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SPOTLIGHT

IκB kinase thwarts aggregation: Phosphorylating TDP-43 for degradation

Cha Yang[1], Yanru Liu[1], and Fenghua Hu[1]

TDP-43 aggregation is a hallmark of neurodegeneration. In this issue, Iguchi et al. (https://doi.org/10.1083/jcb.202302048) report that IκB kinase (IKK), an important mediator of inflammation, phosphorylates cytoplasmic TDP-43 to promote proteasomal degradation, revealing an unexpected link between inflammation and TDP-43 homeostasis.

TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA-binding protein that shuttles between the nucleus and the cytoplasm to regulate transcription, translation, pre-mRNA splicing, and mRNA stability (1) (Fig. 1, A and B). Cytoplasmic aggregation, which often results in nuclear depletion and loss of function of TDP-43, is a common pathological hallmark of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), limbic-predominant age-related TDP-43 encephalopathy (LATE), and Alzheimer's disease (AD). Since such pathology is often associated with disease severity and progression, it is vital to understand molecular mechanisms regulating TDP-43 protein homeostasis (2, 3). Studies have identified many posttranslational modifications of TDP-43 that regulate its homeostasis or function, among which phosphorylation is one of the best studied. Several kinases have been shown to directly phosphorylate TDP-43, including casein kinases 1 and 2 (CK1 and CK2), cell division cycle 7 (CDC7), tau tubulin kinases 1 and 2 (TTBK1 and TTBK2), and the non-receptor tyrosine kinase c-Abl (4, 5) (Fig. 1 A). In addition, phosphorylation of C-terminal Ser-409/410 residues (pSer409/410) of TDP-43 is widely used as a marker for TDP-43 aggregation (4). However, with over 60 potential phosphorylation sites in TDP-43, a comprehensive understanding of their functions and the specific kinases responsible for targeting these phosphorylation sites remains elusive (4).

The IKK complex, comprised of IKKα, IKKβ kinase, and NEMO (IKKγ), is a key mediator of inflammation by phosphorylating IκB (inhibitor of NF-κB) to promote its ubiquitination and proteasomal degradation, which allows nuclear translocation of NF-κB and activation of NF-κB–mediated inflammatory responses (6). Intriguingly, in this issue, Iguchi et al. report that cytoplasmic TDP-43 is another direct substrate of the IKKβ kinase (7). Using mass spectrometry, they identify residues Thr8, Ser92, and Ser180 located at the N-terminus of TDP-43 as IKKβ-dependent phosphorylation sites. They further show that Ser92 is directly phosphorylated by IKKβ in in vitro phosphorylation assays and NEMO functions as a scaffold to interact and recruit TDP-43 to the IKK complex, while IKKα acts as a cofactor (Fig. 1 B). Moreover, they show that the phosphorylation at Thr8 and Ser92 by IKKβ triggers ubiquitination and proteasomal degradation of cytoplasmic TDP-43 (Fig. 1 B). Phosphorylation resistant S92A and T8A mutants are resilient to IKKβ induced degradation. Although IKKβ is known to activate NF-κB (6) and an interaction between TDP-43 and the NF-κB subunit p65 has been previously reported (8), the authors demonstrate that IKK controls TDP-43 protein turnover independently of NF-κB. Interestingly, even though Ser92 is localized in the nuclear localization signal (NLS) of TDP-43, the phosphomimetic S92D mutant of TDP-43 remains localized to the nucleus. However, it is yet to be determined whether S92D indeed does not affect the nuclear localization of TDP-43 or whether the cytoplasmic pool of S92D is rapidly degraded by the proteasome, resulting in its low levels in the cytosol. In addition, the authors find that IKKβ activation specifically decreases the levels of cytoplasmic aggregation-prone TDP-43, either with mutations in NLS and RNA recognition motif 1 (RRM1; 3A2S) or TDP-43 harboring ALS-linked mutations. Since the authors also show that endogenous TDP-43 and overexpressed wild type (WT) TDP-43 can be phosphorylated by IKKβ, it remains unclear whether the specific effect of IKKβ on these mutants is due to their misfolding or due to their mere accumulation in the cytosol, where IKKβ resides. Nevertheless, these results lead the authors to hypothesize that IKK-dependent TDP-43 phosphorylation is a protective event to counter TDP-43 aggregation and alleviate its associated toxicity. In support of this idea, they show that IKKβ reduces both the protein levels and the toxicity of disease-linked TDP-43 mutants in cell culture and mitigates the toxicity of aggregation-prone TDP-43 in the hippocampal neurons in mouse models.

