Title

Monomerization of TDP-43 is a key determinant for inducing TDP-43 pathology in amyotrophic lateral sclerosis

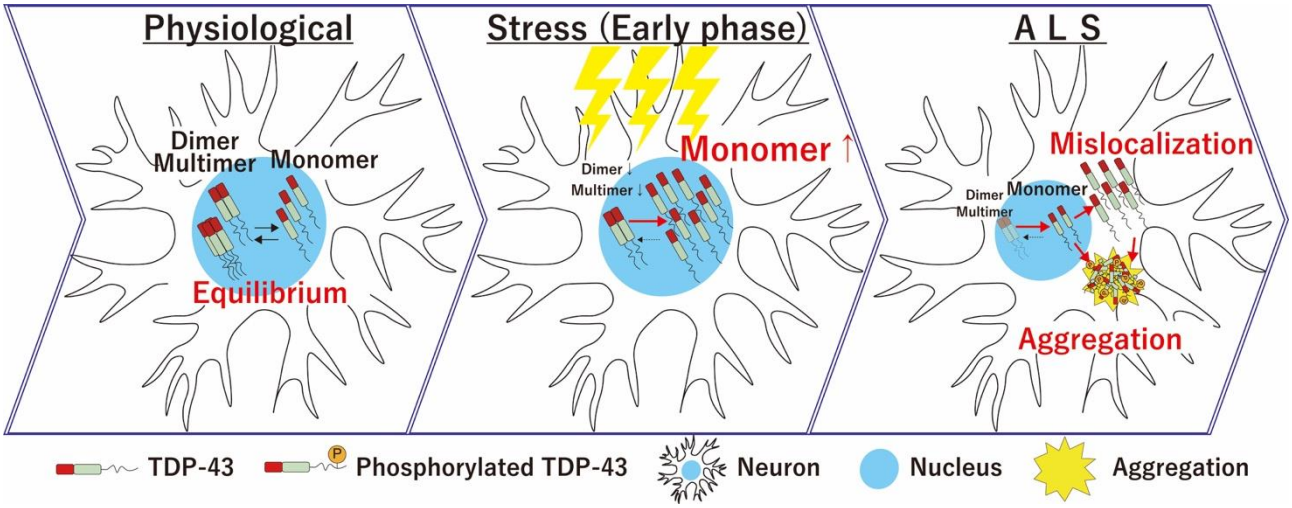
Key Points

• TDP-43 dimerization/multimerization is impaired in the postmortem brains and spinal cords of patients with sporadic amyotrophic lateral sclerosis (ALS).

• Expression of N-terminal dimerization-deficient mutant TDP-43 in Neuro2a cells and induced pluripotent stem cell-derived motor neurons recapitulates TDP-43 pathology.

• TDP-DiLuc, a novel reporter assay to evaluate TDP-43 dimerization/multimerization, could detect decreased N-terminal dimerization of TDP-43 prior to the TDP-43 pathological changes.

• Our findings may be important in future research on early pathogenic events related to TDP-43 and in the search for early diagnostic markers or therapeutic strategies for ALS.



TDP-43 exists in a monomer–dimer/multimer equilibrium in cells. The N-terminal domain of TDP-43 mediates the formation of physiological homodimers. Various types of stresses impair TDP-43 dimerization/multimerization, resulting in increased amounts of monomeric TDP-43. This excess of monomeric TDP-43 is exported from the nucleus, leading to the cytoplasmic mislocalization of TDP-43 and its subsequent phosphorylation and aggregation.

Summary

ALS is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons, resulting in skeletal muscle weakness and death within 3–5 years after onset. The cytoplasmic aggregation of TDP-43, also known as TDP-43 pathology, is the pathological hallmark of ALS. However, the mechanism underlying TDP-43 cytoplasmic mislocalization and subsequent aggregation remains unclear. Herein, we show that TDP-43 dimerization/multimerization is impaired in the postmortem brains and spinal cords of patients with sporadic ALS. Expression of N-terminal dimerization-deficient mutant TDP-43 in Neuro2a cells and induced pluripotent stem cell-derived motor neurons recapitulates TDP-43 pathology, such as cytoplasmic mislocalization and aggregate formation. Furthermore, TDP-DiLuc, a novel reporter assay, could detect decreased dimerization/multimerization of TDP-43 prior to the TDP-43 pathological changes. These findings identified TDP-43 monomerization as a critical determinant inducing TDP-43 pathology in ALS.

Research Background

ALS is a fatal neurodegenerative disease characterized by the progressive loss of motor neurons, resulting in skeletal muscle weakness and death within 3–5 years after onset. Common pathological hallmarks in patients with ALS are the cytoplasmic mislocalization and aggregation of hyperphosphorylated TDP-43, which constitute the so-called TDP-43 pathology. TDP-43 pathology is observed in almost all sporadic ALS cases. The significance of TDP-43 in ALS pathogenesis is also supported by genetic evidence that mutations in the TARDBP gene, which encodes TDP-43, are causative for inherited ALS. Although several mechanisms have been proposed for TDP-43-mediated neurodegeneration, the exact molecular mechanism remains unclear.

