

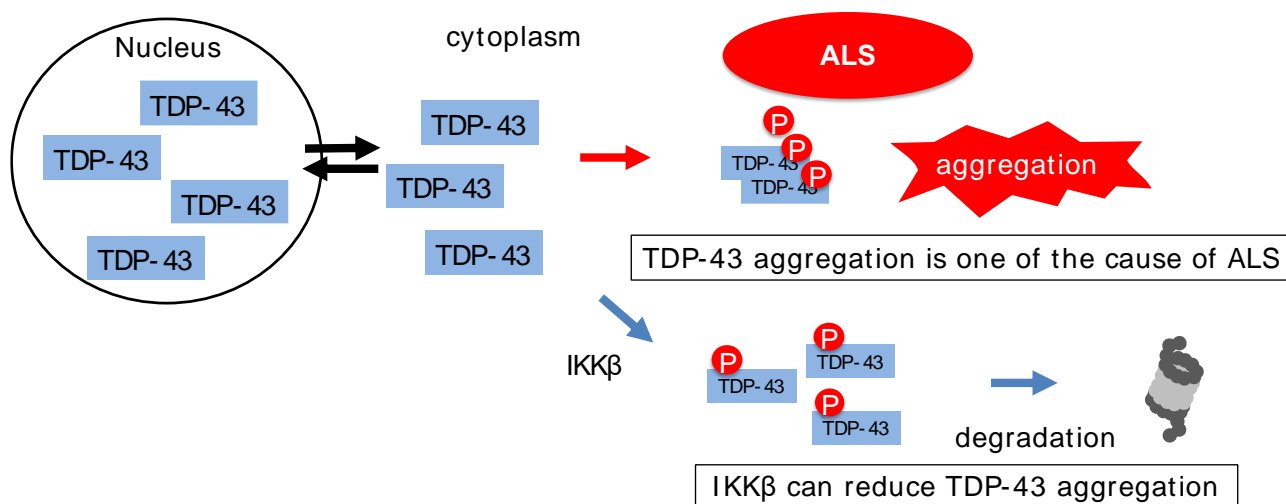
News Release

Title

I κ B kinase phosphorylates cytoplasmic TDP-43 and promotes its proteasome degradation

Key Points

- IKK β phosphorylates N-terminal side of TDP-43
- IKK β -mediated TDP-43 phosphorylation promotes TDP-43 degradation
- IKK β specifically suppresses cytoplasmic TDP-43 expression and reduces its neurotoxicity



TDP-43 shuttles between the nucleus and cytoplasm; in ALS, TDP-43 forms aggregates and accumulates in the cytoplasm, where it is hyperphosphorylated at the C-terminals. IKK β localizes to the cytoplasm and phosphorylates the N-terminals of cytoplasmic TDP-43, leading to its degradation. As a result, it has the effect of reducing TDP-43 aggregation in the cytoplasm.

Summary

A research group led by Professor Masao Katsuno and Associate Professor Yohei Iguchi (first author) of the Department of Neurology, Nagoya University Graduate School of Medicine has demonstrated that I κ B kinase beta (IKK β) selectively inhibits TDP-43 expression in the cytoplasm, which causes neurodegeneration in amyotrophic lateral sclerosis (ALS).

ALS is a progressive neurodegenerative disease that causes muscle atrophy due to selective cell death of motor neurons. In ALS, TDP-43 is found to escape from the nucleus of degenerating neurons and accumulate in the cytoplasm as aggregates. Loss of TDP-43 function in the nucleus and aggregate toxicity in the cytoplasm are thought to be the primary causes of motor neuron death. Therefore, therapeutic strategies that suppress overall TDP-43 expression risk further reduction of TDP-43 function. No therapeutic strategy has been established that specifically reduces aggregation. The group has shown that overexpression of IKK β does not affect TDP-43 functioning in the nucleus, but phosphorylates TDP-43, which is increased in the cytoplasm, leading to its degradation. Although TDP-43 is known to be phosphorylated at the C-terminal serines in ALS pathology, IKK β promotes TDP-43 degradation by phosphorylating serine 92 (Ser92) outside the C-terminal region of TDP-43. In this study, we generated an antibody specific for phosphorylation of TDP-43 Ser92. Immunostaining of ALS autopsy spinal cord with this antibody showed that some of the aggregates in motor neurons were positive for this antibody, suggesting that motor neurons may actively degrade TDP-43 to counteract neurodegeneration in ALS, but it may not be fully functional. Finally, this research group has demonstrated in animal studies that IKK β not only reduces TDP-43 aggregations but also reduces aggregate toxicity.

