Biochemical and Biophysical Research Communications 464 (2015) 236–243

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

A phosphomimetic mutant TDP-43 (S409/410E) induces Drosha instability and cytotoxicity in Neuro 2A cells

Ki Yoon Kim [a, b, 1], Hee-Woo Lee [b, 1], Yu-mi Shim [b], Inhee Mook-Jung [a], Gye Sun Jeon [b, *], Jung-Joon Sung [b, **]

[a] WCU Neurocytomics Group, Department of Biomedical Sciences, Seoul National University Graduate School, Seoul, South Korea
[b] Department of Neurology, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, South Korea

ARTICLE INFO

Article history:
Received 5 June 2015
Accepted 18 June 2015
Available online 20 June 2015

Keywords:
TDP-43
FUS
Drosha
Phosphomimetic mutant TDP-43 (S409/410E)

ABSTRACT

Two DNA/RNA binding proteins, TDP-43 and FUS/TLSU, are involved in RNA processing, and their aberrant mutations induce inherited amyotrophic lateral sclerosis and frontotemporal lobar degeneration with ubiquitinated inclusions. Wild type TDP-43 and FUS (wtTDP-43 and wtFUS) are mainly localized in the nucleus and biochemically interact with the microRNA processing enzyme Drosha. In this study, we investigated Drosha stability in Neuro 2A cells by gain and loss of function studies of wtTDP-43 and wtFUS and cycloheximide mediated protein degradation assay. We also generated three different phosphomimetic mutants of TDP-43 (S379E, S403/404E and S409/410E) by using a site-directed mutagenesis method and examined Drosha stability to elucidate a correlation between the phosphorylated TDP-43 mutants and Drosha stability. Overexpression of wtTDP-43 and/or wtFUS increased Drosha stability in Neuro 2A cells and double knockdown of wtTDP-43 and wtFUS reduced its stability. However, knockdown of wtTDP-43 or wtFUS did not affect Drosha stability in Neuro 2A cells. Interestingly, a phosphomimetic mutant TDP-43 (S409/410E) significantly reduced Drosha stability via prevention of protein–protein interactions between wtFUS and Drosha, and induced cytotoxicity in Neuro 2A cells.

Our findings suggest that TDP-43 and FUS controls Drosha stability in Neuro 2A cells and that a phosphomimetic mutant TDP-43 (S409/410E) which is associated with Drosha instability can induce neuronal toxicity.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) are neurodegenerative diseases [1], and most of the research efforts made to understand the pathogenesis of these diseases have focused on mutations in various genes such as Cu/Zn superoxide dismutase 1 (SOD1), TAF15, EWSR1, FUS, and TDP-43 [2–6]. Recently, many studies demonstrated that mutations in these genes play an important role in the critical pathogenesis of ALS or FTLD-U [2–6].

Fused in sarcoma/translocated in liposarcoma (FUS/TLS) and TAR DNA-binding protein 43 (TDP-43) have recently garnered considerable interest because mutations of FUS and TDP-43 have been identified in patients with ALS-FTLD-U [5–7]. FUS and TDP-43 are ubiquitously expressed and are RNA-DNA binding protein. Both wild type FUS and TDP-43 (wtFUS and wtTDP-43) are mainly located in the nucleus and affect gene expression such as microRNA (miRNA) processing, transcriptional regulation and pre-mRNA splicing [7,8]. WtTDP-43 has two RNA recognition motifs and a glycine-rich region in its C-terminal domain. Most TDP-43 mutations occur in the glycine-rich region that causes neuronal cytoplasmic inclusions in ALS and FTLD-U [8]. According to recent reports, phosphorylated TDP-43 (ser379, ser403/404 and ser409/410) has been detected in patients with ALS and FTLD-U [5,9].