Recent studies have shown that TDP-43 exists in a monomer–dimer/multimer equilibrium under normal physiological conditions. TDP-43 consists of several domains, and an N-terminal domain (NTD) is essential for the physiological dimerization/multimerization of TDP-43. Despite the hypothetical mechanistic link between impaired TDP-43 dimerization/multimerization and ALS pathogenesis, no studies have examined the TDP-43 multimerization status in the central nervous system (CNS) tissues of patients with sporadic ALS.

Research Results

We have shown that the TDP-43 dimerization/multimerization is disrupted in ALS-affected lesions using two independent assays. To evaluate the multimerization status of TDP-43 in human postmortem CNS samples, we performed a crosslinking assay using disuccinimidyl glutarate (DSG) and

revealed that the dimer/monomer ratio of TDP-43 was reduced in the affected tissues of ALS cases (Fig. 1).

We found that the E2G6G antibody, a monoclonal TDP-43 antibody, discriminates N-terminal dimerization-deficient TDP-43 (NDD-TDP-43). Immunohistochemistry using the E2G6G antibody showed that pathological inclusion bodies in the ALS motor neurons consist of NDD-TDP-43 (Fig. 2).

Expression of NDD-TDP-43 mutants in Neuro2a cells and induced pluripotent stem cell-derived motor neurons recapitulates TDP-43 pathology, such as cytoplasmic mislocalization and aggregate formation (Fig. 3).

We developed a split luminescent-based reporter, TDP-DiLuc, for the high-throughput evaluation of TDP-43 dimerization/multimerization in living cells. TDP-DiLuc revealed that various cellular stresses, including transcription inhibition, impaired TDP-43 dimerization/multimerization, which resulted in TDP-43 pathology. Moreover, we analyzed the sequential phenotypic changes using a transcription-inhibited cell model and found that decreased TDP-43 dimerization/multimerization preceded TDP-43 pathological changes.

Research Summary and Future Perspective

In conclusion, we provide the first evidence that TDP-43 monomerization plays an essential role in inducing TDP-43 pathology in ALS. Our findings may be important in future research on early pathogenic events related to TDP-43 and in the search for early diagnostic markers or therapeutic strategies for ALS.

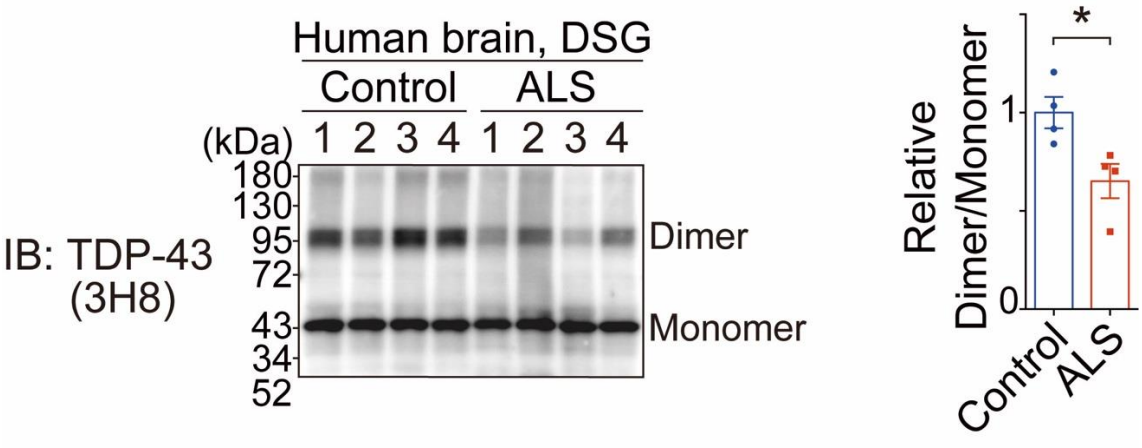


Fig. 1 DSG crosslinking assay on postmortem brains of patients with sporadic ALS

Representative immunoblots of DSG-crosslinked postmortem brain samples visualized with anti-TDP-43 antibody (left panel). Quantification of the dimer/monomer ratio (relative to the mean level of the control samples) of TDP-43 (right panel). n = 4, biologically independent samples.

