The structural integrity of TDP-43 N-terminus is required for efficient aggregate entrapment and consequent loss of protein function

Valentina Romano, Zainuddin Quadri, Francisco E Baralle, and Emanuele Buratti*
International Centre for Genetic Engineering and Biotechnology (ICGEB); Trieste, Italy

ABSTRACT. Nuclear factor TDP-43 has been shown to play a key role in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia, where TDP-43 aggregates accumulate in patient’s affected neurons and this event can cause neuronal dysfunction. A major focus of today’s research is to discover the critical factors that lead to TDP-43 aggregation and the consequences for neuronal metabolism. From a structural point of view, several lines of evidence point toward TDP-43 C-terminus as a key domain able to mediate this process. Regarding this region, we have recently described a novel cellular TDP-43 aggregation model based on 12 tandem repetitions of its 339-366 Q/N rich prion-like domain. In addition, we have shown and confirmed that a minimal TDP-43 construct constituted by the N and C-terminal regions, but lacking both RRM domains, induce aggregation of endogenous TDP-43 and leads to its total loss of function as seen by changes in the alternative splicing of endogenous genes. In this work, we further characterize this model and show the importance of the N-terminus structure in the loss of function process. In addition, from a biochemical point of view we report that, as shown in a previous version of this model (GFP 12×Q/N), the endogenous TDP-43 trapped in the aggregates undergoes the 2 most important post-translational modifications seen in pathological TDP-43 inclusions: ubiquitination and hyperphosphorylation.

KEYWORDS. prion, TDP-43, ALS, post-translational modifications, protein aggregation

ABBREVIATIONS. ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia; GFP, green fluorescent protein; hnRNP, heterogeneous ribonucleoproteins; RRM, RNA recognition motif; TDP-43, TAR DNA binding protein 43; Ub-HA, Ubiquitin-HA

*Correspondence to: Emanuele Buratti; Email: buratti@icgeb.org
Received November 19, 2014; Revised January 19, 2015; Accepted January 21, 2015.
Extra View to: Budini M, Romano V, Quadri Z, Buratti E, Baralle FE. TDP-43 loss of cellular function through aggregation requires additional structural determinants beyond its C-terminal Q/N prion-like domain. Hum Mol Genet 2015; 24(1):9-20; PMID:25122661; http://dx.doi.org/10.1093/hmg/ddu415.
Color versions of one or more figures in this article can be found online at www.tandfonline.com/kprn.
INTRODUCTION

Accumulation of protein aggregates in neurons represents a distinctive feature of several neurodegenerative disorders. Identifying the proteins responsible for these aggregates has therefore become a fundamental step in trying to understand the different neurodegenerative pathologies. For amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) a major breakthrough in this area came in 2006 when the TAR DNA-binding protein (TDP-43) was discovered to be the main component of the ubiquitinated inclusions found in the brains of almost all ALS affected patients and approximately 60% of FTD patients. Since then, TDP-43 aggregation has been found to be a prominent feature of several neurodegenerative diseases including Alzheimer’s disease.

TDP-43 is a 414-aminoacid RNA-binding protein mainly involved in RNA metabolism. In physiological conditions TDP-43 can continuously shuttle between nucleus and cytoplasm thanks to the presence of well defined nuclear localization (NLS) and nuclear export (NES) signals. In ALS and FTD patients, pathological aggregation can occur both in the nucleus and cytoplasm of glia and neuronal cells where the aggregated TDP-43 acquires new biochemical features such as ubiquitination, hyperphosphorylation, mainly at 409/410 serine residues, and is cleaved by proteases to yield C-terminal fragments. Most importantly, loss of soluble nuclear TDP-43 is also observed, an event possibly associated with misregulation of all the molecular processes controlled by TDP-43 inside the nucleus.

The aggregation potential of TDP-43 correlates well with its structural features, since similarities between TDP-43 and other amyloid proteins have been found. In particular, a critical role in TDP-43 aggregation is played by its C-terminal prion-like domain. This region is enriched in glutamine (Q) and asparagine (N) amino acids spanning residues 342–366 and is normally involved in protein-protein interactions, especially with other hnRNP proteins.

Interestingly, this region can spontaneously arrange in a β-sheet rich oligomer in vitro and when expressed in cultured cells or primary neurons has a strong propensity to induce the formation of intracellular aggregates. However, in the originally established model based only on the 12xQ/N region we could not detect loss of TDP-43 function in the cells displaying the aggregates.

