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TDP-43—The key to understanding amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that causes motor neuron degeneration leading to progressive muscle atrophy, weakness, paralysis and death. The majority of ALS (>95%) shows intracellular aggregation of transactive response DNA binding protein (TDP-43) as a prominent pathological feature. TDP-43 is normally a nuclear protein. In ALS, TDP-43 accumulates and aggregates in the cytoplasm (thus forming TDP-43 proteinopathy) and is depleted from the nucleus in CNS cells, including motor neurons and glia. While TDP-43 aggregation can harm cells through a gain of toxicity, it can also cause a loss of TDP-43 function in conjunction with its nuclear depletion. TDP-43 regulates its own expression to maintain itself at a constant level. Perturbation of this level by either increasing or decreasing TDP-43 in animal models leads to neurodegeneration and ALS phenotypes. The evidence supports the hypothesis that TDP-43 dysfunction is a critical driver of neurodegeneration in the vast majority of ALS cases.

Keywords: FTD, FTLD, frontotemporal dementia, gain of function, loss of function, motoneuron, neurological disease

Abbreviations: TDP-43, Transactive response DNA binding protein 43 KD; ALS, amyotrophic lateral sclerosis; SOD1, Cu, Zn superoxide dismutase; FUS, fused in sarcoma; UBQLN2, ubiquilin 2; VCP, vasolin-containing protein; ER, endoplasmic reticulum

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Introduction

Approximately 10% of ALS cases are inherited. The other 90% apparently occur sporadically without a family history. Recently, rapid advances in genetic studies have identified mutations in multiple genes as causes of this disease, including mutations in *SOD1*, *TDP-43, FUS, UBQLN2, optineurin, VCP, c9orf72, profilin1 and* matrin 3.[1,2] Studies on these mutant genes have implicated impaired proteostasis, oxidative stress, damage to endoplasmic reticulum (ER) and mitochondria, disrupted cytoskeleton and dysregulation of RNA processing and function to be involved in the neurodegeneration pathway.[1,3] By further studies of these genes and their mutations, pathways that lead to neurodegeneration in ALS may be elucidated.

Among the mutant genes that have been identified to cause ALS, the gene encoding transactive response DNA binding protein (TDP-43) is the most important. TDP-43 proteinopathy occurs in all the sporadic ALS and the majority of familial cases that originate from a variety of gene mutations.[4,5] Therefore, TDP-43 may be a midstream confluent juncture where multiple upstream origins of ALS converge in the ALS pathogenic pathway. TDP-43 is a heterogeneous nuclear ribonucleoprotein (hnRNP) containing the 2 signature RNA recognition motifs (RRM1 and RRM2). TDP-43 binds to RNA and modulates multiple RNA processes including RNA synthesis, splicing, stability and transport.[5,6] TDP-43 interacts with multiple RNA transcripts and proteins and is likely functioning in multi-protein/RNA complexes.[7-10] Furthermore, TDP-43 may be involved in regulation and biogenesis of miRNAs.[11,12] In addition to its numerous roles in RNA, TDP-43 also binds to DNA and repress transcription of certain genes.[13]

The function of TDP-43 is essential for the survival of mammals and mammalian cells.[14-17] Loss of TDP-43 function in zebra fish and invertebrates also causes developmental abnormalities leading to neurological dysfunction, behavioral alterations and in most cases premature death.[14,18-20] Thus, the normal function of TDP-43 is vital for the survival of cells,

including neurons, and animals. Given the functional importance of TDP-43 in cells and organisms, dramatic changes such as TDP-43 proteinopathy with its nuclear depletion likely signify a change in TDP-43 function that is consequential in the neurodegenerative pathway in ALS.

