The important functional role of TDP-43 plays in amyotrophic lateral sclerosis-frontotemporal dementia

Liam Chen*

Tar DNA-binding protein 43 (TDP-43, encoded by the gene *TARDBP*) neuronal and glial inclusions have unified amyotrophic lateral sclerosis (ALS, ~97% of all cases), a fatal adult onset motor neuron disease characterized by the selective loss of upper and lower motor neurons, and frontotemporal dementia (FTD, and sporadic FTD (~45% of all cases), a common form of dementia characterized by progressive deterioration in behavior, personality and/or language, into one disease spectrum. Although the majority of ALS-FTD cases are sporadic, identification of mutations in the *TARDBP* gene that cause familial ALS, strongly supports the idea that TDP-43 participates in the pathogenesis of ALS-FTD, not merely a secondary phenomenon. That several other genes associated with familial ALS and FTD, including *C9ORF72*, converge on TDP-43 proteinopathy as a key neuropathological hallmark further strengthens this view. In addition, TDP-43 has been implicated in other major forms of neurodegenerative disorders such as Alzheimer's disease (AD) (Josephs et al., 2014), chronic traumatic encephalopathy (Chen, 2018) and multiple system atrophy (Koga et al., 2018). Although strong evidence supports an essentially linear progression of disease triggered by β-amyloid (Aβ) in familial AD, the evidence in sporadic AD cases supports a more multifactorial etiology. While extracellular neuritic plaques and intracellular tau neurofibrillary tangles are well-recognized canonical hallmarks for AD, TDP-43-positive inclusions have recently been identified in 30–70% of brains with pathologically diagnosed AD (Josephs et al., 2014). The morphological characteristics of the TDP-43 deposition are similar across different regions of the brain, predominantly neuronal cytoplasmic inclusions; less commonly dystrophic neurites and only rarely intranuclear inclusions. However, the TDP-43 burden in AD appears to follow a stereotypic topographic progression different from that in ALS-FTD. Importantly, greater cognitive impairment and medial temporal atrophy are associated with greater TDP-43 burden and more extensive TDP-43 distribution. TDP-43 pathology-positive subjects are 10 times more likely to be cognitively impaired at death compared to TDP-43-pathology negative cases (Josephs et al., 2014).

Identified as an essential gene, TDP-43 is required for aspects of neuronal physiology in mice, fruit flies and zebrafish. It contains two RNA recognition motifs and a glycine-rich, prion-like C-terminal domain that is critical for its diverse functions. The level of TDP-43 protein is exquisitely maintained in the cell through an autoregulatory mechanism by which TDP-43 binds to the 3'UTR of its own mRNA. In ALS-FTD, TDP-43 is depleted from the nuclei, but accumulates in ubiquitinated inclusions of affected neurons and glia, suggesting that loss of normal function or, alternatively, gain of a toxic property by cytoplasmic aggregates, play roles in the pathogenesis of these illnesses. Numerous studies have supported the detrimental effects of the TDP-43's cytoplasmic aggregation. Nevertheless this review will focus on the loss-of-function aspect of the protein. Although how this essential RNA-binding protein contributes to the pathogenesis of ALS and FTD-TDP remains elusive, previous work showed that TDP-43 is a founding member of a family of RNA binding proteins that repress cryptic exons to maintain a normal transcriptome (Ling et al., 2015). These cryptic exons are located in distal introns that are normally spliced out due to the presence of adjacent UG microsatellite repeats, the consensus binding site of TDP-43. When TDP-43 function is lost, these cryptic exons stay incorporated and often introduce frameshift or premature stop codons, targeting aberrant transcripts for nonsense-mediated decay, thereby altering the proteome of affected cells. This mechanism of suppressing cryptic exon incorporation has been evolutionarily conserved from invertebrate Drosophila to mice and human (Ling et al., 2015; Donde et al., 2019a). Importantly, incorporation of aberrant cryptic exons are observed in ALS-FTD patients (Ling et al., 2015). Interestingly, one illuminating patient, a 74-year-old female with FTD due to *C9orf72* repeat expansion who underwent temporal lobe resection for epilepsy 5 years prior to her first dementia symptom was reported recently (Vatsavayai et al., 2016). Archival surgical resection tissue demonstrated RNA foci, dipeptide repeat protein inclusions, and loss of nuclear TDP-43 but without TDP-43 inclusions despite florid TDP-43 inclusions at autopsy 8 years after first symptoms, raising important questions about the timing and significance of TDP-43-associated events and highly implicating that loss of nuclear function of TDP-43 may play an early pathogenic role. Indeed we have reported for the first time that cryptic exon incorporation occurred not only in AD brains exhibiting TDP-43 pathology, but also in neurons lacking cytoplasmic inclusion but exhibiting nuclear clearance of TDP-43 (Sun et al., 2017). Our data strongly suggest that nuclear clearance of TDP-43 associated with its inability to repress cryptic exons may indicate an early pathogenic event that is much more widespread than currently appreciated.