To address this issue, we have recently been able to engineer a stable cellular model (Fig. 1A) that causes loss of TDP-43 function. To achieve this, we have linked the tandem repetitions of the Q/N rich region downstream to an otherwise wild-type Flag-TDP-43 protein (Flag-TDP-12X-Q/N) and the resulting construct was used to generate a stable HEK-293 cell line. Expression of this construct induced a widespread formation of cellular aggregates. In many cells, this was accompanied by the complete clearance of TDP-43 from the nucleus and alteration of several splicing events known to be regulated by TDP-43. Moreover, through a fine mapping of the Flag-TDP-12X-Q/N protein we demonstrated that the first 75 residues of TDP-43-12X-Q/N resulted essential in binding the endogenous protein and in mediating the loss-of-function effect. Indeed, by deleting the N-terminal region of this molecule, TDP-43 splicing activity was recovered (Fig. 1A lanes 5 and 6) even in the presence of aggregates (as shown by the immunofluorescence in Fig. 1A). This result demonstrated that at least in our system efficient trapping of TDP-43 and its loss function were enhanced by the N-terminal domain, rather than by the aggregation process itself. Recently, moreover, Qin and colleagues have found that the N-terminal region of TDP-43 is characterized by a mix of folded and unfolded structures that coexist in a tightly regulated equilibrium by salt and protein concentration. Most importantly, the well-folded conformation appears to assume an ubiquitin-like structure able to bind ssDNA and RNA molecules, probably allowing TDP-43 oligomerization that may favor some of the physiological cellular...
functions of this protein. This suggests that any events capable of shifting the equilibrium between these species could enhance the formation of the unfolded form, leading to loss of TDP-43 oligomerization/function and a higher propensity to form insoluble and pathological aggregates.

In this work, therefore, we show that the N-terminal intact structure is needed for efficient capture of TDP-43 in the aggregates and
consequent loss of function. In fact deleting parts of it makes the aggregation system behave like in the case of GFP 12×Q/N\textsuperscript{12} with no or marginal loss of TDP-43 functionality. We have also better characterized the molecular features of the aggregates formed by the TDP-12X-Q/N stable cell line, further confirming the importance of TDP-43 C-terminus in inducing aggregation.

**RESULTS**

**Flag-TDP-12X-Q/N Aggregates are Positive for Ubiquitination**

The Flag-TDP-12X-Q/N cellular model has been previously shown to recapitulate both the aggregation and the loss of nuclear TDP-43 function in a way that is similar to what has been observed in patients.\textsuperscript{16} For this reason, it was also considered important to check for the presence of other characteristic features of these TDP-43 aggregates. First of all, therefore, the presence of ubiquitination was investigated by immunoprecipitation. For this experiment, we took advantage of the stable cell line overexpressing the Flag-TDP-12X-Q/N protein that can sequester endogenous TDP-43 in a highly efficient manner. As control, we used a stable cell line that overexpressed a Flag-TDP-43-F4/L mutant which was previously shown not to induce aggregation in our system and, most importantly, is also not able to autoregulate the level of endogenous TDP-43 mRNA\textsuperscript{18} (thus allowing to see the eventual ubiquitination levels of the endogenous protein).

To check for this modification, both cell lines were then transiently transfected with a construct able to express ubiquitin protein conjugated with a hemagglutinin epitope (Ub-HA). Flag-TDP-12X-Q/N and Flag-TDP-43-F4/L protein expression was induced or not with tetracycline for 72 hours and immunoprecipitation was carried out using an anti-TDP-43 antibody. Finally, the samples were analyzed by Western blot using an anti-HA antibody. In Figure 1B (left panel) it is possible to observe that ubiquitination was evident only in the immunoprecipitated samples overexpressing Flag-TDP-12X-Q/N, but not in those over-expressing Flag-TDP-43-F4/L protein (Fig. 1B, compare line 4 with 2). A prominent positive band was observed at approximately 80 kDa, which corresponds to Flag-TDP-12X-Q/N migration pattern. Because the immunoprecipitation was performed using an anti-TDP-43 antibody, the smear observed above the 80 kDa band (Fig. 1B, line 4) should correspond to ubiquitinated Flag-TDP-12X-Q/N species with higher MW. On the other hand, the smear observed below the 80 kDa band (Fig. 1B, lane 4 arrows) should represent the higher MW species coming from ubiquitinated endogenous TDP-43. As expected, no ubiquitinated bands could be observed in the induced Flag-TDP-43-F4/L sample that does not induce aggregation (Fig. 1B, lane 2) and, also as expected, no signal could be detected in the non induced Tet- lanes for both Flag-TDP-12X-Q/N and Flag-TDP-43-F4/L (Fig. 1B, lanes 1 and 3).

