Molecular Therapy
Commentary

New Therapeutic Avenue for ALS: Avoiding a Fatal Encounter of TDP-43 at the Mitochondria

Eloise Hudry[1]

http://dx.doi.org/10.1016/j.ymthe.2016.12.001

Amyotrophic lateral sclerosis (ALS) is the most common fatal neuromuscular disease in adults, and very limited options for clinical improvement are currently available.[1] The etiology of ALS is complex and heterogeneous, with multiple genetic associations reported so far.[2] Among these, the gene coding for TAR DNA-binding protein 43 (TDP-43) RNA/DNA binding protein was identified as the principal component of ubiquitinated cytoplasmic inclusions, which are one of the main pathological hallmarks observed in most familial and sporadic cases of ALS as well as in frontotemporal dementia with ubiquitin inclusions (FTD-U).[3] While this finding has put TDP-43 at the center of the molecular events leading to ALS neurodegenerative processes, whether the clinical manifestations of the disease are directly related to the abnormal depletion of TDP-43 from the nucleus or its pathologic accumulation in the cytoplasm remains highly debated. In this issue of *Molecular Therapy*, Wang and colleagues[4] establish that specific inhibition of TDP-43 recruitment to mitochondria suffices to alleviate ALS-type symptoms in a relevant mouse model of the disease that is heterozygous for the human mutant TDP-43^{M337V} transgene. The present work is a direct follow-up on a recent study published by the same group in *Nature Medicine*,[5] describing how TDP-43 mitochondrial accumulation is a central neurotoxic event in the disease.

Identifying the Most Relevant Pre-clinical Model

Evaluating the potential benefit of therapeutic agents toward ALS has been challenging due to the lack of optimal pre-clinical models that would recapitulate the clinical symptoms of the disease while also being relevant to its most common sporadic form. The first ALS transgenic mouse lines, genetically manipulated to express various mutant forms of the human superoxide dismutase 1 (SOD1) gene, have proven very useful to decipher some mechanisms of the disease, such as the non-cell-autonomous processes featured in ALS. While most of those models exhibit adult-onset neuromotor deficits and motor neuron loss, cortical motor neurodegeneration is not always observed, which is an important discrepancy considering that it constitutes a necessary feature to diagnose the disease.[6] Additionally, because SOD1 mutations are only characteristic of a very low percentage of familial ALS patients, the relevance of the SOD1-based mouse models have been questioned, a worry reinforced by the failure of numerous clinical trials based on treatments successfully tested in SOD1 mouse models.[7] On the other hand, even though TDP-43 mutations have only been reported in rare familial cases and are characteristic of a very specific variant of ALS (ALS-TDP-43), ubiquitinated cytoplasmic TDP-43 inclusions are found in the vast majority of sporadic cases as well as in fronto-temporal dementia,[3] emphasizing a central role for TDP-43 neurotoxicity in driving ALS pathological symptoms. However, TDP-43-based transgenic mouse models often show a very aggressive phenotype and premature death, which has therefore prevented the assessment of subtler and eventually more relevant phenotypic traits. In this issue of *Molecular Therapy*, Wang and colleagues[4] extensively characterize the progression of cognitive deficits and the loss of motor-coordinated functions in TDP-43^{M337V} hemizygous adult mice, which actually precede cortical neuron loss (spinal motor neurons remaining unaffected). Importantly, TDP-43^{M337V} mice show similar expression of total TDP-43 as compared to age-matched controls, suggesting that these pathological hallmarks do not directly result from a possible confounding effect of overexpression. While this phenotype may be considered to be mild, the rather slow progression of the symptoms actually reflects the course of the disease in many ALS and behavioral-variant FTD cases, which also exhibit cognitive impairment (sometimes before the appearance of obvious motor dysfunctions).[8] Additionally, a recent clinical report by Prudlo and colleagues[9] has specifically established a significant association between cognition and the extent of TDP-43 pathology in ALS, with a significant difference shown in the extent of TDP-43 pathology between ALS-FTD from ALS cases without dementia. Those observations therefore add to the relevance of using the TDP-43^{M337V} hemizygous mice as a more complete and complex rodent model of ALS to test the efficacy of certain therapeutic agents over the course of the disease.