It is not surprising to see that many important genes and cellular pathways are modulated by TDP-43, from mitochondrial function, cellular stress response to transcription and genomic regulation, given the observed cell death associated with TDP-43 loss of function (Jeong et al., 2017). To test whether depletion of TDP-43 in motor neurons is sufficient to cause age-dependent neurodegeneration through cell-autonomous mechanism, our group crossbred the conditional *Tardbp* knockout mice with *ChAT-IRES-Cre* mice to generate mice lacking TDP-43 in spinal motor neurons (Donde et al., 2020). These mice failed to grow after 13 week of age and reached end-stage between 7–8 months of age. Moreover, they exhibited tremors, abnormal gait and muscle weakness. Significantly, pathological analysis of *ChAT-IRES-Cre;tardbp*$^{F/F}$ mice showed striking age-dependent neurodegeneration, particularly motor neuron loss and axonal degeneration, consistent with the view that loss of TDP-43 in motor neurons is a major contributing factor in the pathogenesis of ALS. Importantly, we found a reduction in ATG7 protein levels in the lumbar spinal cord of *ChAT-IRES-Cre;tardbp*$^{F/F}$ mice. Together with *ATG4B* and *Tecpr1* identified through analysis of TDP-43-deficient transcriptomes from different tissues (Jeong et al., 2017), these three autophagy-related genes strongly suggest that disruption of autophagy may be a shared downstream pathway when TDP-43 is depleted in different species and/or cell types. Indeed, *Atg7* mRNA and protein were markedly diminished in *Drosophila* model deficient in TBPH, the fruit fly homolog of human *TARDBP* (Donde et al., 2020). Remarkably, ATG7 level was marked decreased in the motor cortex of postmortem brain tissues from a clinical ALS-FTD cohort compared to that in age matched controls. Correspondingly accumulation of SQSTM1/p62, a multi-functional receptor protein that targets ubiquitinated substrates for degradation, can only be seen in neurons exhibiting nuclear depletion of TDP-43 in human ALS-FTD brains. Taken together, these findings strongly support the idea that ATG7 is a highly conserved target of the TDP-43 through evolution and autophagy is a critical pathway dysregulated in ALS-FTD.

As no compensatory mechanism exists for TDP-43 repression when such function is lost in motor neurons, complementing TDP-43 splicing repression via gene therapy approach, such as the FDA-approved AAV platform for human disease, would be a rational mechanism based therapeutic strategy for TDP-43 proteinopathies. We took a genetic approach to test whether a chimeric protein termed CTR, consisting of the RNA-recognizing N-terminal domain of TDP-43 fused with an unrelated, structurally distinct but well characterized splicing repressor, RAVER1, could complement the loss of TDP-43 function in motor neurons if delivered by AAV9 to mice lacking TDP-43 in spinal motor neurons. Perinatal unilateral intracerebroventricular injection of AAV9 carrying the CTR chimeric construct under a ubiquitous chicken beta-actin hybrid promoter selected for its small size and robust long-term expression resulted in CTR protein expression in 50–60% of cervical and lumbar mouse motor neurons that persisted to at least 8 months. While viral transduction was broadly distributed throughout the CNS, no behavioral or pathological evidence of acute or chronic toxicity from CTR protein expression was