Moreover, a western blot using an anti-TDP-43 antibody was also performed. As shown in Figure 1B (right panel, lanes 5-8), immunoprecipitated endogenous TDP-43 and exogenous Flag-TDP-43-F4/L and Flag-TDP-12X-Q/N proteins were detected at the expected molecular weight.

Taken together, these data indicate that aggregates induced by Flag-TDP-12X-Q/N are being ubiquitinated and that also the sequestered endogenous TDP-43, trapped in these aggregates undergoes the same modification.

**Flag-TDP-12X-Q/N Aggregates Induce Hyperphosphorylation of Endogenous TDP-43**

In addition to ubiquitination, hyperphosphorylation represents one of the main pathological signature of several neurodegenerative disorders, such as tauopathies or α-synucleopathies.\textsuperscript{19} In brain tissues deriving from patients affected by ALS and FTLD, but not in controls, TDP-43 has been found hyperphosphorylated on 5 serine residues\textsuperscript{8}: S379, S403, S404, S409, S410. Although the functional significance of this hyperphosphorylation is not completely understood, it is certainly a feature that
discriminates between a physiological and a pathological state.

To investigate whether hyperphosphorylation was present in the endogenous sequestered TDP-43, we performed an immunofluorescence experiment of Flag-TDP-12X-Q/N cells, induced with tetracycline for 72 hours, using a specific antibody against the phosphorylated residues 409/410 (anti-pS409/pS410). As shown in Figure 1C, a positive signal was detected for endogenous TDP-43 co-localizing with the aggregates. In this respect, it should be noted that the 12 repetitions of the Q/N rich region have been cloned at the residue 403 of the Flag-TDP-43 wild type protein. Therefore, the anti-pS409/pS410 antibody cannot cross-react with any possible phosphorylation of the Flag-TDP-12X-Q/N protein.

**TDP-43 Prion-Like Domain is Essential in Determining Aggregation**

In our previous work we have demonstrated that the TDP-43 prion-like domain is the minimal region essential in promoting protein aggregation. To better investigate which functional domains of TDP-43 are required for aggregation we previously engineered a minimal Myc-TDP-Δ1Δ2 construct in which the 2 RRM s were deleted and showed that none of the RRM s is necessary.

---

**FIGURE 2.** A shows an immunofluorescence of HEK-Flip in and HEK-Flag-TDP-12X-Q/N cell lines. Cells were transiently transfected with MYC-TDP-Δ1Δ2, MYC-TDP-Δ1Δ2-ΔN or MYC-TDP-Δ1Δ2-ΔC constructs and induced for 72 hours with tetracycline. An anti-Myc (red) antibody was used in order to identify the distribution of the transfected plasmids. Cell nuclei were stained using TO-PRO3 reagent. A merge between anti-Myc/TO-PRO3 is showed. B shows a cell lysate fractionation experiment. In this experiment, HEK-Flip in and HEK-Flag-TDP-12X-Q/N cell lines were transfected with MYC-TDP-Δ1Δ2, MYC-TDP-Δ1Δ2-ΔN or MYC-TDP-Δ1Δ2-ΔC plasmids and induced for 72 hours. Then a cell lysate fractionation was performed and an anti-Myc antibody was used in a western blot experiment to identify protein localization in soluble and/or pellet fractions.
to induce aggregation and causing loss-of-function effects.\cite{12,16} To further extend this observation, we have now generated 2 more different mutants that carried deletion in the N-terminal (Myc-TDP-Δ1Δ2-ΔN) or in the C-terminal (Myc-TDP-Δ1Δ2-ΔC) domain. Stably expressing Flag-TDP-12X-Q/N cells were then transiently transfected with Myc-TDP-Δ1Δ2, Myc-TDP-Δ1Δ2-ΔN or Myc-TDP-Δ1Δ2-ΔC. As control, transfection in empty HEK-293-Flip in cells was also performed. As shown in Figure 2A, left panels, all the 3 Myc-TDP-43 constructs retained an homogeneous distribution in HEK-293-Flip in cells. However, when transfected in Flag-TDP-12X-Q/N cells, Myc-TDP-Δ1Δ2 and Myc-TDP-Δ1Δ2-ΔN were included in the cellular aggregates. This effect could not be observed for Myc-TDP-Δ1Δ2-ΔC that remained diffuse in the cell without being sequestered by the inclusions (Fig. 2A, right panels). To further confirm these results, a cell lysate fractionation was also performed to separate the soluble from insoluble fractions by ultracentrifugation (Fig. 2B). In accordance to the results obtained from the immunofluorescence experiment, Myc-TDP-Δ1Δ2 and Myc-TDP-Δ1Δ2-ΔN partially shifted from the soluble to the insoluble fraction in the presence of Flag-TDP-12X-Q/N aggregates. In contrast, Myc-TDP-Δ1Δ2-ΔC localized in the soluble fraction in both cell lines (Fig. 2B, compare left and right panels).

