Structure/function analysis of TDP-43 neurotoxicity in *C. elegans*

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MAYO CLINIC

ABSTRACT

TDP-43 is a conserved RNA binding protein with known roles in mRNA splicing and stability. Cytoplasmic deposition of TDP-43 has been linked to multiple neurodegenerative diseases, including ALS and frontotemporal lobar dementia (FTLD). We have engineered pan-neuronal expression of human TDP-43 protein in *C. elegans*, with the goal of generating a convenient *in vivo* model of TDP-43 neurotoxicity. Full-length (wild type) human TDP-43 expressed in *C. elegans* is nuclear as is observed in human cells. Transgenic worms with neuronal human TDP-43 expression exhibit an uncoordinated phenotype and have abnormal motorneuron synapses. By using this uncoordinated phenotype as a read-out of TDP-43 neurotoxicity, we have investigated the contribution of specific TDP-43 domains as well as TDP-43 sub-cellular localization to toxicity. Deletion of either RNA recognition domain (RRM1 or RRM2) completely blocks neurotoxicity, as does deletion of the C-terminal region. These deleted TDP-43 variants still accumulate in the nucleus, although their subnuclear distribution is altered. In contrast, N-terminal deletions result in the formation of toxic cytoplasmic aggregates. Mutation of the TDP-43 nuclear localization signal (NLS) results in cytoplasmic deposition of full-length TDP-43, which is not toxic. Mutations that alter two TDP-43 caspase cleavage sites (D89/219E), however, do not reverse TDP-43 toxicity. Our results demonstrate that TDP-43 neurotoxicity can result from either nuclear activity of the full-length protein or accumulation of cytoplasmic aggregates composed of C-terminal fragments. These results suggest that there may be (at least) two different mechanisms of TDP-43 neurotoxicity.

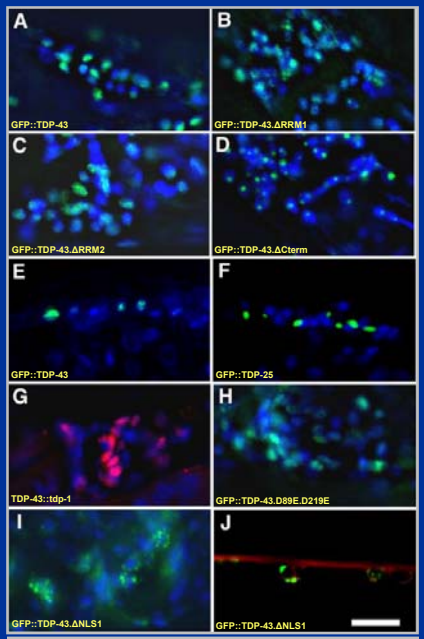

Figure 1: *snb-1*-driven full-length and variant hTDP-43 constructs. **A** to **I** fixed and counterstained with DAPI. **A**. Nerve ring area of eGFP::hTDP-43 transgenic worm (CL1626). **B**. Nerve ring of eGFP::hTDP-43 RRM1 deletion (Δaa106 to 175) transgenic worm (CL1702). **C**. Nerve ring of eGFP::hTDP-43 RRM2 deletion (Δaa193 to 257) transgenic worm (CL1705). **D**. Nerve ring of eGFP::hTDP-43 C-terminal deletion (hTDP-43 1-257, strain CL1710). **E**. Ventral cord region of eGFP::hTDP-43 transgenic worm (CL1626). **F**. Ventral cord region of eGFP::TDP-25 expressing worm (F1 transgenic animal). **G**. Nerve ring of hTDP-43/TDP-1 fusion construct probed with anti-hTDP-43 monoclonal antibody (red). **H**. Nerve ring of eGFP::hTDP-43 caspase-cleavage resistant (D89E, D219E) transgenic worm. **I**. Nerve ring of eGFP::hTDP-43 NLS1 mutant transgenic worm (strain CL1687). **J**. Ventral cord region of live eGFP::hTDP-43 NLS1 mutant worm (CL1687) containing co-injected *rgef-1*/DsRed2 marker to highlight cytoplasm in axons and cell bodies. Size bar = 5μm.

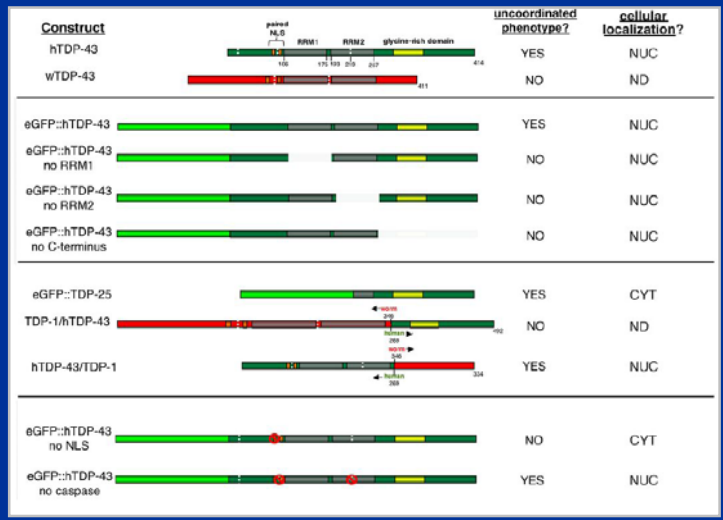

Figure 2: Summary of temperature inducible *snb-1* driven constructs expressed in *C. elegans*; their respective binary phenotypic outcome (unc/non-unc) and their cellular localisation.

