Identification of target mRNA transported to axons by TDP-43
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Abstract
TDP-43 abnormally deposits in neurons of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin-positive inclusion bodies (FTLD). TDP-43 is an RNA-binding protein supposed to transport mRNA outside the nucleus. To know whether dysfunction of specific mRNA transport to axons by TDP-43 is associated with the neurodegeneration in ALS and FTLD, we identified target mRNA transported by TDP-43 and analyzed their localization and function at the site.

Down-regulation of TDP-43 decreased mRNA of ribosomal proteins in axons. TDP-43 colocalized with and transported the mRNA in axons. These mRNAs were translated locally, and overexpression of ribosomal proteins suppressed the eye degeneration in TDP-43 transgenic flies.

In ALS and FTLD, neurodegeneration may occur due to impaired protein synthesis in axons as a result of transport deficiency of mRNA of ribosomal proteins by TDP-43.

Purpose
While abnormal deposition of TDP-43 is a hallmark in neurons of ALS and FTLD, the pathogenic mechanism of the diseases caused by TDP-43 is largely unknown. TDP-43 is an RNA-binding protein that regulates transcription and splicing of pre-mRNA, nucleocytoplasmic export and transport of mature mRNA. We hypothesized that the failure of specific mRNA transport to axons by TDP-43 is the cause of neurodegeneration in ALS/FTLD, and we aimed to identify target mRNA transported to axons by TDP-43.

Methods
We detected mRNA decreased by shRNA-based TDP-43 down-regulation in neurites of mouse cortical neurons using microarray analysis. We next analyzed the identified mRNA for transport by TDP-43, localization and local translation in axons of cortical neurons.

Cluster and pathway analysis

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<tr>
<th>No.</th>
<th>shRNA#1</th>
<th>shRNA#2</th>
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MAPP Name               | Z score | Adjusted P |
------------------------|---------|------------|
Cytoplasmic ribosomal proteins | 18.449  | 20.144     |
Translation factors       | 3.308   | 2.593      |

mRNA of ribosomal proteins specifically decreases in axons

Quantitative PCR, Ratio to Control shRNA in each fraction

TDP-43 binds and transports mRNA of ribosomal proteins

RNA immunoprecipitation
mRNA in TDP-43-Myc precipitates
Myc IP/Control IgG IP

Colocalization of Rplp1 mRNA and TDP-43 protein

Visualization of Rplp1 mRNA by MS2-MS2bs system

mRNA of ribosomal proteins is locally translated in axons

Translational stimulation of mRNA by BDNF in axons

IB: Rps6
IB: Rpl26
IB: GAPDH

Overexpression of ribosomal proteins mitigates disrupted axonal extension in TDP-43 KD neurons

Overexpression of ribosomal proteins suppresses eye degeneration in TDP-43 flies

Human TDP-43 transgenic flies

EGFP (cont.)
Rps6
Rpl26
Rpl41

Conclusion
• We identified mRNA of ribosomal proteins as targets transported into axons by TDP-43.
• TDP-43 is supposed to control the formation of ribosomes and local translation in axons by transporting these mRNAs.
• Failure of the function may be linked to the pathogenesis of ALS/FTLD.