The "selective degradation of cytoplasmic TDP-43 by IKK β ," which was elucidated in this study, is expected to be applied to disease-modifying therapy to inhibit ALS progression.

Research Background

Most ALS patients develop the disease in middle age or later without any special triggers or prodromal signs. In the early stages of the disease, muscle weakness is restricted to a focal area, but gradually the entire body becomes weak, and in an average of 3 to 5 years, patients are unable to breathe on their own due to paralysis of the respiratory muscles. More than 90% of patients with ALS are sporadic, meaning that they have no relatives with the disease, and the cause of the disease has not been identified. However, pathological and biochemical analysis of the brain and spinal cord of ALS patients has revealed that TDP-43, a protein that normally resides in the nucleus, escapes from the nucleus and

forms aggregates in the cytoplasm of ALS motor neurons (Fig. 1A). Since then, researchers including our own have conducted cell and animal studies and now consider TDP-43 "loss of function" in the nucleus and "aggregate toxicity" in the cytoplasm to be the primary causes of motor neuron death (Fig. 1B). The current feasible therapeutic strategy is to supplement TDP-43 function or to reduce aggregation. One possible strategy to reduce aggregation would be to use gene therapy to decrease the overall expression of TDP-43 protein, but this would risk further exacerbating the TDP-43 "loss of function".

TDP-43 aggregated in ALS motor neurons is hyperphosphorylated at the C-terminal serine, and C-terminal phosphorylation antibodies are commonly used as pathological diagnostic markers. However, the phosphorylation itself does not seem directly involved in TDP-43 metabolism. There are many amino acids in TDP-43 that can be phosphorylated, but phosphorylation outside of the C-terminus has not been fully investigated.

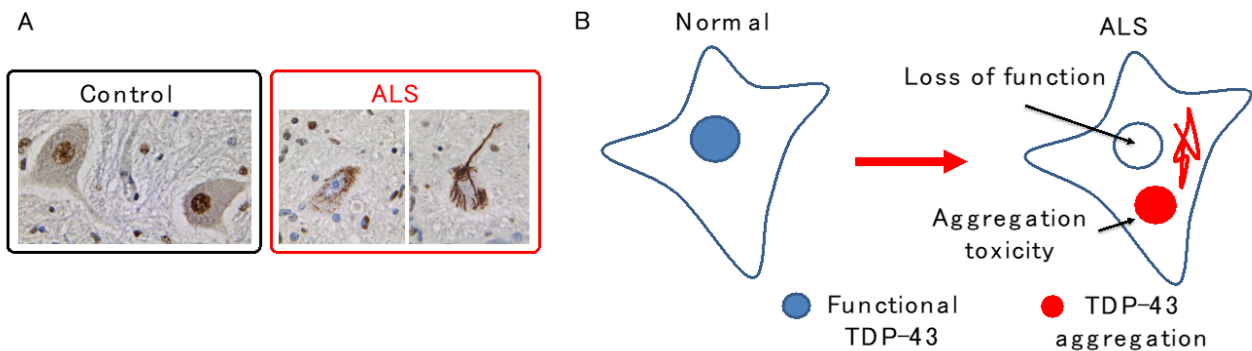


Figure 1. A. Immunostaining of spinal motor neurons. TDP-43 is normally predominantly localized in the nucleus, but forms aggregate in the cytoplasm in ALS pathology. B. "Loss of function" of TDP-43 in the nucleus and "aggregate toxicity" of TDP-43 in the cytoplasm are considered the major cause of the degeneration of motor neurons in ALS.

Research Results

In this study, we first elucidated that overexpression of $IKK\beta$ has no effect on wild-type TDP-43 but reduces the mutated cytoplasmic aggregate TDP-43 in experiments using Neuro2a cells (Fig. 2). Furthermore, proteomics and in vitro kinase assays revealed that $IKK\beta$ phosphorylates multiple amino acids on the amino-terminal side of TDP-43, especially Ser92, and promotes proteasomal degradation of TDP-43 itself by direct phosphorylation (Fig. 3). Notably, $IKK\beta$ localizes primarily to the cytoplasm and thus has the effect of reducing TDP-43 aggregation by leading to degradation of increased TDP-43 in the cytoplasm, without reducing normal TDP-43 functioning in the nucleus.

Immunostaining of ALS spinal cord revealed C-terminal phosphorylation in all

aggregates of motor neurons, while Ser92 phosphorylation was also found in some aggregates (Fig. 4). It is possible that the active degradation of TDP-43 is partially mediated in ALS, but that its inadequate function leads to disease progression.