SUMMARY OF RESULTS

- Pan-neuronal expression of full length nuclear hTDP-43 in *C. elegans* produces uncoordinated movement.
- GABAergic motorneuron synaptic dysregulation and axonal fasciculation is observed, but not motorneuron loss.
- This phenotype is alleviated by deletion of the functional domains RRM1, RRM2 and the C terminal domain.
- This phenotype is alleviated by mutagenesis of the hTDP-43 NLS.
- The unc phenotype is recapitulated by pan-neuronal cytoplasmic expression of the ALS relevant C terminal fragment TDP-25.
- The mechanisms of nuclear hTDP-43 and cytoplasmic hTDP-25 derived neurotoxicity can be disseminated from each other using the caspase-cleavage resistant construct hTDP-43.D89E, D219E.

CONCLUSIONS

We report two mechanisms of hTDP-43 neurotoxicity in *C. elegans*, from:

1. Pan-neuronal expression of full length nuclear hTDP-43.

2. Cytoplasmic aggregation of the ALS relevant C terminal fragment TDP-25.

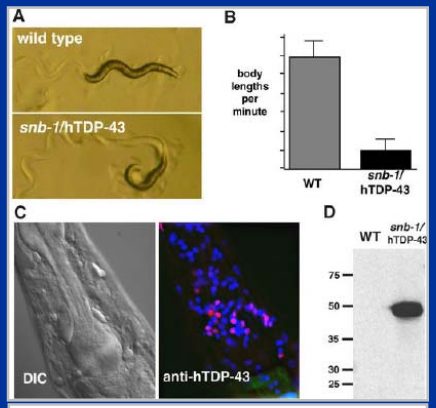

Figure 3: A. Binary phenotypes for wild type and *snb-1*/hTDP-43 transgenic worms. **B**. Quantification of movement defects in *snb-1*/hTDP-43 worms (strain CL2609). **C**. Nerve ring of fixed and permeabilized *snb-1*/hTDP-43 worm probed with anti-hTDP-43 antibody (ProteinTech anti-TARDBP polyclonal antibody). Red, anti-hTDP-43; blue, DAPI staining of nuclei; green, intestinal GFP from transformation marker plasmid. **D**. Immunoblot of extracts from wild type and *snb-1*/hTDP-43 transgenic worms (strain CL1682) probed with anti-TDP-43 monoclonal antibody M01.

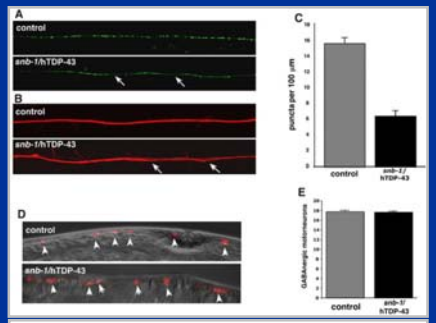

Figure 4: Neuropathology in *snb-1*/hTDP-43 transgenic worms. **A.** GABAergic motor neuron synapses in dorsal cord of living control (CL1685) and *snb-1*/hTDP-43 (CL1681) worms using an *unc-25*/SNB-1::GFP reporter transgene (*juIs1*). **B**. Dorsal cord axonal processes in wild type and *snb-1*/hTDP-43 worms, visualized by co-injection with *rgef-1*/DsRed2, a reporter that accumulates in all axonal processes (32). Note defasciculations in *snb-1*/hTDP-43 axonal bundles (arrows). **C**. Quantification of dorsal GABAergic synapses in control (CL1685) and *snb-1*/hTDP-43 (CL1681) worms, N=30 **D**. Visualization of GABAergic motor neurons using *unc-47*/DsRed2 reporter transgene (*hdIs22*). **E**. Quantification of GABAergic motor neurons in control and *snb-1*/hTDP-43 worms, N=30.

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This work was supported by:

Mayo Clinic Foundation (L.P.), the National Institutes of Health/National Institute on Aging [R01AG026251 and P01-AG17216-08 (L.P.)], the National Institutes of Health/National Institute of Neurological Disorders and Stroke [R01 NS 063964-01 (L.P.) and R01 NS063964 (C.D.L.)], the Amyotrophic Lateral Sclerosis Association (L.P.) and the Department of Defense USAMRMC PR080354 (L.P).