MiRNAs are small noncoding RNAs that regulate post-transcriptional gene regulation. miRNAs (~22 nucleotides) are involved in developmental and pathological processes in animals [10,11]. Processing of miRNAs is initiated by RNA polymerase II to

* Corresponding author. Department of Neurology, Seoul National University Hospital 28 Yongon-Dong, Chongno-gu, Seoul, 110-744, Republic of Korea.
** Corresponding author. Department of Neurology, Seoul National University Hospital 28 Yongon-Dong, Chongno-gu, Seoul, 110-744, Republic of Korea.
E-mail addresses: gyesun@daum.net (G.S. Jeon), jjsaint@snu.ac.kr (J.-J. Sung).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.06.125
0006-291X/© 2015 Elsevier Inc. All rights reserved.

generate primary-miRNAs (pri-miRNAs). In the nucleus, two microprocessors, Drosha, an RNase III enzyme, and DGCR8, a double-stranded RNA-binding protein, cleave pri-miRNAs to precursor-miRNAs (pre-miRNAs) [12–14]. In the last few years, wtFUS and wtTDP-43 have been shown to interact with Drosha and regulate miRNA biogenesis [8,15]. Specifically, loss of wtTDP-43 induces destabilization of Drosha under neuronal differentiation [16]. However, the precise role of wtTDP-43 in Drosha protein stability remains less characterized.

In this report, we investigate Drosha stability via gain- and loss of function studies of wtTDP-43 and wtFUS. We have also developed three different phosphomimetic mutants of TDP-43 (S379E, S403/404E and S409/410E) to elucidate the correlation between the phosphorylated TDP-43 mutants and Drosha stability. Surprisingly, TDP-43 (S409/410E), a phosphomimetic mutant, causes the destabilization of a conspicuous amount of Drosha protein and induces cytotoxicity in Neuro 2A cells.

2. Materials and methods

2.1. Cell culture

Neuro 2A cells were grown in Dulbecco's modified Eagle's medium (Hyclone) supplemented with 10% FBS (Hyclone), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen).

2.2. Mutagenesis of TDP-43

wtTDP-43 plasmid DNAs were mutated by using mutagenesis KIT (TOYOBO) and PCR carried out with mutagenesis primers (Supplementary Table 1). PCR product was sequenced.

2.3. Quantitative real Time-PCR (qRT-PCR)

Total RNA was extracted from Neuro 2A cells by TRIzol reagent (MRC). RNA was measured in a spectrophotometer at 260-nm absorbance. RNA analysis was conducted as follows. Fifty nanograms of RNA were used as a template for quantitative RT-PCR (qRT-PCR) amplification, using SYBR Green Real-time PCR Master Mix (Toyobo). Primers were standardized in the linear range of cycle before the onset of the plateau. Primer sequences are given in Supplementary Table 2. Mouse GAPDH was used as an internal control. Two-step PCR thermal cycling for DNA amplification and real-time data acquisition were performed with an ABI StepOnePlus™ Real-Time PCR System using the following cycle conditions: 95 °C for 1 min × 1 cycle, and 95 °C for 15s, followed by 60 °C for 1 min × 45 cycles. Fluorescence data were analyzed by the ABI StepOnePlus software and expressed as C_t the number of cycles needed to generate a fluorescent signal above a predefined threshold. The ABI StepOnePlus software set baseline and threshold values.

2.4. RNA interference experiments and Western blot analysis

30 nM of siRNA duplex were transfected in Neuro 2A cells with RNAiMax transfection reagent (Invitrogen) according to the manufacturer's instructions. siRNAs were synthesis from COSMO GENETECH. Target sequences of mouse siTDP-43 and siFUS were 5′-GUUAGAAAGAAGUGGAAGATT-3′ and 5′-GAAUUCUCUGGGAAUCCUAdTdT-3'. Neuro 2A cells were collected at 72 h after siRNA transfection. Neuro 2A cells were collected at 72 h after GFP-wtTDP-43, V5-wtFUS and Flag-Drosha transfection with Lipofectamine 2000 (Invitorgen). The cells were dispersed by pipetting in lysis buffer (10 mM Tris at pH 7.4, 1 mM ethylenediaminetetra acetic acid [EDTA] at pH 8.0500 mM NaCl, and 0.5% Triton X-100) and incubated for 30 min on ice. Primary antibodies used in this study are goat anti-GFP antibody (Rockland), mouse anti-V5 antibody (Invitrogen), rabbit anti-Drosha antibody (abCam), rabbit anti-TDP-43 (Proteintech), rabbit anti-FUS (abCam), mouse anti-Flag (SIGMA, St Louis, MO, USA), rabbit anti-Bax (Cell signaling) and mouse anti β-actin (Millipore).