In addition, experiments expressing the aggregate form of TDP-43 and IKK β in mouse hippocampal neurons have shown that IKK β has a mitigating effect on TDP-43 aggregate toxicity (Fig. 5).

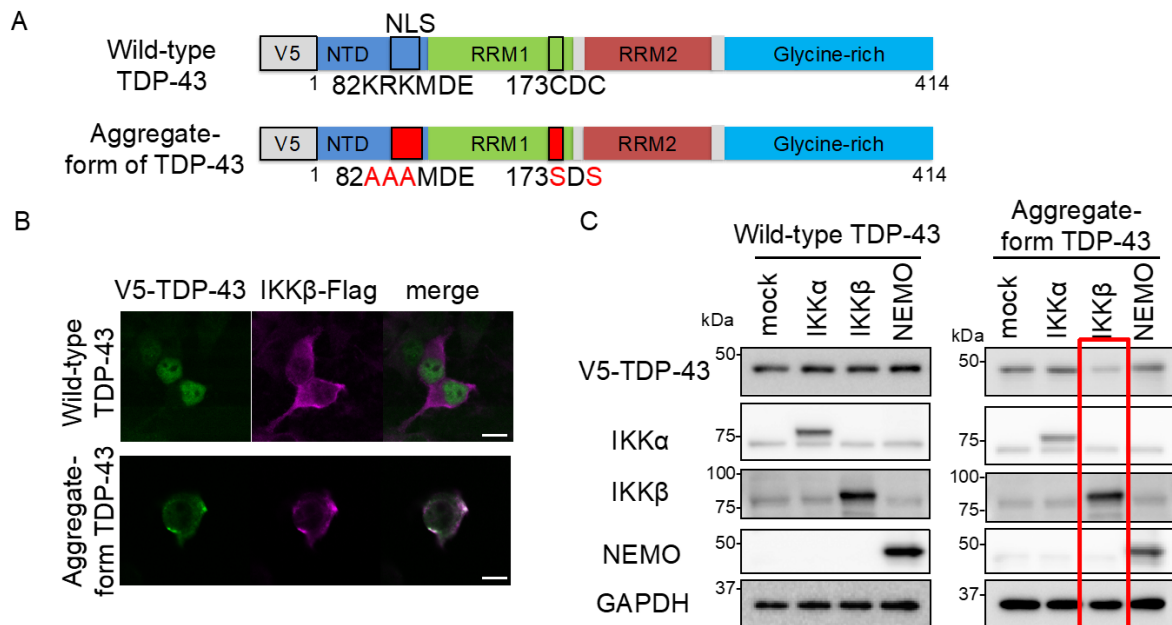


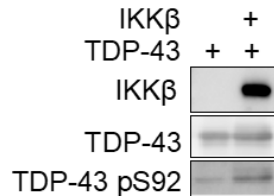
Figure 2. A. Wild-type and aggregate-prone TDP-43, in which nuclear localization signal (NLS) and RNA binding motifs (RRM) are mutated. B. TDP-43 and IKK β were expressed simultaneously in Neuro2a cells; IKK β is cytoplasmically localized and does not coexist with wild-type TDP-43, but does well with aggregate-prone TDP-43. Scale bar is 10 μ m. C. Western blot analysis using proteins extracted from Neuro2a cells. TDP-43 was expressed with the IKK complex subunits IKK α , IKK β , or NEMO, respectively. IKK β has no effect on wild-type TDP-43, but decreased the expression of aggregate-prone TDP-43 (red box).

A

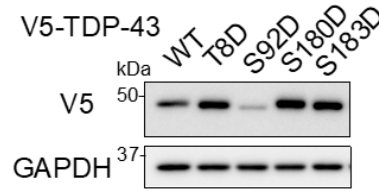
| Fragment sequence | Position | score | | Probability |
|--|----------|-----------------|-----------------|-------------|
| | | IKK β -WT | IKK β -SA | |
| VTEDEND ⁸ EPIEIPSEDDGTVLLSTVTAQFPGACGLR | 8 | 78 | 0 | 100 |
| KMDETDA ⁹² SSAVK | 92 | 51 | 16 | 87.8 |
| LPNSK ^{180/183} QSQDEPLR | 180/183 | 62 | 0 | 100 |

B

in vitro kinase assay



C



D

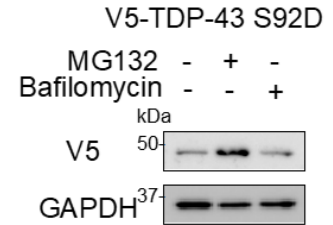


Figure 3. A. Proteomics identified the 8th threonine (Thr8), 92nd serine (Ser92), and 180/183rd serine (Ser180/183) as IKK β -specific phosphorylation sites. B. In vitro kinase assay confirms that IKK β directly phosphorylates TDP-43 Ser92. C. Phospho-mimetic mutation for each phosphorylation site was generated and expressed in Neuro2a cells for Western blot analysis. Administration of proteasome inhibitor (MG132), but not autophagy inhibitor (Bafilomycin), to Neuro2a cells restored TDP-43 expression.