[1]Department of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY, USA.

Correspondence to Fenghua Hu: fh87@cornell.edu.

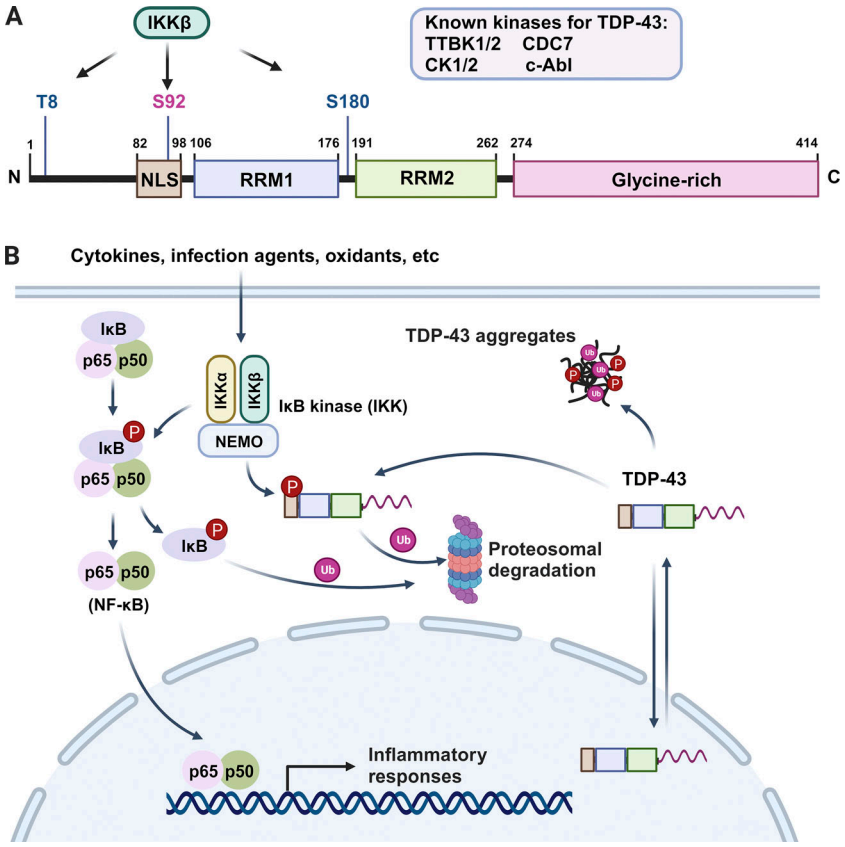

Figure 1. **IKK phosphorylates cytoplasmic TDP-43 to promote proteasome-dependent turnover.** **(A)** TDP-43 is a DNA/RNA-binding protein comprised of an N-terminal domain harboring an NLS, two RRMs, and a C-terminal glycine-rich region. Many kinases have been shown to phosphorylate TDP-43, including CK1, CK2, CDC7, TTBK1, TTBK2, and c-Abl, to regulate its solubility, localization, and function. In this study by Iguchi et al. (7), IKKβ is shown to phosphorylate TDP-43 at T8, S92, and S180. **(B)** Upon the activation of IKK by the potential triggers, NEMO recruits cytoplasmic TDP-43 to the IKK complex. IKKα acts as a cofactor, and IKKβ directly phosphorylates TDP-43. Consequently, TDP-43 is ubiquitinated and degraded via the proteasome, thus reducing the level and toxicity of aggregation-prone TDP-43. Activation of IKK also leads to phosphorylation and proteasomal degradation of IκB, allowing nuclear translocation of NF-κB (comprised of p65 and p50) and activation of inflammatory responses. P: phosphorylation; Ub: ubiquitination. The figure was created by BioRender.