FIGURE 3. A shows a schematic representation of the Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN1-25, Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN26-50 and Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN51-75 proteins. RT-PCR in B shows the pattern of alternative splicing of POLDIP3 mRNA in induced (Tet + lanes) and not induced (Tet- lanes) HEK-293 stable cell lines following addition of Tetracycline.
The N-terminal Domain Structural Integrity is Essential to Induce TDP-43 loss-of-function Effects

Since the discovery of TDP-43 as the main signature of ALS and FTLD pathogenesis, the role of the N-terminal domain of the protein has remained quite unclear. However, very recently it was uncovered the importance of the extreme N-terminus of TDP-43 in protein oligomerization and loss of function.16,20 We have in fact previously demonstrated that by deleting the first 75 residues of TDP-43 the splicing function of the protein was completely restored (Fig. 1A).16 Very recently, Qin and colleagues17 have also reported that the N-terminal region of TDP-43 appears to assume an ubiquitin-like structure that is important for this protein to perform its physiological cellular functions. Therefore, in order to find whether the integrity of this structure is important to cause the observed loss of function effect we then moved to map the N-terminal domain of Flag-TDP-12X-Q/N protein.

As a starting point, we considered the minimal construct Flag-TDP-12X-Q/N-Δ1Δ2-ΔCterm that was known to cause loss of function effects.16 Serial deletions of the first 75 residues in 3 different portions were then performed to generate the mutants Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN1-25, Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN26-50, Flag-TDP-12X-Q/N-Δ1Δ2-ΔC-ΔN51-75 (Fig. 3A). The FlpIn system was then used to make corresponding HEK-293 stable cell lines where protein expression was made inducible following the addition of Tetracycline. Protein expression was induced for 72 hours and then RNA extraction and RT-PCR were performed in order to analyze splicing effect on POLDIP3 mRNA. As shown in Figure 3B, none of the mutants was able to induce TDP-43 loss of function in comparison with Flag-TDP-12X-Q/N, meaning that the N-terminal domain structure needs to be completely intact in order to bind and sequester endogenous protein within inclusions.

DISCUSSION

The present work outlines novel molecular and structural features of our recently described Flag-TDP-12X-Q/N aggregation model12 that is based on the Q/N prion-like domain of TDP-43.

First of all, we focused our attention on establishing whether the aggregates obtained using our system shared other characteristic features of the pathological inclusions. Among these features, ubiquitination and phosphorylation represent major traits of TDP-43 aggregation in ALS and FTD patients and can be considered as the common denominator of almost all the neurodegenerative diseases featured by accumulation of misfolded proteins, such as, PD, AD or Huntington’s disease (HD).21–23 Phosphorylated TDP-43 has been associated to both a protective and a toxic effect24,25 and its implications are still unknown. On the other hand, the role of ubiquitination is much better known and is particularly important to target aggregated proteins for proteasomal degradation.

Our results show that the Flag-TDP-12X-Q/N protein induces endogenous TDP-43 aggregation which becomes ubiquitinated and hyperphosphorylated on serines 409/410 (Fig. 1B and C), as it is observed in patients. Taken together, both results further support the evidence that our model very well resembles the main features of ALS pathology.

