PERSPECTIVE

TDP-43 overexpression impairs presynaptic integrity

The pathological mechanisms associated with the trans-activating response DNA/RNA binding protein (TDP)-43 remain largely enigmatic. Accumulation, insolubility and post-translational modification of nuclear and cytoplasmic TDP-43 are evident in many neurodegenerative diseases. TDP-43 constitutes one of the major molecular pathologies associated with RNA metabolism. Human neuronal TDP-43 overexpression suppresses mRNA translation and expression of housekeeping synaptic proteins, which facilitate the release of neurotransmitters from presynaptic vesicles into the synaptic cleft, resulting in increased levels of vesicular glutamate. TDP-43 also alters the tricarboxylic (TCA) cycle of mitochondria, leading to oxidative stress and lactate accumulation. Evidence of presynaptic defect is also supported by reduced levels of glutamine, which is indicative of glutamate entry and detoxification in active astrocytes. Astrocytes also appear to be less active in the presence of neuronal TDP-43 overexpression, suggesting inadequate function, perhaps due to less involvement in glutamate clearance. However, reduction and nucleocytoplasmic re-localization of neuronal TDP-43 reverse its detrimental effects on presynaptic function and improve cell survival.

TDP-43 is a 414 amino acid polypeptide involved in regulation of the expression of thousands of genes *via* DNA/RNA binding and alternative splicing of pre-mRNAs (Polymenidou et al., 2011). Neurons in the spectrum of disorders of motor neuron disease (MND) and frontotemporal lobar degeneration (FTLD-TDP) are marked by ubiquitin-positive inclusions that mainly consist of TDP-43 (Neumann et al., 2006). In MND, TDP-43 may mediate mRNA expression, capping, transport, splicing and other forms of processing in long motor neurons that support distant neuromuscular junctions and synapses. Therefore, maintenance of synaptic functions in excitatory glutamatergic neurons within the brain and spinal cord may be critical for TDP-43 biological function. TDP-43 has been found in stress granules that can control local protein expression *via* silencing mRNA translation, indicating that TDP-43 may be involved in local protein expression in post-synaptic terminals (Wang et al., 2008; Pascual et al., 2012). However, the role of TDP-43 is also critical in pre-synaptic terminals.

Our laboratory demonstrated that overexpression of human wild type neuronal TDP-43 can mimic the pathologies of the spectrum of disorders of MND-FTLD-TDP in a transgenic animal model that displays cognitive, psychiatric and motor symptoms (Wenqiang et al., 2014; Heyburn et al., 2016). Homozygous TDP-43 mice mimic MND pathology, including weakness, paralysis and hunch back, but hemizygous littermates exhibit symptoms that are reminiscent of the FTLD-TDP phenotype, including anxiety and learning and memory deficits (Heyburn et al., 2016). These phenotypes are associated with neuronal TDP-43 accumulation and suppression of pre-synaptic protein expression *via* reduction of synapsin and synaptotagmin mRNAs. This reduction in presynaptic protein levels is associated with cell death and an increase of glutamate concomitant with reduction of glutamine levels, indicating lack of glutamate detoxification *via* conversion to glutamine in astrocytes. Synaptic proteins and glutamate/glutamine levels were restored when TDP-43 accumulation was reduced and its nucleocytoplasmic distribution was altered (Hebron et al., 2014; Wenqiang et al., 2014), indicating TDP-43 role in synaptic maintenance *via* possible control of synaptic protein expression (Polymenidou et al., 2011). Overexpression of neuronal TDP-43 and glutamate accumulation were also associated with astrocytic inactivity as evident in reduction of glial fibrillary acidic protein (GFAP) expression and attenuation of glutamine and aspartate levels, suggesting either reduced efficiency or lack of involvement of astrocytes to detoxify glutamate. Reduction of astrocytic activity and glutamate accumulation were independent of changes in excitatory amino acid transporters (EAAT)-1 and EAAT2 (Hebron et al., 2014; Heyburn et al., 2016). However, the change in amino acid homeostasis was associated with elevation of γ-amino butyric acid (GABA) neurotransmitter levels suggesting conversion of glutamate into GABA instead of glutamine, perhaps as an alternate cellular quality control mechanism to detoxify glutamate.

Synaptic activity may increase TDP-43 level in post-synaptic dendritic spines (Wang et al., 2008). However, TDP-43 overexpression did not change the number of dendritic spines but reduced mitochondrial TCA cycle metabolism and increased oxidative stress (Hebron et al., 2014; Heyburn et al., 2016). Mitochondria are predominantly present at the pre-synaptic terminal of the synapse. However, reduction of soluble and nuclear TDP-43 significantly increased dendritic spines and reversed TCA cycle defects (Wenqiang et al., 2014; Heyburn et al., 2016), suggesting that pre-synaptic mitochondrial integrity may contribute to restoration of glutamate metabolism and could affect brain plasticity *via* increase of excitatory post-synaptic spine density.

Inactive astrocytes and synaptic glutamate accumulation were not associated with significant changes of brain inflammatory markers including microglial morphology or number, suggesting that TDP-43 suppression of expression of key synaptic proteins that mediate vesicular neurotransmitter release may trap glutamate in synaptic vesicles, preventing its effects on inflammation. Alternatively, neuronal expression of TDP-43 may reduce astrocytic function, attenuating production of inflammatory markers that provoke microglial response to exacerbate inflammation and cell death.