Mitochondria as the Center of TDP-47 Neurotoxicity in ALS … and Potentially Other Neurological Disorders?

While the fundamental role of TDP-43 in ALS no longer requires demonstration, the exact mechanisms through which this protein leads to neurodegeneration remain highly debated. Because of its primary role in regulating post-transcriptional RNA processing (splicing, transportation, and translation) and its mislocalization in the cytoplasm, it has been proposed that TDP-43 proteinopathies originate from either the loss of function in the nuclear compartment or the gain of function in the cytoplasmic compartment (or both). However, several previous studies have demonstrated that the nuclear depletion of TDP-43 does not necessarily lead to pathogenic effects,[10] but a specific increase in the levels of cytoplasmic TDP-43 was sufficient to induce

[1]Alzheimer's Disease Research Unit, Harvard Medical School and Massachusetts General Hospital, 114 16th St., Charlestown, MA 02129, USA

Correspondence: Eloise Hudry, PhD, Alzheimer's Disease Research Unit, Harvard Medical School and Massachusetts General Hospital, 114 16th St., Charlestown, MA 02129, USA
E-mail: ehudry@mgh.harvard.edu

neurotoxicity, even though the presence of inclusions did not necessarily correlate with a more severe phenotype.[11, 12] Those findings logically resonate with the recent results of Wang and colleagues[5] published last year in *Nature Medicine*, specifically emphasizing the deleterious impact of TDP-43 accumulation in mitochondria, where it inhibits the translation of the respiratory complex subunits ND3 and ND6. In the present study, the relevance of this neurotoxic pathway is further confirmed, showing in particular that chronic infusion with PM1, a small inhibitor of the TDP-43 mitochondrial motif, could alleviate mitochondrial dysfunction, neuronal loss, motor coordination, and cognitive deficits characterizing the TDP-43^{M337V} hemizygous model. Whether or not the PM1 peptide impacts other deleterious functional changes induced by TDP-43 or its binding with various partners remains unresolved, but these results certainly emphasize the clinical importance of mitochondrial recruitment of TDP-43. Considering that TDP-43 cytoplasmic accumulation is detected in degenerating neurons in a broad spectrum of neurodegenerative diseases, including Alzheimer's disease[13] and Parkinson's disease,[14] the results also raise the question as to whether or not preventing TDP-43 recruitment in mitochondria could have a much broader therapeutic impact on multiple neurological disorders.

TDP-43 and FUS: Pathological Similarities for Common Therapeutic Approaches?

Mutations in another DNA/RNA binding protein, FUS/TLS (fused in sarcoma/translocated in liposarcoma), have also been identified in 4% of familial ALS cases and in 1% of sporadic ALS cases.[15] Similarly to TDP-43, FUS physiologically regulates RNA processing and has been involved in RNA maturation, transcription, splicing, and micro-RNA processing. Additionally, neuropathological studies of brain and spinal cord from patients with FUS/TLS mutations have reported an abnormal depletion of FUS/TLS in the nuclear compartment and while it is enriched in the cytoplasm and aggregates to form cytoplasmic tau-negative inclusions.[15] Despite those puzzling commonalities, FUS/TLS aggregates do not present any cross-reactivity with TDP-43 foci, and FUS/TLS and TDP-43 have been reported to affect the expression levels of distinct sets of RNAs.[16] Those results argue in favor of possible common neurodegenerative processes at the same time as distinct molecular pathological mechanisms of both TDP-43 and FUS/TLS. One may therefore wonder whether FUS/TLS also happens to be mislocalized at the mitochondria in the context of ALS and if this may open new opportunities for the treatment of certain forms of the disease associated with FUS/TLS mutations.

