates from NT, hemizygous and homozygous TDP-43$_{PrP}$ mice using an antibody that detects total TDP-43. Note that TDP-43 fragments (~35 kDa and 25 kDa) in spinal cord co-migrate with C-terminal fragments generated by staurosporine (STP)-induced caspase activation in human neuroglioma cells expressing hTDP-43. NT= non-transgenic, hemi= hemizygous, homo=homozygous.

Neuropathology in TDP-43$_{PrP}$ mice

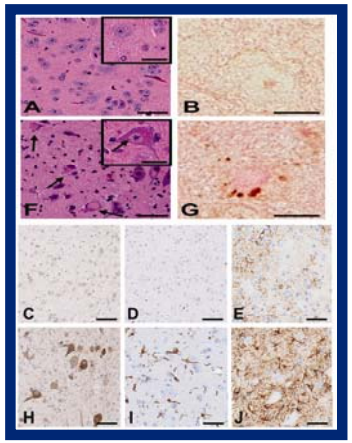

Figure 2. Neuropathology in TDP-43$_{PrP}$ mice. (A,F) Hematoxylin and eosin staining in spinal cord sections of 1 month old non-transgenic (NT) and homozygous TDP-43$_{PrP}$ mice. Eosinophilic aggregates in spinal cord motor neurons from TDP-43$_{PrP}$ mice (F, arrows and insert) are not observed in NT mice (A). (B,G) IHC analysis of spinal cord neurons in TDP-43$_{PrP}$ mice (G) and NT mice (B) using an antibody for the detection of TDP-43 phosphorylated at serines 403/404 and eosin counterstain. (C,H) Abnormal ubiquitin immunoreactivity was present in the cytoplasm and nucleus of neurons in TDP-43$_{PrP}$ mice (H) but not in NT mice (C). Enhanced IBA-1 (I, D) and GFAP (J, E) immunoreactivity indicative of activated microglia and reactive astrogliosis, respectively, were observed in TDP-43$_{PrP}$ (I, J), but not NT mice (D, E).

TDP-43 Pathology in TDP-43$_{PrP}$ mice

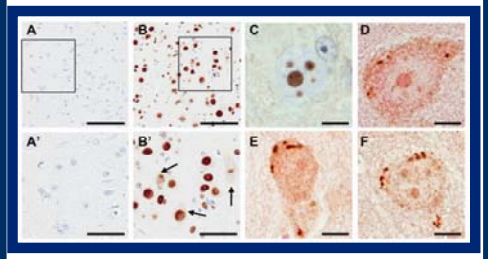

Figure 3. TDP-43 Pathology in TDP-43$_{PrP}$ mice. (A,B) Immunostaining in spinal cord sections of a 1-month old NT and homozygous TDP-43PrP mice using an antibody to hTDP-43 shows hTDP-43 in nuclei of TDP-43PrP mice (B and B'), with occasional cytoplasmic staining (arrows). hTDP-43 was not observed in NT mice (A and A'). (C-F) IHC analysis of spinal cord (C,D,E) or cortical (F) neurons using an antibody for the detection of TDP-43 phosphorylated at serines 403/404 and hematoxylin (C) or eosin (D,E,F) counterstain. Shown in panel C are nuclear bodies immunoreactive for pTDP-43 within a spinal cord motor neuron, while cytoplasmic pTDP-43-immunoreactive inclusions are shown in panels D,E and F.

Axonal Degeneration and Myelin Degeneration in TDP-43$_{PrP}$ mice

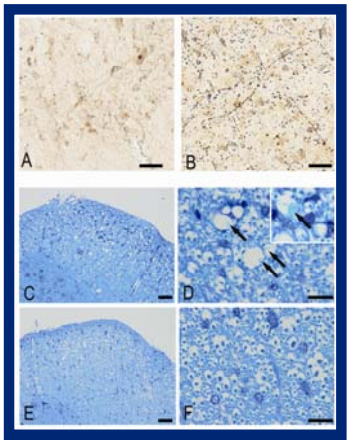

Figure 4. Axonal degeneration and myelin degeneration in TDP-43$_{PrP}$ mice. Silver staining of neurites and neuronal cell bodies revealed argyrophilic degenerating neurites and neurons in spinal cord of symptomatic TDP-43$_{PrP}$ mice (B) compared to NT controls (A). Toluidine blue stains show myelin vacuolization, with myelin ovoids (arrows and inset) in anterolateral funiculi of spinal cords of symptomatic TDP-43$_{PrP}$ mice (C, D) but not in NT mice (E, F).

