Aggregation party in ALS

By Lev Osherovich, Senior Writer

Aggregation and mislocalization of TDP-43 are at the heart of amyotrophic lateral sclerosis, but it is difficult to manipulate the protein directly because of its broad role in cellular survival. **University of Pennsylvania** researchers now have found evidence that TDP-43 interacts with ataxin 2, an intracellular protein linked to another neurodegenerative disease, spinocerebellar ataxia, and think that inhibiting ataxin 2 activity could provide a handle for preventing the toxicity caused by mislocalized TDP-43.¹

Mutations in TAR DNA binding protein 43 (TDP-43; TARDBP) lead to certain rare cases of hereditary amyotrophic lateral sclerosis (ALS), and in a majority of ALS patients the normally soluble protein mislocalizes into aggregates.² But tinkering with TDP-43 levels can disrupt neurological development, complicating efforts to study the protein's function in mice.

A team led by Aaron Gitler, assistant professor of cell and developmental biology at the University of Pennsylvania, chose a simpler approach to understanding TDP-43's role in the cell. Instead of mouse studies, the group screened for genes that affected TDP-43's aggregation and toxicity in yeast, then extended the findings through a series of more complex models of TDP-43 pathology ranging from flies to cell culture to ALS patients.

The yeast work was done with a screening platform Gitler helped develop while he was a postdoc in the laboratory of Susan Lindquist, a professor of biology at the **Massachusetts Institute of Technology** and the **Whitehead Institute for Biomedical Research**. Lindquist is a cofounder of **FoldRx Pharmaceuticals Inc.**, a company that develops drugs to treat diseases caused by protein misfolding. The screening platform is licensed to FoldRx.

FoldRx is being acquired by **Pfizer Inc.**

"We've provided evidence for a potentially novel risk factor for ALS," said Gitler. "This underscores the power of simple model systems like yeast and flies in identifying modifiers of disease."

"This is a validation of yeast as a model system and of TDP-43 as a target," said FoldRx CEO Richard Labaudinière.

Call to ATXN2

Gitler's team previously reported that overexpression of TDP-43 kills yeast cells.³ In a new report published in *Nature*, his group identified

13 yeast genes whose overexpression increased toxicity caused by TDP-43 compared with that seen in vector-treated controls. Among these was *pbp1*, which encodes an mRNA-associated protein that strongly resembles human ataxin 2 (ATXN2; SCA2).

The team also found that *pbp1* deletion decreased TDP-43 toxicity compared with wild-type *pbp1* expression.

Gitler's team then collaborated with UPenn biology professor Nancy Bonini to test whether tinkering with ATXN2 gene levels increased or decreased TDP-43 toxicity in a fly model of neurodegeneration. Overexpression of an ALS-linked allele of TDP-43 in fly eyes and motor neurons led to cell degeneration and loss of motility compared with those in wild-type controls.

Just as in yeast, increasing levels of the fly homolog of ATXN2 exacerbated TDP-43's toxicity, whereas halving the homolog's expression reduced TDP-43's effects compared with what was seen in wild-type controls.

Together, the yeast and fly findings argued for an interaction between ATXN2 and TDP-43 genes, which could be due to mutual involvement of the two proteins in a common biological process or direct physical interaction between the resulting proteins.

The team thus turned to mammalian cell culture and patient tissue studies to tease out the most likely mechanism behind the ATXN2 and TDP-43 interaction.

In humans, mutations in ATXN2 cause spinocerebellar ataxia, which involves the degeneration of neurons that control muscle motion. Although spinocerebellar ataxia is clinically distinct from ALS, Gitler suspected the interactions between TDP-43 and ATXN2 in simple model organisms

pointed to a direct role for both proteins in ALS pathogenesis.

To test this idea, Gitler's team examined the localization of ATXN2 in spinal cord neurons from ALS patients. Immunostaining experiments showed that ATXN2 formed large intracellular inclusions in about 27% of neurons from 6 ALS patients but was distributed evenly throughout the cell bodies of about 95% of neurons from healthy controls.

Thus, Gitler suspected that problems with ATXN2 and TDP-43 could synergize to exacerbate ALS. Indeed, the team found that mutations in ATXN2 that predict greater risk of spinocerebellar ataxia were about three times more common in ALS patients than in healthy controls.