2.5. Lactate dehydrogenase (LDH) release assay

At the end of treatment, cell culture medium was collected and briefly centrifuged. The supernatants were transferred into wells in 96-well plates. Equal amounts of lactate dehydrogenase assay substrate (SIGMA), enzyme and dye solution were mixed. A Half volume of the above mixture was added to one volume of medium supernatant. After incubating at room temperature for 30 min, the reaction was terminated by the addition of 1/10 volume of 1N HCl to each well. Spectrophotometrical absorbance was measured at a wavelength of 490 nm and reference wavelength of 690 nm.

2.6. Immunoprecipitation (IP)

Cells were harvested at 48 h post-transfection and lysed in HEPES lysis buffer (20 mM HEPES, pH 7.2, 50 mM NaCl, 0.5% Triton X-100, 1 mM NaF and 1 mM DTT) supplemented with protease inhibitor cocktail (Roche). Immunoprecipitations were performed using the indicated primary antibody and protein A/G-agarose beads (Santa Cruz) at 4 °C. Lysates and immunoprecipitates were examined using the indicated primary antibodies followed by detection with the related secondary antibody and the SuperSignal chemiluminescence kit (Pierce).

2.7. Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). Data analysis was performed by Student's *t* test or one-way ANOVAs followed by Mann–Whitney and Kruskal–Wallis tests. Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Wild type TDP-43 and FUS are associated with Drosha protein stability in Neuro 2A cells

Since depletion of wtTDP-43 is known to be involved in Drosha stability under retinoic acid treatment condition [15], we examined whether overexpressed wtTDP-43 could maintain Drosha stability. To investigate our question, we found that the Drosha protein level was suitably increased by transfected wtTDP-43 in Neuro 2A cells (Fig. 1A). To confirm that wtTDP-43 regulates mRNA level of Drosha, we performed quantitative real-time PCR (qRT-PCR) analysis and identified that Drosha mRNA did not show significant change under overexpressed wtTDP-43 (Fig. 1B). To determine Drosha stabilization, we carried out Cycloheximide (CHX) mediated protein degradation assay after wtTDP-43 transfection in Neuro 2A cells. When CHX (10 ug/ml) was treated for the indicated period of time, Drosha stability observed when wtTDP-43 is overexpressed was stronger than controls (Fig. 1C). It seems likely that the stability of Drosha protein correlated with wtTDP-43. According to recent reports, wtFUS also interacts with both wtTDP-43 and Drosha and regulates miRNA processing [8,15]. To determine whether wtFUS stabilizes Drosha, similar to that observed with wtTDP-43, we measured protein and mRNA levels of Drosha under overexpressed wtFUS condition. Drosha protein expression was moderately increased but Drosha mRNA expression was not changed by