Abnormal Aggregation of Mitochondria in TDP-43$_{PrP}$ mice

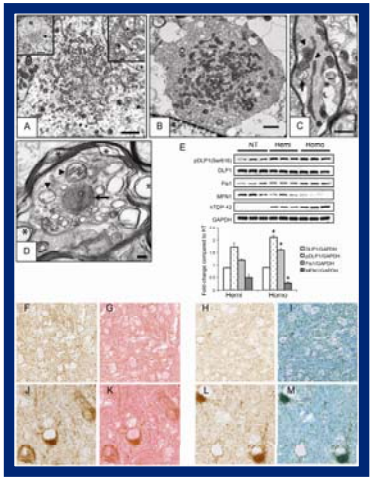

Figure 5. Ultrastructural evidence of abnormal aggregation of mitochondria in TDP-43$_{PrP}$ mice. The upper left inset of panel A depicts a low power image of a motor neuron in the anterior horn of a homozygous TDP-43$_{PrP}$ mouse containing a cytoplasmic aggregate (arrow) and a peripherally located nucleus (N; Bar represents 5μm). Enlargement of the aggregate (panel A, proper) reveals clustered mitochondria (Bar represents 2μm). A large, abnormal mitochondrion with disorganized inner cristae (arrow) is shown in the right upper inset. Also observed are mitochondria with paucity of cristae and vacuoles within the mitochondrial matrix (arrowheads; Bar represents 0.2μm). (B) The accumulation of mitochondria of various shapes and sizes, and small and large vesicles is observed in a swollen dendrite (Bar represents 1μm). (C) Abnormally shaped (arrowheads) and degenerated (arrow) mitochondria, as well as autophagic vacuoles (*) are present within a swollen axon of a spinal cord neuron (Bar represents 0.25μm). (D) An axon with a vacuolated myelin sheath (*), containing degenerating mitochondria (arrowheads), many vesicles/vacuoles and tightly packed neurofilaments (arrow) is shown (Bar represents 2μm). (E) Immunoblot analysis of mitofusin 1 (MFN1), Fis1, DLP1 and Ser616-phosphorylated DLP1 expression in brain lysates of non-transgenic, hemizygous and homozygous TDP-43$_{PrP}$ mice. Densitometric analysis of Western blots is shown. While total DLP1 levels did not change, phosphorylation of DLP1 at Ser616 was significantly increased in homozygous TDP-43$_{PrP}$ mice compared to non-transgenic mice. Similarly, expression of Fis1, another component of the fission machinery was significantly upregulated in TDP-43$_{PrP}$ mice. In contrast, mitofusin 1 (MFN1) expression was significantly decreased in TDP-43$_{PrP}$ mice. (F,J) Following IHC against the mitochondrial marker, COX-IV, spinal cord sections were counterstained with eosin (G,K). Notice the COX-IV-positive aggregates, which are also eosinophilic, in TDP-43$_{PrP}$ mice (J,K) but not non-transgenic mice (F,G). (G,H) Likewise, COX-IV-positive aggregates (L) stained blue following staining with toluidine blue (M) in TDP-43$_{PrP}$ mice, supporting the presence of high phospholipid levels associated with mitochondria. No similar staining was observed in non-transgenic mice (H,I).

CONCLUSIONS

- Moderate overexpression of hTDP-43 results in TDP-43 truncation and the formation of aggregates of phosphorylated TDP-43.
- Moderate overexpression of hTDP-43 results in elevated levels of cytoplasmic and nuclear ubiquitin, axonal degeneration, reactive gliosis, gait abnormalities and early lethality.
- Over-expression of hTDP-43 plays a critical role in mitochondrial dynamics.

ACKNOWLEDGEMENTS

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