Secondly, in this work we have also taken advantage of our original deletion analysis to obtain more information regarding the involvement of other TDP-43 domains in aggregates formation and using a MYC-TDP-Δ1Δ2 mutant we have further confirmed the key role played by the C-terminus of TDP-43 in triggering aggregates formation.

Finally we have also found that the structural integrity of the N-terminus of TDP-43 is essential in binding endogenous protein and inducing loss of function in the splicing process (Fig. 3B). Our results are well in line with the recent work described by Qin et al.,17 who on the basis of structural studies have suggested that there is a tightly regulated equilibrium between a functional well-folded and a dysfunctional unfolded conformation of the N-terminal domain. Our data, together with their observations, support the idea that the structure and the
stability of N-terminal domain play a key role in changing protein characteristics and inducing pathogenesis.

In summary, this work further demonstrates that the Flag-TDP-12X-Q/N model has a great potential to assess additional molecular properties behind TDP-43 aggregation thanks to its ability to recapitulate the key points of the pathology: loss of nuclear function and post-translational modifications of the endogenous TDP-43 trapped in the aggregates.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

FUNDING

This work was supported by AriSLA (TARMA), Thierry Latran Fondation (REHNPALS), the EU Joint Programme-Neurodegenerative Diseases JPND (RiMod-FTD, Italy, Ministero della Sanita’).

REFERENCES

1. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314:130-3; PMID:17023659; http://dx.doi.org/10.1126/science.1134108

2. Chen-Plotkin AS, Lee VM, Trojanowski JQ. TAR DNA-binding protein 43 in neurodegenerative disease. Nat Rev Neurol 2010; 6:211-20; PMID:20234357; http://dx.doi.org/10.1038/nrneurol.2010.18

3. Buratti E, Dork T, Zuccato E, Pagani F, Romano M, Baralle FE. Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. EMBO J 2001; 20:1774-84; PMID:11285240; http://dx.doi.org/10.1093/emboj/20.7.1774

4. Buratti E, Brindisi A, Pagani F, Baralle FE. Nuclear factor TDP-43 binds to the polymorphic TG repeats in CFTR intron 8 and causes skipping of exon 9: a functional link with disease penetrance. Am J Hum Genet 2004; 74:1322-5; PMID:15195661; http://dx.doi.org/10.1086/420978

5. Mercado PA, Ayala YM, Romano M, Buratti E, Baralle FE. Depletion of TDP 43 overrides the need for exonic and intronic splicing enhancers in the human apoA-II gene. Nucleic Acids Res 2005; 33:6000-10; PMID:16254078; http://dx.doi.org/10.1093/nar/gki897

6. Ayala YM, Zago P, D’Ambrogio A, Xu YF, Petrucci I, Buratti E, Baralle FE. Structural determinants of the cellular localization and shuttling of TDP-43. J Cell Sci 2008; 121:3778-85; PMID:18957508; http://dx.doi.org/10.1242/jcs.038950

7. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2006; 351:602-11; PMID:17084815; http://dx.doi.org/10.1016/j.bbrc.2006.10.093

8. Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Ann Neurol 2008; 64:60-70; PMID:18546284; http://dx.doi.org/10.1002/ana.21425

9. Lee EB, Lee VM, Trojanowski JQ. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat Rev Neurosci 2012; 13:38-50.

10. Zhu L, Xu M, Yang M, Yang Y, Li Y, Deng J, Ruan L, Liu J, Du S, Liu X, et al. An ALS-mutant TDP-43 neurotoxic peptide adopts an anti-parallel beta-structure and induces TDP-43 redistribution. Hum Mol Genet 2014; 23:6863-77; PMID:25113748; http://dx.doi.org/10.1039/fmg/ddu409

11. Fuentealba RA, Udan M, Bell S, Wegorzewska I, Shao J, Diamond MI, Weihl CC, Baloh RH. Interaction with polyglutamine aggregates reveals a Q/N-rich domain in TDP-43. J Biol Chem 2010; 285:26304-14; PMID:20554523; http://dx.doi.org/10.1074/jbc.M110.125039

12. Budini M, Buratti E, Stuani C, Guaraccia C, Romano V, De Conti L, Baralle FE. Cellular model of TAR DNA-binding protein 43 (TDP-43) aggregation based on its C-terminal Gln/Asn-rich region. J Biol Chem 2012; 287:7512-25; PMID:22235134; http://dx.doi.org/10.1074/jbc.M111.288720