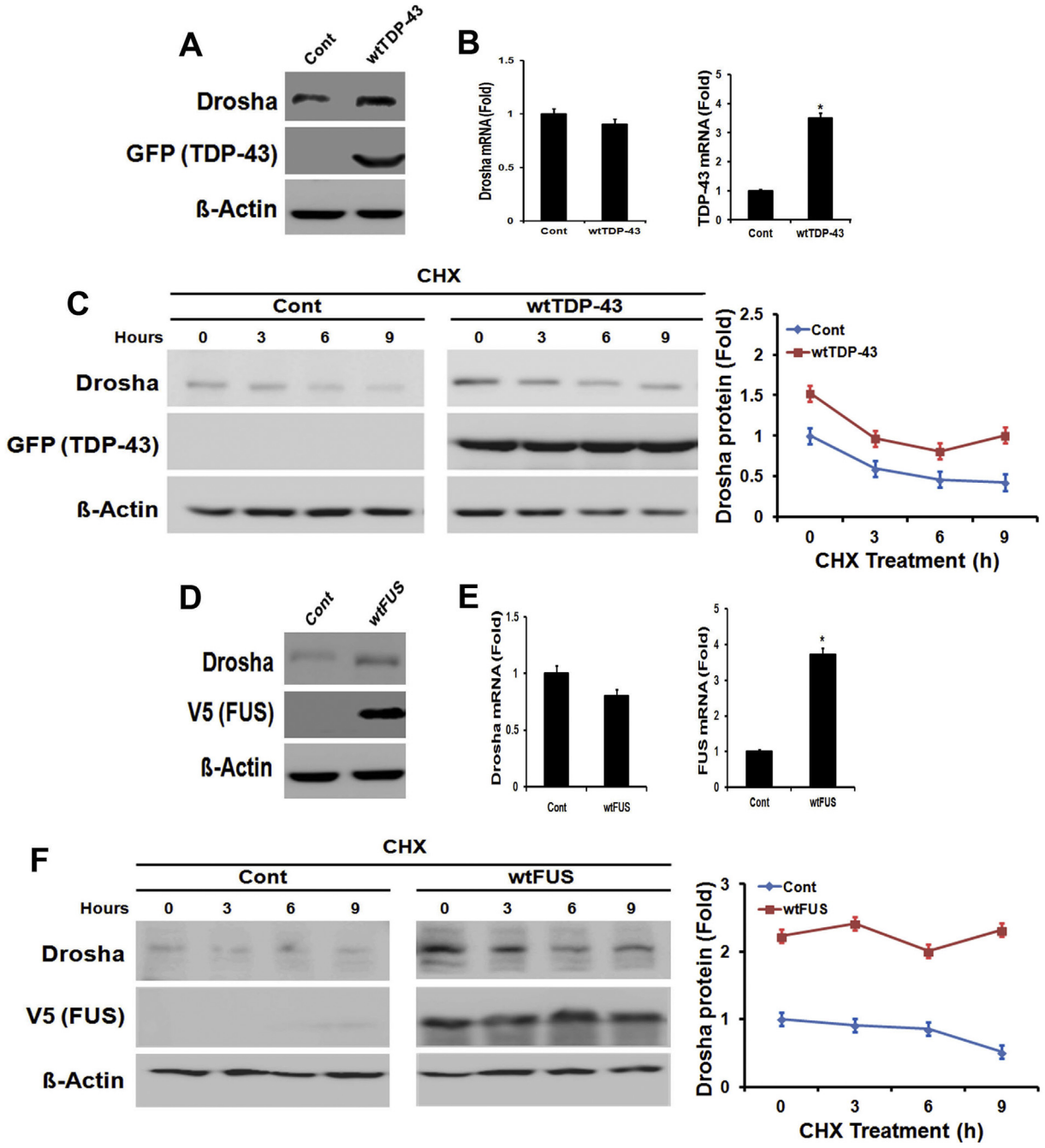

Fig. 1. wtTDP-43 and wtFUS regulate Drosha stability. (A) Overexpression of TDP-43 increased Drosha protein stability in Neuro 2A cells. (B) qRT-PCR analysis demonstrated that Drosha transcripts were not changed by transfected wtTDP-43. The data represent the average ± SEM of three separate experiments. (C) Western blot and quantification analysis showed that Drosha stability was increased by wtTDP-43 after 10 ug/ml of Cycloheximide (CHX) stimulation up to 9 h. (D) Overexpressed wtFUS stabilized Drosha. (E) qRT-PCR analysis showed that the mRNA of Drosha was not changed by overexpressed wtFUS. Significantly different at *, $p < 0.05$; **, $p < 0.005$. (F) Western blot and quantification analysis results represented that Drosha stability by wtFUS was higher that controls under treated CHX. All data represent the average ± SEM of five separate experiments.

overexpressed wtFUS (Fig. 1D, E). We also performed the CHX chase study under overexpressed wtFUS condition and found that Drosha stability was still stronger than controls (Fig. 1F). Because Drosha stability was induced by wtTDP-43 and wtFUS, we also co-transfected wtTDP-43 and wtFUS and treated the Nenro 2A cells with CHX for 9 h. We found that simultaneously overexpressed wtTDP-43 and wtFUS stabilized Drosha protein (Supplementary Fig. 1). These results suggested that wtTDP-43 and wtFUS affected the stability of the Drosha protein.