REFERENCES

1. Zinman, L., and Cudkowicz, M. (2011). Emerging targets and treatments in amyotrophic lateral sclerosis. Lancet Neurol. *10*, 481–490.
2. Renton, A.E., Chiò, A., and Traynor, B.J. (2014). State of play in amyotrophic lateral sclerosis genetics. Nat. Neurosci. *17*, 17–23.
3. Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science *314*, 130–133.
4. Wang, W., Arakawa, H., Wang, L., Okolo, O., Siedlak, S.L., Jiang, Y., Gao, J., Xie, F., Petersen, R.B., and Wang, X. (2016). Motor-coordinative and cognitive dysfunction caused by mutant TDP-43 could be reversed by inhibiting its mitochondrial localization. Mol Ther. *25*, this issue, 127–139.
5. Wang, W., Wang, L., Lu, J., Siedlak, S.L., Fujioka, H., Liang, J., Jiang, S., Ma, X., Jiang, Z., da Rocha, E.L., et al. (2016). The inhibition of TDP-43 mitochondrial localization blocks its neuronal toxicity. Nat. Med. *22*, 869–878.
6. Joyce, P.I., Fratta, P., Fisher, E.M., and Acevedo-Arozena, A. (2011). SOD1 and TDP-43 animal models of amyotrophic lateral sclerosis: recent advances in understanding disease toward the development of clinical treatments. Mamm. Genome *22*, 420–448.
7. Mitsumoto, H., Brooks, B.R., and Silani, V. (2014). Clinical trials in amyotrophic lateral sclerosis: why so many negative trials and how can trials be improved? Lancet Neurol. *13*, 1127–1138.
8. Goldstein, L.H., and Abrahams, S. (2013). Changes in cognition and behaviour in amyotrophic lateral sclerosis: nature of impairment and implications for assessment. Lancet Neurol. *12*, 368–380.
9. Prudlo, J., König, J., Schuster, C., Kasper, E., Büttner, A., Teipel, S., and Neumann, M. (2016). TDP-43 pathology and cognition in ALS: A prospective clinicopathologic correlation study. Neurology *87*, 1019–1023.
10. Arnold, E.S., Ling, S.C., Huelga, S.C., Lagier-Tourenne, C., Polymenidou, M., Ditsworth, D., Kordasiewicz, H.B., McAlonis-Downes, M., Platoshyn, O., Parone, P.A., et al. (2013). ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43. Proc. Natl. Acad. Sci. USA *110*, E736–E745.
11. Igaz, L.M., Kwong, L.K., Lee, E.B., Chen-Plotkin, A., Swanson, E., Unger, T., Malunda, J., Xu, Y., Winton, M.J., Trojanowski, J.Q., and Lee, V.M. (2011). Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. J. Clin. Invest. *121*, 726–738.
12. Barmada, S.J., Skibinski, G., Korb, E., Rao, E.J., Wu, J.Y., and Finkbeiner, S. (2010). Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. J. Neurosci. *30*, 639–649.
13. McAleese, K.E., Walker, L., Erskine, D., Thomas, A.J., McKeith, I.G., and Attems, J. (2016). TDP-43 pathology in Alzheimer's disease, dementia with Lewy bodies and ageing. Brain Pathol., Published online August 6, 2016. http://dx.doi.org/10.1111/bpa.12424.
14. Nakashima-Yasuda, H., Uryu, K., Robinson, J., Xie, S.X., Hurtig, H., Duda, J.E., Arnold, S.E., Siderowf, A., Grossman, M., Leverenz, J.B., et al. (2007). Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. Acta Neuropathol. *114*, 221–229.
15. Kwiatkowski, T.J., Jr., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T., et al. (2009). Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science *323*, 1205–1208.
16. Lagier-Tourenne, C., Polymenidou, M., Hutt, K.R., Vu, A.Q., Baughn, M., Huelga, S.C., Clutario, K.M., Ling, S.C., Liang, T.Y., Mazur, C., et al. (2012). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat. Neurosci. *15*, 1488–1497.