Also, ALS patients who carried the ATXN2 mutations had a more severe form of the disease with earlier onset and more rapid progression than patients with wild-type ATXN2.

Aggregation combo

Although Gitler's genetic studies point to a connection between ATXN2 and TDP-43, it is not yet clear how this interaction leads to disease, much less to how to modulate the interaction.

"I hadn't imagined that there would be an interaction between these two proteins, so the fact that they've found this in an unbiased yeast screen is significant," said Steven Finkbeiner, senior investigator and

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—Richard Labaudinière,
FoldRx Pharmaceuticals Inc.

associate director at the **Gladstone Institute of Neurological Disease**, director of the Taube-Koret Center for Huntington's Disease Research and professor of neurology and physiology at the **University of California, San Francisco**.

If the two proteins physically interact, it is possible that aggregation of TDP-43 recruits ATXN2 into aggregates or vice versa.

In fact, Gitler's team included data in the *Nature* paper supporting this idea. They "looked at spinocerebellar ataxia patients and found some evidence of TDP-43 aggregation," said Virginia Lee, professor of pathology and laboratory medicine at the **University of Pennsylvania School of Medicine** and a coauthor of the paper.

Alternatively, TDP-43 aggregation may trigger overall problems with the proteostasis system—the network of chaperones that keeps protein folding in balance. For example, TDP-43 aggregation could overtax a neuron's protein folding machinery, triggering the aggregation of ATXN2 and potentially other proteins.

"There's some question about whether they've excluded the possibility of stress on the proteostasis system as a cause for this interaction," said Finkbeiner.

Either way, a possible therapeutic strategy could be to block the interaction between TDP-43 and ATXN2 or to stabilize one or both proteins in a conformation that resists misfolding. The latter approach is where FoldRx has had success with tafamidis, a small molecule that stabilizes transthyretin (TTR) protein. That compound is under review in Europe to treat TTR amyloid polyneuropathy (ATTR-PN).

"Small molecules that disrupt protein-protein interactions are very difficult to find. It's more realistic to target a folding pathway that decreases the interaction between two proteins," said Labaudinière. "Our drugs bind to the protein to stabilize it. Continuing to work on the yeast platform, you could screen for small molecules that inhibit TDP-43 toxicity."

Labaudinière said FoldRx has used the yeast screening platform to look for small molecule modulators of protein misfolding in Parkinson's disease (PD) but has not yet tackled ALS.

RNA approach

Another possibility is that TDP-43 and ATXN2, which are both RNA-binding proteins, interact via binding to RNA molecules that could act as bridges between the two proteins.

In support of this idea, Gitler's team found that disrupting the RNA-binding domain of TDP-43 prevented the protein's interaction with ATXN2 in cell culture, immunoprecipitation and colocalization studies.

"We've provided evidence for a potentially novel risk factor for ALS."

—Aaron Gitler,
University of Pennsylvania

Gitler noted that the yeast homolog of ATXN2 is part of a complex that regulates translation of mRNAs. If mRNA is the glue that holds the two proteins together, targeting that mRNA with antisense or small interfering RNA agents may prevent recruitment of TDP-43 to ATXN2 and could reduce toxicity.

"One idea would be to target the mRNA that sequesters the protein, but we don't know what that mRNA is," said Gitler. "The other question is how to target that mRNA."

Finkbeiner was cautious about the RNA-targeting approach, noting that both proteins are likely to bind to many different RNAs as part of their normal function and that TDP-43's RNA binding activity is likely essential for cellular survival.

"From what we know of TDP-43, it could be involved in any number of processes in RNA biogenesis or translation," he said. Although targeting the RNAs bound by TDP-43 or ATXN2 is "definitely a possibility worth thinking about, loss of function of TDP-43 is deleterious, so the cure may be worse than the disease itself."

For now, Gitler plans to characterize some of the other genetic modifiers of TDP-43 toxicity from his yeast screen to get a better handle on the biology around the ALS-linked protein. He also hopes to test the effect of lowering ATXN2 levels in a mouse model of TDP-43-driven ALS.

Meanwhile, Lee is examining whether ATXN2 plays a role in frontotemporal dementia, another neurological disorder linked to TDP-43 mislocalization.

UPenn has filed a patent application covering the findings described in the *Nature* paper.

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