13. Mompean M, Buratti E, Guaraccia C, Brito RM, Chakrabarty A, Baralle FE, Laurents DV. Structural characterization of the minimal segment of TDP-43 competent for aggregation. Arch Biochem Biophys 2014; 545:53-62; PMID:24440310; http://dx.doi.org/10.1016/j.abb.2014.01.007
14. Furukawa Y, Kaneko K, Watanabe S, Yamanaka K, Nukina N. A seeding reaction recapitulates intracellular formation of Sarkosyl-insoluble transactivation response element (TAR) DNA-binding protein-43 inclusions. J Biol Chem 2011; 286:18664-72; PMID:21454603; http://dx.doi.org/10.1074/jbc.M111.231209
15. Guo W, Chen Y, Zhou X, Kar A, Ray P, Chen X, Rao EJ, Yang M, Ye H, Zhu L, et al. An ALS-associated mutation affecting TDP-43 enhances protein aggregation, fibril formation and neurotoxicity. Nat Struct Mol Biol 2011; 18:822-30; PMID:21666678; http://dx.doi.org/10.1038/nsmb.2053
16. Budini M, Romano V, Quadri Z, Buratti E, Baralle FE. TDP-43 loss of cellular function through aggregation requires additional structural determinants beyond its C-terminal Q/N prion-like domain. Hum Mol Genet 2015; 24:9-20; PMID:25122661; http://dx.doi.org/10.1093/hmg/ddu415
17. Qin H, Lim LZ, Wei Y, Song J. TDP-43 N terminus encodes a novel ubiquitin-like fold and its unfolded form in equilibrium that can be shifted by binding to ssDNA. Proc Natl Acad Sci U S A 2014; 111:18619-24; PMID:25503365; http://dx.doi.org/10.1073/pnas.1413994112
18. Ayala YM, De Conti L, Avendano-Vazquez SE, Dhir A, Romano M, D’Ambrogio A, Tollervey J, Ule J, Baralle M, Buratti E, et al. TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J 2011; 30:277-88; PMID:21131904; http://dx.doi.org/10.1038/emboj.2010.310
19. Inukai Y, Nonaka T, Arai T, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle FE, Akiyama H, Hisanaga S, et al. Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS. FEBS Lett 2008; 582:2899-904; PMID:18656473; http://dx.doi.org/10.1016/j.febio.2008.07.027
20. Zhang YJ, Caulfield T, Xu YF, Gendron TF, Hubbard J, Stetler C, Sasaguri H, Whitelaw EC, Cai S, Lee WC, et al. The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation. Hum Mol Genet 2013; 22:3112-22; PMID:23575225; http://dx.doi.org/10.1093/hmg/ddt166
21. Tepper K, Biernat J, Kumar S, Wegmann S, Timm T, Hubschmann S, Rebeck L, Mandelkow EM, Muller DJ, Mandelkow E. Oligomer Formation of Tau Hyperphosphorylated in Cells. J Biol Chem 2014; 289:34389-407; PMID:25339173; http://dx.doi.org/10.1074/jbc.M114.611368
22. Dzamko N, Zhou J, Huang Y, Halliday GM. Parkinson’s disease-implicated kinases in the brain; insights into disease pathogenesis. Front Mol Neurosci 2014; 7:57; PMID:25009465; http://dx.doi.org/10.3389/fnmol.2014.00057
23. Watkin EE, Arbez N, Waldron-Roby E, O’Meally R, Ratovitski T, Cole RN, Ross CA. Phosphorylation of mutant huntingtin at serine 116 modulates neuronal toxicity. PloS One 2014; 9:e88284; PMID:24505464; http://dx.doi.org/10.1371/journal.pone.0088284
24. Zhang YJ, Gendron TF, Xu YF, Ko LW, Yen SH, Petrucelli L. Phosphorylation regulates proteasomal-mediated degradation and solubility of TAR DNA binding protein-43 C-terminal fragments. Mol Neurodegeneration 2010; 5:33; PMID:20804554; http://dx.doi.org/10.1186/1750-1326-5-33
25. Li HY, Yeh PA, Chiu HC, Tang CY, Tu BP. Hyperphosphorylation as a defense mechanism to reduce TDP-43 aggregation. PloS One 2011; 6:e23075; PMID:21850253; http://dx.doi.org/10.1371/journal.pone.0023075