TDP-43 May be Increased in ALS Leading to Neurotoxicity from Both Gain and Loss of Functions

The involvement of TDP-43 in ALS and its related disease frontotemporal dementia (FTD) was initially discovered by its identification as a component in the ubiquitin-positive intracellular inclusions.[21,22] The subsequent identification of TDP-43 mutations in familial ALS cases further cemented its role as a causal gene for ALS.[23,24] Although mutations in the TDP-43 gene cause only ~4% of familial ALS, the mutations also cause TDP-43 proteinopathy that is indistinguishable from the TDP-43 proteinopathy observed in the majority of ALS cases, including all sporadic and the majority of the familial cases.[4,25] Therefore, over 95% of ALS cases involve TDP-43 proteinopathy. A uniform feature that accompanies the aggregates is a depletion of TDP-43 from the nucleus.[4,21,22] The twin presence of TDP-43 cytoplasmic aggregates and nuclear depletion has led to the proposal that both a gain of toxicity in the cytoplasm and a loss of function in the nucleus contributes to the disease.[26]

Numerous studies have provided evidence for a gain of toxicity by the ALS-linked TDP-43 mutants. These studies demonstrate that overexpression of mutant TDP-43 cause neurodegeneration in various model systems, including cell culture, *C. Elegans*, *Drosophila*, zebrafish and rodents.[27,28] In general, the neurodegeneration is widespread in the central nervous system (CNS) and not restricted to motor neurons. One puzzling and less discussed observation among these studies is that overexpression of the wild type TDP-43 causes similar neurodegenerative phenotypes as the mutants.[29-33] The confusion over the "wild type gene toxicity" stems from the conception that the wild type gene performs the normal function, and therefore, should not cause the disease.

However, humans with the wild type TDP-43 do develop ALS and these cases comprise >95% of all ALS, including the sporadic and the most of the familial cases. This fact makes the finding that overexpression of the wild type TDP-43 relevant for the ALS disease mechanism. Indeed recent studies suggest that TDP-43 level is increased in sporadic and familial ALS.[34,35] Additionally, TDP-43 mutants show various degrees of prolonged half-life and enhanced stability,[36,37] which could also lead to an elevated level of TDP-43.[38,39] The fact that overexpression of the wild type TDP-43 causes neurodegeneration in animal models suggest that such an increase in TDP-43 level can result in neurotoxicity in humans.

To prevent such toxicity, controlling TDP-43 expression level is essential for cell survival. Indeed, TDP-43 expression is normally autoregulated. An increase in TDP-43 inhibits its own expression whereas a low level relieves the inhibition and in turn increases TDP-43 expression.[40,41] A defect in this regulation may cause an elevated level of TDP-43, which could lead to consequences of both gain- and loss-of-function types.

TDP-43 functions in multi-protein RNA complexes.[7-9,42] An elevated level of TDP-43 could increase the stoichiometric ratio between TDP-43 and other components in these complexes. This could lead to the formation of partial or incomplete complexes which contain TDP-43 and some but not all of the other components. These partial complexes may not be functional (loss of function), or perform unregulated abnormal functions that lead to cellular toxicity (gain of function).[43] For example, an elevated level of TDP-43 could increase the uncomplexed TDP-43 (monomer or dimer), which could increase the opportunity for TDP-43 to engage in aberrant interactions including ectopically binding to RNAs that it normally does not bind (gain of function) and self-aggregation.

TDP-43 self-aggregation or proteinopathy is a hallmark in ALS pathology and can produce consequences with both gain-of-function and loss-of-function characters. TDP-43 has a putative prion-like domain in its C-terminal glycine-rich region and is aggregation-prone.[44-46] An increase in TDP-43 may exhaust the proteins that TDP-43 normally complex with, leaving the excess TDP-43 to freely self-associate and form aggregates. Once formed, the initial aggregates could act as seeds for further expansion of aggregates, thereby absorbing more TDP-43 into the aggregates and depleting functional TDP-43 in cells.[43] Furthermore, the aggregates could be released from dying cells or by exosomes and internalized by neighboring cells, where they could propagate TDP-43 aggregation. This has been proposed to be a mechanism whereby TDP-43 aggregation and ultimately the clinical symptoms spread from the initial focal area to the broad areas of the CNS.[47,48] Recent evidence that TDP-43 aggregates from patients' brain possess prion characteristics and are capable of seeding and inducing TDP-43 aggregates in cultured cells supports this idea.[49]