observed in *ChAT-IRES-Cre;Tardbp*$^{F/+}$ mice within the CNS or any major organ system. Two cohorts of *ChAT-IRES-Cre;tardbp*$^{F/F}$ mice injected with the CTR fusion protein gained greater weight as compared to their untreated breeder-matched knockout controls. Treated mice showed a robust extension of their lifespan, with a median survival increase of 29 weeks, performed better on the hanging wire and accelerating rotarod tests, with a delayed onset and slower progression of motor deficits. As predicted, treatment with the CTR protein significantly re-repressed the aberrant cryptic exon splicing events as determined by quantitative RT-PCR (Donde et al., 2019). Similarly, the CTR fusion protein and a control protein with just the N-terminal fragment of TDP-43 alone was expressed using the binary GAL4/UAS expression system under control of either a restricted motor neuron specific driver *D42-Gal4*, or an *Hsp70-Gal4* driver to achieve ubiquitous expression in *Drosophila*. Whereas N-terminal fragment of TDP-43 alone expression under both drivers failed to show any rescue effect, expression of CTR in motor neurons was efficient to mitigate the motility defects and extend the life span to almost 30 days, close to one half of a normal life span (Donde et al., 2019). Expression of CTR ubiquitously by *Hsp70-Gal4* further extended the life span to over 45 days. Taken together, our results support the notion that splicing repression is a major function of TDP-43 in motor neurons and a general strategy designed to repress cryptic exon incorporation would be a valuable therapeutic approach.

Consistent with our findings, study from other groups have shown that depletion of nuclear TDP-43 leads to neuronal dysfunction and death. *TARDBP* knockout mice show embryonic lethality (Kraemer et al., 2010) and partial knockdown of TDP-43 causes motor deficits and motor neuron loss in mice (Yang et al., 2014). Indeed, a recent cell-based study also identified incorporation of cryptic exon-containing *ATG4B* mRNA, accompanied by reduced ATG4B protein in the context of *TARDBP* knockdown (Torres et al., 2018). Our findings provide first direct *in vivo* evidence that TDP-43 mediated splicing repression is a major function of TDP-43 in motor neurons. Our findings strongly argue that compromised transcriptomic integrity following the loss of TDP-43 mediated splicing repression represents a key pathogenic mechanism underlying motor neuron degeneration. Moreover, our findings establish TDP-43 loss of function as the central determinant that unify two major pathogenic mechanisms in ALS-FTD: RNA dysregulation and compromised autophagy function (**Figure 1**). Nuclear TDP-43 depletion could be an early pathogenic event, possibly preceding its cytoplasmic aggregation, and while promising therapeutic options for reducing TDP-43 aggregation mediated toxicity are being developed, further effort is needed to clarify the different contributions of toxic gain of function and nuclear loss of function of TDP-43 to the pathogenesis of neurodegenerative disease.

Liam Chen*
Neuropathology Division, Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
***Correspondence to:** Liam Chen, MD, PhD, lchen99@jhmi.edu.
https://orcid.org/0000-0001-5553-5473 (Liam Chen)
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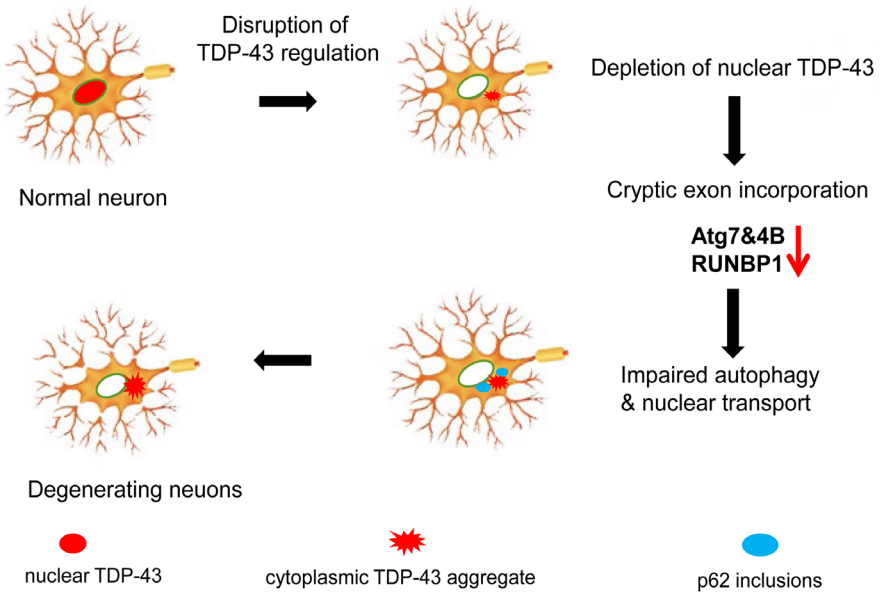

