News Release

Title
Two independent mechanisms of TDP-43 aggregation in ALS

Key Points
- An efficient cell-based screening system for TDP-43 aggregation was developed.
- Two independent mechanisms of TDP-43 aggregation: LLPS and aggresome were identified in ALS.
- The findings will lead to a novel therapeutic strategy for ALS.

Summary
Assistant Prof. Seiji Watanabe and Prof. Koji Yamanaka (Department of Neuroscience and Pathobiology, RIEM/Nagoya University Graduate School of Medicine) identified a new mechanism of TDP-43 aggregation in amyotrophic lateral sclerosis (ALS).

Cytoplasmic inclusion of TAR DNA-binding protein 43 (TDP-43) is a pathological hallmark of ALS and a subtype of frontotemporal lobar degeneration (FTLD). However, a mechanism(s) of TDP-43 pathology is not fully elucidated. To identify intracellular mechanisms responsible for TDP-43 aggregation, we established an efficient cell-based screening system for TDP-43 aggregation. We found that microtubule-related proteins (MRPs) and RNA-binding proteins (RBPs) co-aggregated with TDP-43. These two types of proteins sequestered TDP-43 through independent mechanisms: a liquid-liquid phase separation (LLPS) and an aggresome formation. Moreover, in sporadic ALS patients, approximately half of skein-like TDP-43 inclusions were co-localized with HDAC6, but round and granular type inclusion were not. Our findings suggest that two pathways (LLPS and aggresomes) independently induce TDP-43 aggregation and that both the mechanisms are involved in TDP-43 pathology in sporadic ALS patients. The findings will lead to a novel therapeutic strategy for ALS.
**Research Background**

Cytoplasmic inclusion of TAR DNA-binding protein 43 (TDP-43) is a pathological hallmark of amyotrophic lateral sclerosis (ALS) and a subtype of frontotemporal lobar degeneration (FTLD). Recent studies have suggested that the formation of cytoplasmic TDP-43 aggregates is dependent on a liquid-liquid phase separation (LLPS) mechanism. However, it is unclear whether TDP-43 pathology is induced through a single intracellular mechanism such as LLPS.

**Research Results**

To identify intracellular mechanisms responsible for TDP-43 aggregation, we established a TDP-43 aggregation screening system using a cultured neuronal cell line stably expressing EGFP-fused TDP-43 and a mammalian expression library of the inherited ALS/FTLD causative genes, and performed a screening. We found that microtubule-related proteins (MRPs) and RNA-binding proteins (RBPs) co-aggregated with TDP-43. MRPs and RBPs sequestered TDP-43 into the cytoplasmic aggregates through distinct mechanisms such as microtubules and an LLPS, respectively. The MRPs-induced TDP-43 aggregates were co-localized with aggresomal markers and dependent on histone deacetylase 6 (HDAC6), suggesting that aggresome formation induced the co-aggregation. However, the MRPs-induced aggregates were not affected by 1,6-hexanediol, an LLPS inhibitor. On the other hand, the RBPs-induced TDP-43 aggregates were sensitive to 1,6-hexanediol, but not dependent on microtubules or HDAC6. In sporadic ALS patients, approximately half of skein-like TDP-43 inclusions were co-localized with HDAC6, but round and granular type inclusion were not. Moreover, HDAC6-positive and HDAC6-negative inclusions were found in the motor neurons of same ALS patient, suggesting that the two distinct pathways are both involved in TDP-43 pathology. Our findings suggest that at least two distinct pathways (i.e., aggresome formation and LLPS) are involved in inducing the TDP-43 pathologies.

**Research Summary and Future Perspective**

Our findings suggest that two pathways (LLPS and aggresomes) independently induce TDP-43 aggregation and that both the mechanisms are involved in TDP-43 pathology in sporadic ALS patients. The strategy focused on each aggregation mechanism will help to develop a future therapeutic strategy for ALS.
Aggresome Formation and Liquid-liquid Phase Separation Independently Induce Cytoplasmic Aggregation of TAR DNA-binding protein 43

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