3.2. Double knock down of wtTDP-43 and wtFUS and mtTDP-43 (S409/410E) reduces the level of Drosha protein in Neuro 2A cells

To confirm that Drosha protein is stabilized by wtTDP-43 and wtFUS, we performed RNA interference (RNAi) experiments. Drosha stabilization was not changed at all when either wtTDP-43 or wtFUS were downregulated (Fig. 2A). We hypothesized that wtTDP-43 and wtFUS may cooperatively affect the stability of Drosha due to the stabilizing effects seen in the results of the CHX

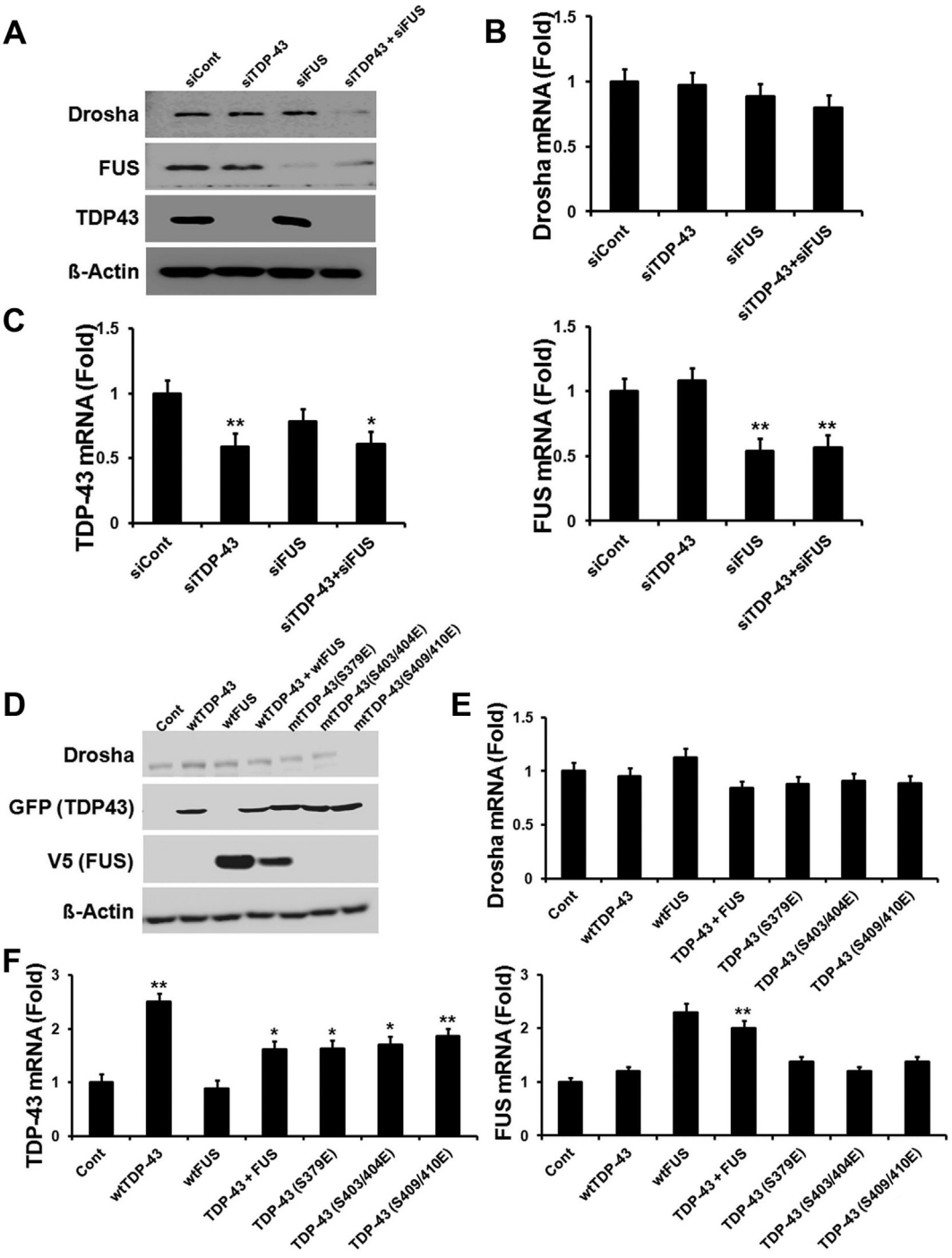

Fig. 2. Drosha protein stability regulated by double knock down of wtTDP-43 and wtFUS and TDP-43 (S409/410E). (A) Double knock down of wtTDP-43 and wtFUS decreased Drosha protein but decreased wtTDP-43 and wtFUS did not show significant change of Drosha protein in Neuro 2A cells. (B) qRT-PCR analysis showed that Drosha mRNAs did not change significantly by double knock down of wtTDP-43 and wtFUS. Significantly different at *, $p < 0.05$; **, $p < 0.005$. (C) qRT-PCR analysis showed down regulation of wtTDP-43 and wtFUS under siTDP-43 and siFUS overexpressed condition. (D) Western blot results showed that the Drosha slightly increased by wtTDP-43 and wtFUS but was significantly down regulated by TDP-43 (S409/410). (E) qRT-PCR analysis showed that the Drosha transcripts did not change by wtTDP-43, wtFUS and mutation of TDP-43 (S379E, S403/404E and S409/410). (F) qRT-PCR confirmed the over expression wtTDP-43, wtFUS and mutated TDP-43 (S379E, S403/404E and S409/410E). These data represent the average ± SEM of three separate experiments.