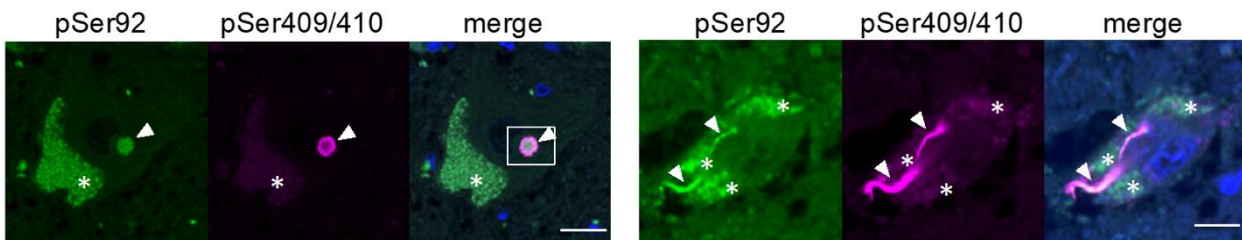


Figure 4. Fluorescent immunostaining was performed on ALS spinal cord using phospho-specific antibody against TDP-43 Ser92 (pSer92) and against TDP-43 C-terminus (pSer409/410), which are commonly used as pathological diagnostic markers (green: pSer92; purple: pSer409/410). All aggregates in the motor neurons were positive for pSer409/410, while some aggregates were also positive for pSer92 (arrowheads). * is autofluorescence due to lipofuscin. Scale bar is 10 μ m.

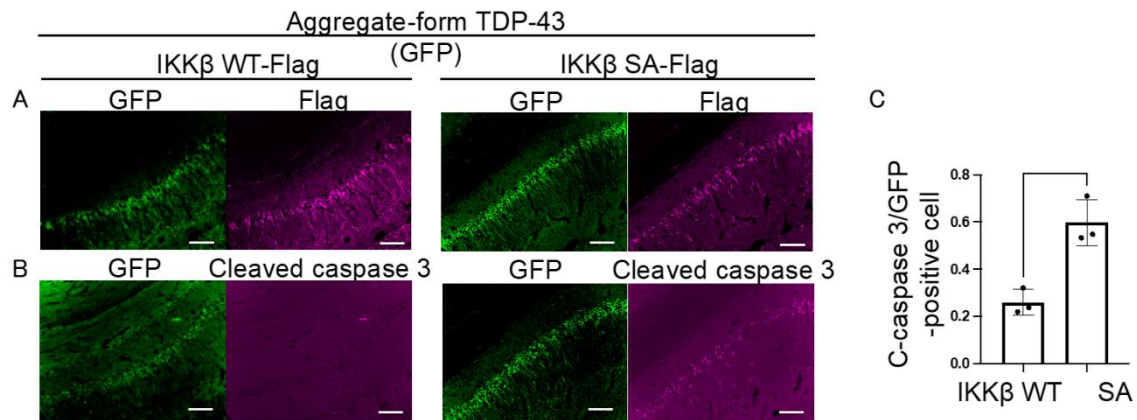


Figure 5. A. Aggregate-prone TDP-43-GFP and IKK β -Flag were expressed simultaneously in mouse hippocampus to test the effect of IKK β on aggregate toxicity (green: GFP; purple: Flag); WT: wild type; SA: inactive type; B, C. A cell death marker, cleaved caspase 3-positive neurons were significantly reduced by IKK β expression. Scale bar is 100 μ m.

Future Perspective

This study showed that IKK β has the effect of promoting cytosolic TDP-43 degradation and reducing TDP-43 aggregate toxicity. In the future, we plan to test the long-term therapeutic effects of IKK β in a mouse model of ALS pathology.

Publication

Journal of Cell Biology

I κ B kinase phosphorylates cytoplasmic TDP-43 and promotes its proteasome degradation

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DOI: 10.1083/jcb.202302048

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