Figure 1 | **TDP-43 loss of function leads to neurodegeneration.**
Risk factors such as mutant genes, age and stress may cause loss of nuclear TDP-43 which leads to cryptic exon activation. Dysregulated genes such as *ATG7*, *ATG4B* and *RANBP1*—function in autophagy and nuclear import, respectively, could undermine the cell's ability to restore TDP-43 to the nucleus, leading to further exacerbation of TDP-43 loss-of-function, producing a feed forward loop. TDP-43: Tar DNA-binding protein 43.

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References

Chen L (2018) What triggers tauopathy in chronic traumatic encephalopathy? Neural Regen Res 13:985-986.
Donde A, Sun M, Ling JP, Braunstein KE, Pang B, Wen X, Cheng X, Chen L, Wong PC (2019) Splicing repression is a major function of TDP-43 in motor neurons. Acta Neuropathol 138:813-826.
Donde A, Sun M, Jeong YH, Wen X, Ling J, Lin S, Braunstein K, Nie S, Wang S, Chen L, Wong PC (2020) Upregulation of ATG7 attenuates motor neuron dysfunction associated with depletion of TARDBP/TDP-43. Autophagy 16:672-682.
Jeong YH, Ling JP, Lin SZ, Donde AN, Braunstein KE, Majounie E, Traynor BJ, LaClair KD, Lloyd TE, Wong PC (2017) Tdp-43 cryptic exons are highly variable between cell types. Mol Neurodegener 12:13.
Josephs KA, Whitwell JL, Weigand SD, Murray ME, Tosakulwong N, Liesinger AM, Petrucelli L, Senjem ML, Knopman DS, Boeve BF, Ivnik RJ, Smith GE, Jack CR, Jr., Parisi JE, Petersen RC, Dickson DW (2014) TDP-43 is a key player in the clinical features associated with Alzheimer's disease. Acta Neuropathol 127:d811-824.
Koga S, Lin WL, Walton RL, Ross OA, Dickson DW (2018) TDP-43 pathology in multiple system atrophy: colocalization of TDP-43 and alpha-synuclein in glial cytoplasmic inclusions. Neuropathol Appl Neurobiol 44:707-721.
Kraemer BC, Schuck T, Wheeler JM, Robinson LC, Trojanowski JQ, Lee VM, Schellenberg GD (2010) Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. Acta Neuropathol 119:409-419.
Ling JP, Pletnikova O, Troncoso JC, Wong PC (2015) TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. Science 349:650-655.
Sun M, Bell W, LaClair KD, Ling JP, Han H, Kageyama Y, Pletnikova O, Troncoso JC, Wong PC, Chen LL (2017) Cryptic exon incorporation occurs in Alzheimer's brain lacking TDP-43 inclusion but exhibiting nuclear clearance of TDP-43. Acta Neuropathol 133:923-931.
Torres P, Ramirez-Nunez O, Romero-Guevara R, Bares G, Granado-Serrano AB, Ayala V, Boada J, Fontdevila L, Povedano M, Sanchis D, Pamplona R, Ferrer I, Portero-Otin M (2018) Cryptic exon splicing function of TARDBP interacts with autophagy in nervous tissue. Autophagy 14:1398-1403.
Vatsavayai SC, Yoon SJ, Gardner RC, Gendron TF, Vargas JN, Trujillo A, Pribadi M, Phillips JJ, Gaus SE, Hixson JD, Garcia PA, Rabinovici GD, Coppola G, Geschwind DH, Petrucelli L, Miller BL, Seeley WW (2016) Timing and significance of pathological features in C9orf72 expansion-associated frontotemporal dementia. Brain 139:3202-3216.
Yang C, Wang H, Qiao T, Yang B, Aliaga L, Qiu L, Tan W, Salameh J, McKenna-Yasek DM, Smith T, Peng L, Moore MJ, Brown RH, Jr., Cai H, Xu Z (2014) Partial loss of TDP-43 function causes phenotypes of amyotrophic lateral sclerosis. Proc Natl Acad Sci U S A 111:E1121-1129.

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