experiments performed when the two wild type genes (TDP-43 and FUS) were co-transfected into the Neuro 2A cells (Supplementary Fig. 1). As expected, the simultaneous down regulation of wtTDP-43 and wtFUS dramatically reduced the Drosha protein level (Fig. 2A), However, the Drosha mRNA level was not reduced (Fig. 2B). The reduced levels of wtTDP-43 and wtFUS were confirmed by qRT-PCR (Fig. 2C). Because abnormal TDP-43 such as phosphorylated TDP-43 (ser379, ser403/404 and ser409/410) has been detected in ALS and FTD-U [17], we investigated the correlation between abnormal phosphorylation of TDP-43 and Drosha. To

investigate this, we generated phosphomimetic mutants of TDP-43 (S379E, S403/404E and S409/410E) (Supplementary Fig. 2), we transfected wtTDP-43, wtFUS and tree mutants of TDP-43 (S379E, S403/404E and S409/410E) into the Neuro 2A cells. While both wtTDP-43 and wtFUS stabilized Drosha protein, two of the TDP-43 mutants (S379E and S403/404E) did not affect Drosha stability and TDP-43 mutant (S409/410E) dramatically decreased Drosha stability (Fig. 2D). We carried out qRT-PCR to verify the variation in Drosha transcripts when expressed with phosphomimetic mutants of TDP-43 (S379E, S403/404E and S409/410E) and other genes (wtTDP-43 and wtFUS). qRT-PCR analysis confirmed that Drosha mRNA level was not significantly changed by overexpression of these genes (wtTDP-43, wtFUS, S379E, S403/404E and S409/410E) (Fig. 2E). The exogenous induction of wtTDP-43, wtFUS and mutated TDP-43 (S379E, S403/404E and S409/410E) in Neuro 2A cells was confirmed by qRT-PCR (Fig. 2F).