A Partial Loss of TDP-43 Function Causes Neurodegeneration and ALS Phenotypes

While numerous gain-of-function models have been generated by overexpression of TDP-43, relatively few loss-of-function models have been created. In *Drosophila* and zebrafish, knocking out or knocking down of TDP-43 caused similar neurodegenerative phenotypes as those caused by overexpression of TDP-43, thus suggesting that overexpression of TDP-43 may cause TDP-43 dysfunction by a dominant-negative mechanism.[43,50] However, confirmation of these findings in mammalian models has been hampered by technical difficulties as a result of the early embryonic lethality of the homozygous TDP-43 knockouts.[15,16,51,52] Even the heterozygous knockouts failed to provide a partial loss of function model because the TDP-43 level was unaltered from the wild type animals due to the aforementioned autoregulatory mechanism. To overcome this difficulty, 2 groups attempted to knock out TDP-43 specifically in motor neurons. Wu and colleagues disrupted the *TDP-43* gene by a Cre-loxP conditional knockout approach. They reported that the mice developed

motor neuron degeneration phenotypes after induction of TDP-43 knockout specifically in motor neurons by the HB9-Cre driver.[53] Specifically, they showed that the mice developed kyphosis, motor dysfunction, muscle weakness/atrophy, motor neuron loss and astrogliosis in the spinal cord. Iguchi and colleagues used a similar Cre-loxP approach but a different Cre driver. After induction of TDP-43 knockout in motor neurons by the VAChT-Cre driver, they also observed degenerative changes in motor neurons including atrophy of motor neurons and their axons, and skeletal muscle denervation.[54] However, they failed to confirm motor neuron loss and premature death of these mice as reported by Wu and colleagues. While the reasons for the discrepancies remain unknown, the data on motor neuron loss and the analysis on the phenotypes by Wu and colleagues were incomplete and could be subjected to alternative interpretations. For example, Wu and colleagues concluded that there was motor neuron loss based on the counting of the ChAT-positive and Nissl-stained motor neurons. However, a reduction of TDP-43 expression is known to cause a reduction in ChAT expression.[55] Therefore, a reduction in ChAT expression, rather than an actual loss of motor neurons, could have led to a reduced counting of motor neurons. Similarly, a reduction in the size of motor neurons or rough endoplasmic reticulum, rather than a true loss of motor neurons, could have caused a lower motor neuron counting in Nissl stained spinal cord sections. Both of these possibilities could have been ruled out if the ventral roots were examined and quantified. As to the phenotypes, kyphosis, motor dysfunction, muscle weakness and atrophy could all have been caused by metabolic disturbances resulting from TDP-43 knockout in cells outside of the motor neurons such as pancreatic β cells and various other systems.[56-58]

While the motor neuron specific TDP-43 knockout model addresses the question of whether a loss of TDP-43 function causes motor neuron degeneration in a cell-autonomous manner, it does not fully mimic the condition in ALS patients. In the human disease, TDP-43 proteinopathy and its accompanying nuclear depletion occur in both neurons and glia.[4,21,22,59] Moreover, TDP-43 is not completely depleted from these cells. Therefore, to evaluate the impact of loss of TDP-43 function in ALS, a model with partial loss of TDP-43 function in both neurons and glia is desirable. To fulfill this need, Yang and colleagues generated an in vivo model with a partial loss of TDP-43 function by creating a transgenic mouse line that expresses an artificial microRNA that knocks down TDP-43 expression (amiR-TDP43).[60] They demonstrated that the transgenic mice express the transgene ubiquitously and that TDP-43 was knocked down in multiple organs. Despite the widespread transgene expression and TDP-43 knockdown, the mice displayed predominantly neurological phenotypes and neurodegeneration in cortical layer V and spinal cord motor neurons. These data suggest that the CNS cells, particularly motor neurons, possess a heightened sensitivity to TDP-43 dysfunction. In light of the prominent TDP-43 proteinopathy in ALS, this result strengthens the notion that TDP-43 plays a central role in the vast majority of ALS cases.