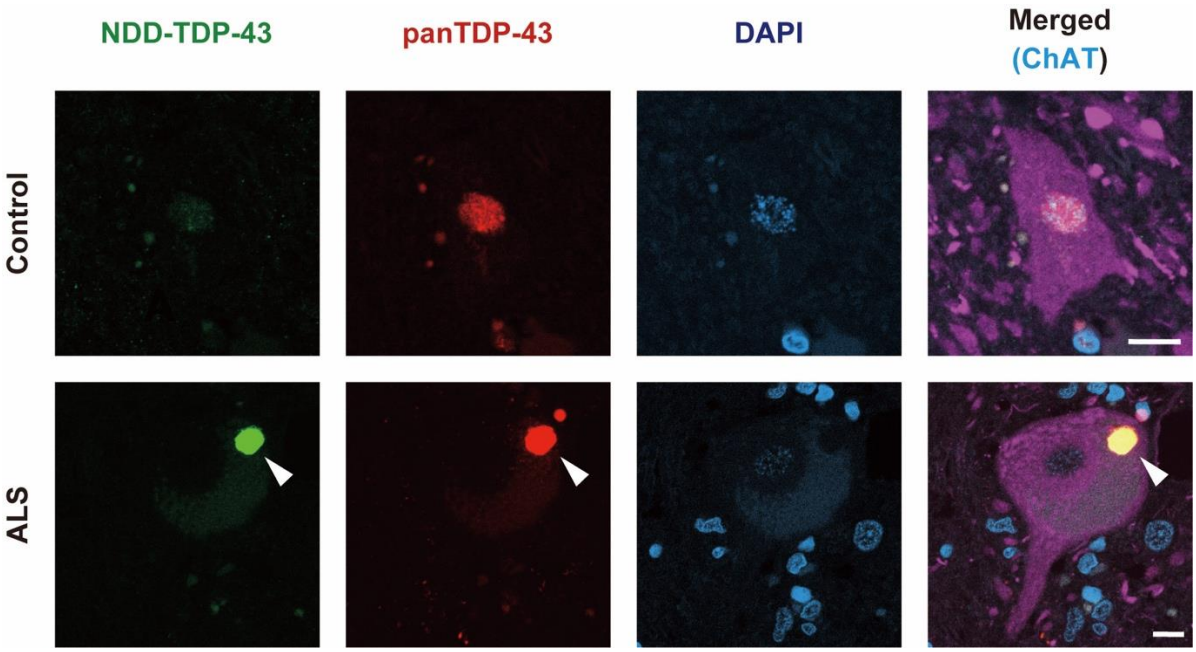


Fig. 2 Immunocytochemistry of ALS spinal motor neuron using the E2G6G antibody

Representative images of spinal motor neurons from a control and a patient with sporadic ALS, immunostained with E2G6G antibody which can discriminate N-terminal dimerization-deficient TDP-43, anti-panTDP-43 (3H8), and anti-choline acetyltransferase (ChAT. motor neuron marker). Arrowheads indicate pathological inclusion bodies. Scale bar, 10 μm.

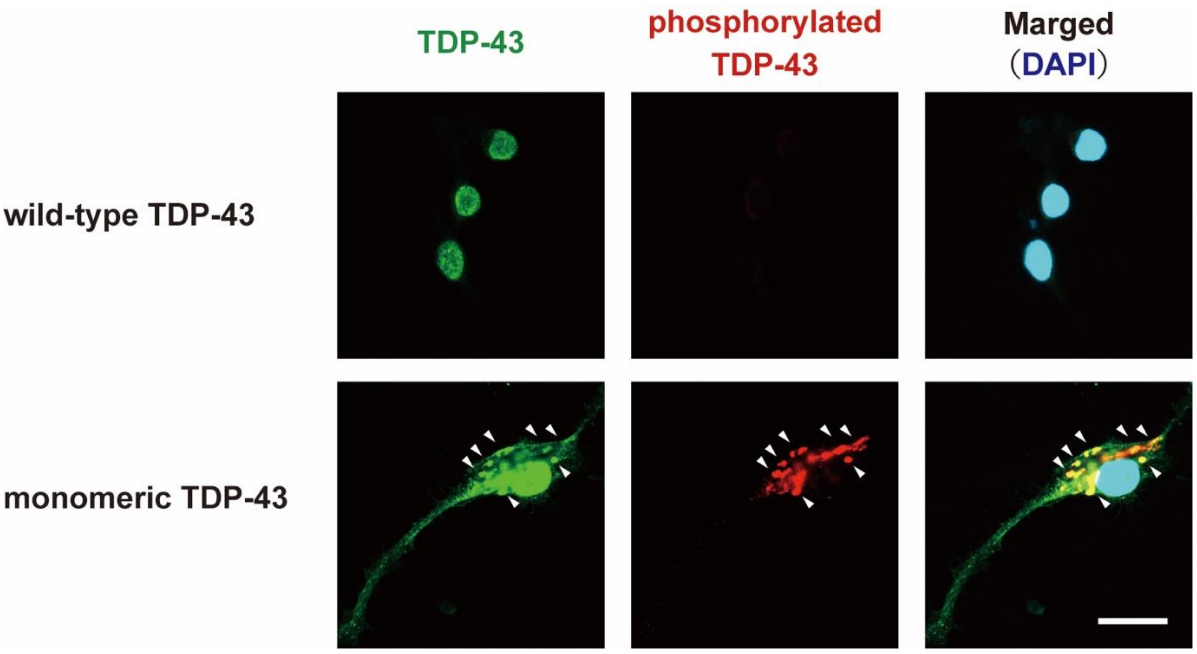


Fig. 3 Expression of N-terminal dimerization-deficient mutant TDP-43 recapitulates TDP-43 pathology

Representative images of Neuro2a cells transiently expressing TDP-43 WT or an N-terminal dimerization-deficient mutant. Arrowheads indicate aggregates.

Publication

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