Taken together, we propose that TDP-43 overexpression leads to suppression of mRNA translation of key synaptic proteins triggering a feedforward response that leads to reduction of astrocytic function, independent of microglial or other inflammatory effects, due to lack of glutamate supply for conversion into glutamine (**Figure 1**). Our model also suggests that TDP-43 overexpression impairs pre-synaptic integrity and shifts the metabolism of glutamate, the brain most abundant excitatory amino acid, from conversion to glutamine in astrocytes to production of inhibitory GABA, altering excitatory-inhibitory neurotransmitter balance and synchrony. Finally, reduction of nuclear and soluble TDP-43 may alter its nucleocytoplasmic localization and aberrant effects on mRNAs, reversing suppression of synaptic mRNA translation. This leads to restoration of the pre-synaptic function, including mitochondrial activity, astrocytic monitoring of glutamate toxicity and balance of excitatory-inhibitory neurotransmission.

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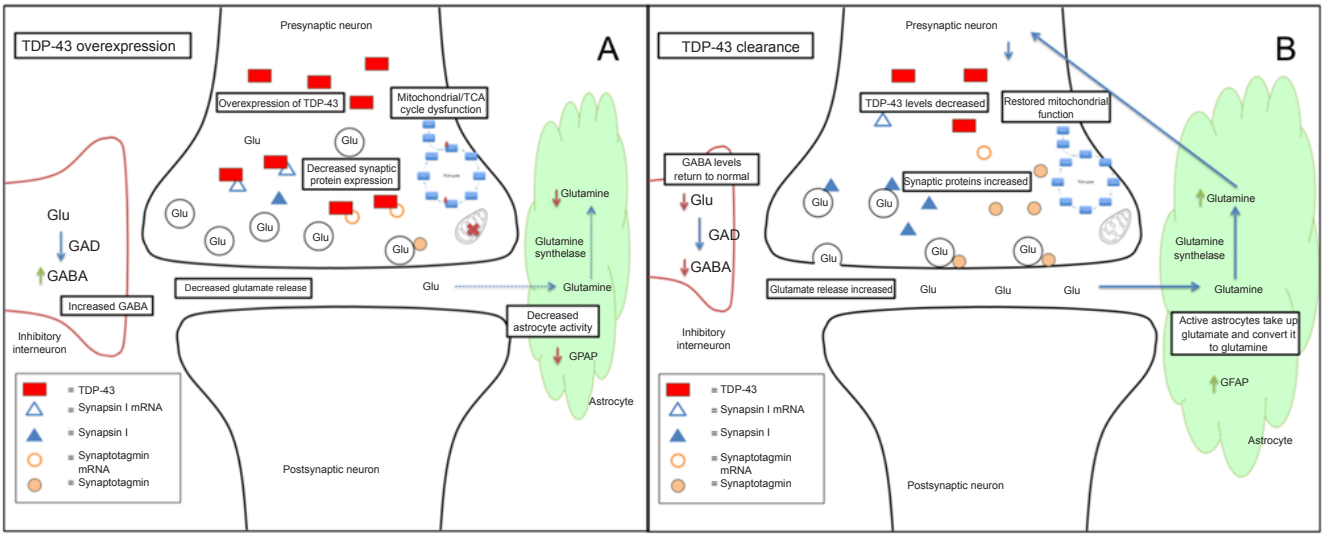

Figure 1 Schematic diagram of TDP-43 control of synaptic function.
(A) Overexpression of the DNA/RNA binding protein TAR DNA-binding protein 43 (TDP-43) leads to increased binding to a number of targets, including synapsin I and synaptotagmin mRNA. TDP-43 suppresses expression of the synapsin and synaptotagmin proteins, which are essential for the formation and release of synaptic vesicles in the presynaptic neuron. Loss of synaptic proteins may lead to a decrease in the amount of glutamate released from the presynaptic neuron. One of the many functions of astrocytes is to detoxify the environment *via* uptake of synaptic glutamate. Decreased glutamate release leads to a down-regulation of astrocyte activity, and decreased glial fibrillary acidic protein (GFAP) expression. Glutamate from the presynaptic neuron is cycled between the excitatory neuron and inhibitory interneurons, which convert glutamate into γ-amino butyric acid (GABA) *via* glutamic acid decarboxylase (GAD), leading to an increase in GABA levels when TDP-43 is overexpressed. TDP-43 overexpression also causes an increase in oxidative stress and a decrease in the tricarboxylic (TCA) cycle intermediates succinate and citrate, indicating mitochondrial dysfunction. (B) Reduction of TDP-43 levels leads to loss of suppression of the synaptic proteins synapsin I and synaptotagmin. These synaptic proteins facilitate glutamate release from the presynaptic neuron. Glutamate is taken up by astrocytes, which then convert glutamate to glutamine. Glutamine is cycled back into the presynaptic neuron where it can be re-converted into glutamate for later release. Because the glutamate-glutamine cycle is restored, GABA levels also return to normal levels. Reduction of TDP-43 overexpression restores synaptic function and mitochondrial function.

on a pending U.S. patent application to use tyrosine kinase inhibitors as a treatment for neurodegenerative diseases.

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