TDP-43 Toxicity: Effects of Neighboring Cells on Motor Neurons

A role of glial cells in motor neuron degeneration in ALS has been well established in models for familial ALS that are caused by mutations in Cu, Zn superoxide dismutase (SOD1). Abundant experimental literature suggests that mutant SOD1 expression in both neurons and glia contribute to motor neuron degeneration.[61-65] By comparison, research on the role of glia expressing mutant or wild type TDP-43 is at an early stage and the results have been contradictory. Some studies suggest that glial cells expressing mutant TDP-43 do not play a role in neuronal degeneration, and therefore, the neurodegeneration caused by mutant TDP-43 is determined by a cell-autonomous mechanism.[39,66] Other studies suggest that astrocytes expressing mutant TDP-43 can exert toxicity on motor neurons and cause motor neuron degeneration.[67-69] In the amiR-TDP43 transgenic mice that exhibited motor neuron vulnerability, TDP-43 knockdown was detected in astrocytes but not in motor neurons in the spinal cord, despite both cell types expressing the transgene.[60] Therefore, the massive motor neuron loss in these TDP43 knockdown transgenic mice suggests that TDP-43 dysfunction in astrocytes is a significant driver of motor neuron degeneration, consistent with the absence of motor neuron loss when TDP-43 is specifically knocked out in motor neurons.[54] These findings are relevant to sporadic ALS where TDP-43 proteinopathy occurs in glial cells in addition to neurons.[21,22]

Dysregulation and Dysfunction of TDP-43: Relevance to ALS

To determine whether dysregulation and dysfunction of TDP-43 is relevant to ALS, let us consider 2 questions: Does alteration of TDP-43 function impact neuronal function and survival, and do dysregulation and dysfunction of TDP-43 occur in ALS? Based on our discussions above, the answer to the first question is a clear yes. A large number of experiments in the literature have already demonstrated that either overexpression or knockdown of TDP-43 causes neurodegeneration and ALS phenotypes.[27,28,43,60] Therefore, any alterations in the level of TDP-43 function are detrimental to neuronal health and can seriously impact neuronal function and survival. The answer to the second question is less certain largely because few studies on human ALS have addressed this problem. However, emerging evidence has lent support to a yes answer. First, some studies suggest that the level of TDP-43 is elevated in the spinal cord of sporadic ALS patients and in cultured cells derived from patients with TDP-43 mutations.[34,38,39] Second, several studies have shown that ALS-associated TDP-43 mutants have an increased half-life[8,36] and enhanced stability,[37] which could lead to an increased TDP-43 level. Third, some studies suggest that the alternative splicing of TDP-43-regulated genes are altered in human CNS tissues from ALS cases,[60,70] thus suggesting that TDP-43 function is altered in ALS.

Conclusion

The vast majority of ALS patients develop TDP-43 proteinopathy and its accompanying nuclear depletion. TDP-43 mutations can cause familial ALS, with proteinopathy and nuclear depletion that is indistinguishable from sporadic ALS. Thus, although the origin for pathogenesis in sporadic ALS is unknown, the involvement of TDP-43 is likely a converging point with its familial ALS counterpart. The emerging evidence that the level of TDP-43 is elevated in human sporadic ALS may be pointing to the origin from which TDP-43 proteinopathy eventually develops. The expression level of TDP-43 is tightly maintained by an auto-regulatory mechanism. Strong evidence has indicated that perturbation of this level, by either an increase or a decrease, is detrimental to CNS cells and can cause cell degeneration and ALS phenotypes. Currently, the evidence for the existence of TDP-43 functional perturbation in ALS patients is emerging but further research is needed in this area. Taken together, the current literature supports the hypothesis that TDP-43 dysfunction is a critical driver of neurodegeneration in ALS and other CNS diseases with TDP-43 proteinopathy.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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