These findings are novel and exciting, revealing an unexpected role of a key regulator of inflammation in regulating TDP-43 proteinopathy. This work has also raised many new questions and avenues for research. First of all, when the IKKβ-dependent phosphorylation happens remains to be determined. In addition to aggregation-prone TDP-43, endogenous TDP-43 and WT TDP-43 appear to be phosphorylated by IKKβ as well. Using the antibodies developed to specifically recognize phosphorylation of Ser92 (pSer92), Iguchi et al. (7) report the detection of pSer92 signals in spinal motor neurons of sporadic ALS patients. Intriguingly, they find that pSer92 signals completely merge with pSer409/410 signals in dot-like TDP-43 inclusions but tend to be at the center of large, round, and skein-like inclusions with pSer409/410 staining at the outer edge. pSer92 signals are not detected in granular TDP-43 inclusions. These observations suggest that different types of TDP-43 inclusions might be subject to distinct phosphorylation modifications. The accumulation of pSer92 signals in many types of TDP-43 inclusions suggests that pSer92 does not necessarily lead to clearance of TDP-43 aggregates and there could be dynamic interactions between different phosphorylation events and other posttranslational modifications to regulate TDP-43 homeostasis. It will be interesting to investigate pSer92 signals in other diseases with TDP-43 aggregation, such as FTLD, AD, and LATE to better understand the role of pSer92 in disease progression and its potential as a diagnostic marker for TDP-43 proteinopathy.

Furthermore, Iguchi et al. (7) specifically investigate the phosphorylation of TDP-43 by IKK in neurons, despite the IKK complex being best known for its role in inflammation in immune cells. Thus, it remains uncertain whether IKKβ regulates TDP-43 homeostasis in non-neuronal cells in the central nervous system. Given its key role in inflammation, further research and investigation are warranted to delineate the specific role of IKKβ in TDP-43 phosphorylation across various cell types in the CNS, especially microglia, and to ascertain the significance of this phosphorylation in the broader context of neurodegenerative diseases. Finally, since IKKβ diminishes cytoplasmic TDP-43 aggregates and their associated toxicity in their study, Iguchi et al. (7) propose modulating N-terminal phosphorylation of TDP-43 by IKK as a potential therapeutic strategy in treating TDP-43 proteinopathy. While activating IKKβ could promote the degradation of cytoplasmic TDP-43, this strategy would need to be carefully evaluated for potential adverse effects considering many of IKK's pivotal cellular functions (6). Depending on pathophysiological conditions, IKKβ/NF-κB activation in neurons could be either beneficial or toxic. While the activation of IKKβ/NF-κB could potentially promote neuronal survival, neurite outgrowth, synaptogenesis, and neuronal plasticity (9), chronic overexpression of a constitutively active IKKβ in mouse forebrain neurons induces learning deficits and apoptosis independent neurodegeneration (10). Moreover, loss of normal TDP-43 function, rather than cytoplasmic TDP-43 aggregation, has been shown to drive neurodegeneration in many cases (2, 3). Whether promoting the degradation of cytoplasmic TDP-43 through IKKβ activation could help restore normal TDP-43 function is still a question. Additionally, the fact that pSer92 has been detected in both endogenous and overexpressed WT TDP-43 and the S92D mutant of TDP-43 is degraded much faster than WT TDP-43 indicates that the IKKβ phosphorylation could potentially promote the degradation of endogenous cytosolic WT TDP-43, which might lead to decreased levels of TDP-43 and loss of TDP-43 functions in the cytosol.

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