Screening methods for drug candidates using TDP-43 cellular models of neurodegenerative disease and Several compounds as drug candidate for neurodegenerative disease.

Frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-TDP)

- FTLD is the second most common form of cortical dementia in the population below the age of 65 years.
  Progressive atrophy is found in frontal lobe and temporal lobe of the brain.

Amyotropic Lateral Sclerosis (ALS)

- ALS is the most common motor neuron diseases, characterized by progressive weakness and muscular wasting, resulting in death within a few years.

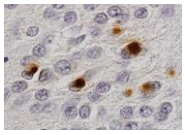
Abstract

1. Two types of TDP-43 cellular model mimic intracellular aggregates found in brains of FTLD-TDP and ALS.
2. Several compounds were selected by the cellular model.

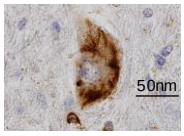
=> Our cellular models are efficient screening method for drug candidates of neurodegenerative diseases.

Neuronal cytoplasmic inclusions in brains of FTLD-TDP and ALS

FTLD Neuronal Cytoplasmic inclusions



ALS Skein-like inclusions



Pathological features

- ◆Ubiquitin-positive tau-negative neuronal cytoplasmic inclusions are common pathological features in FTLD and ALS.
- ◆TDP-43 is the major component of ubiquitin-positive tau-negative inclusions in FTLD and ALS.
  (*Arai et al, Biochem Biophys Res Commun. 2006 Dec 22;351(3):602-11. Epub 2006 Oct 30.*)

TDP-43 (TAR DNA-binding protein of 43 kDa)

- TDP-43 belongs to the heterogeneous nuclear ribonucleoprotein family.
- TDP-43 has multiple functions in RNA splicing and transport.
- Missense mutations in the TDP-43 gene have been identified in familial and sporadic ALS cases. These findings indicated that abnomality of TDP-43 protein causes neurodegeneration.

*NLS: Nuclear localization signal #RRM: RNA-recognition motif

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Cellular model for TDP-43 intracellular aggregates

- Two types of deletion mutant are prepared.
- The mutant is expressed in neuroblast SH-SY5Y

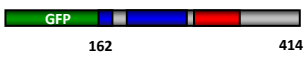
1 μg vector ＋ 3 μL FuGENE6
Culture for 3 days

- Anti-phosphorylated TDP-43 antibody
- Anti-ubiquitin abtibody

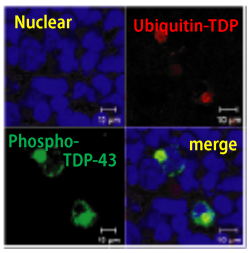
- Observation with confocal laser microscope.
- Immunoblot analysis.

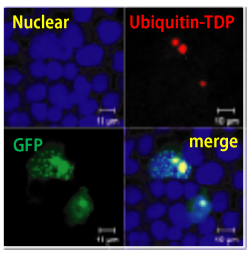
Type1 : 

Mutant TDP-43 lacks both NLS and NLS-like sequence.

TYPE2 : 

TDP-43 C-terminal fragment observed in patients.



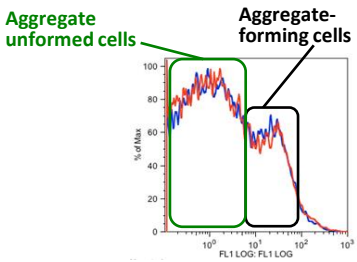


TDP-43 intracellular aggregates were phosphorylated and ubiquitinated observed in patient's brain.

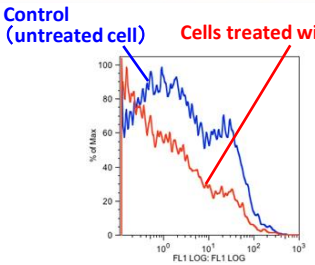
Search for drug candidate compounds

1. Screening by FAC scan

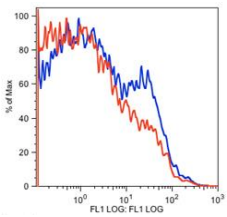
Selection of compounds suppressing TDP-43 aggregation from Prestwick Chemical Library®.



=>no difference

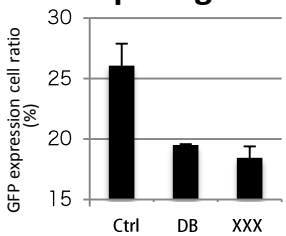


=> Cytotoxicity



⇒Inhibition of aggregation

2. Comparing with positive control.

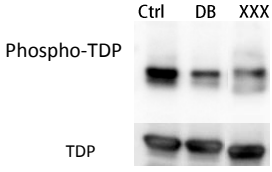


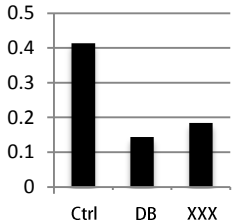
Immunostaining

: p <0.01 (Against Control)(%)

Ctrl = DMSO (Solvent)
DB=Dimebon (Positive Control)

Western blotting





We find **several compounds** which suppress **TDP-43 aggregation.**

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