3.3. TDP-43 (S409/410E) reduces the Drosha protein level and disturbs the protein–protein interaction between Drosha and wtFUS in Neuro 2A cells

To determine a specific correlation mechanism between TDP-43 (S409/410E) and Drosha, we verified the interaction between Drosha and TDP-43 (S409/410E) by using Co-immunoprecipitation analysis. We confirmed that wtTDP-43 and wtFUS interacted with Drosha and that two mutants (S379E and S403/404E) also bound to Drosha and wtFUS, but, remarkably, found that TDP-43 (S409/410E) did not interact with Drosha and wtFUS (Fig. 3A). In order to

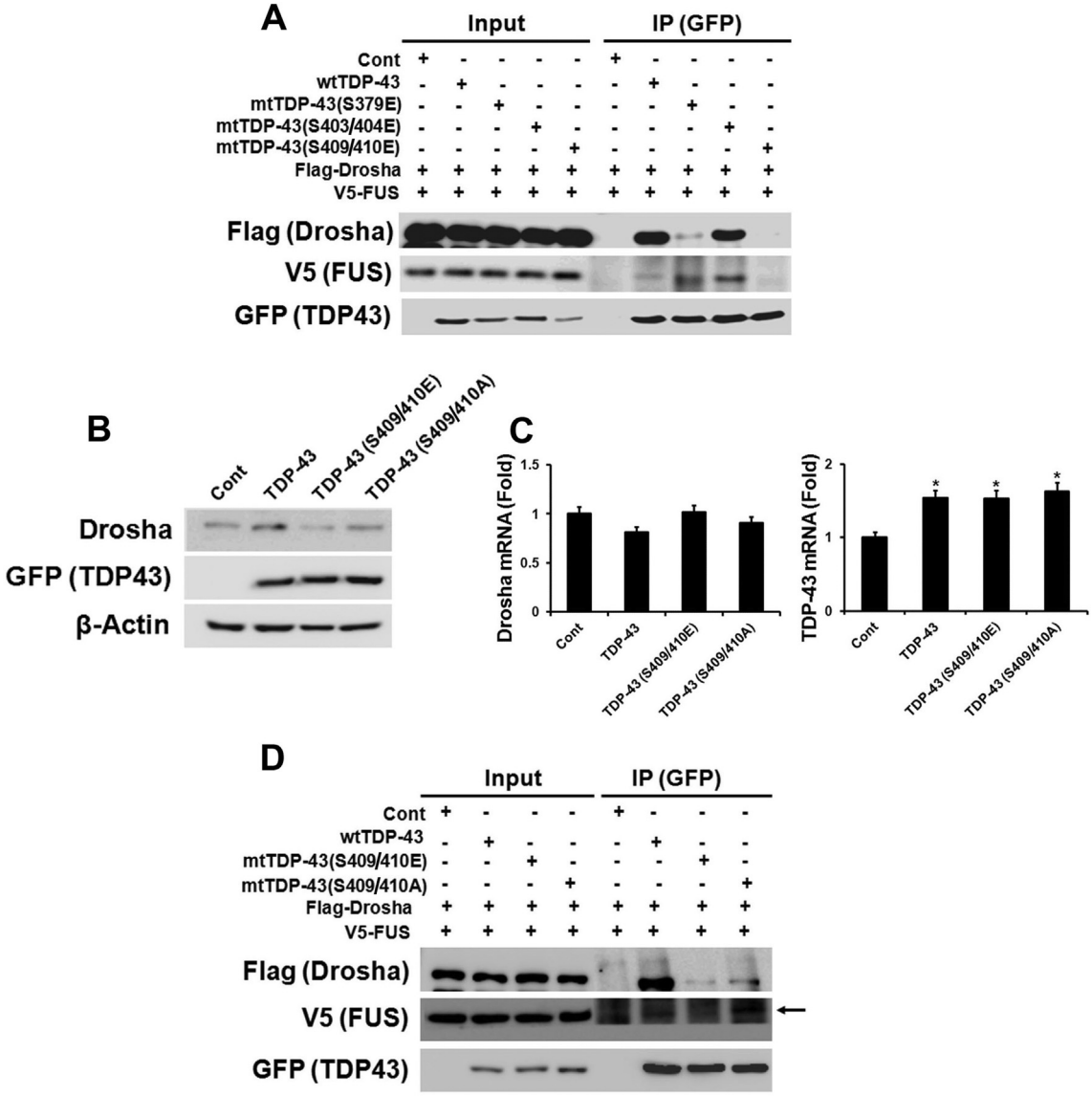

Fig. 3. Protein–protein interaction of Drosha and wtFUS is disturbed by TDP-43 (S409/410E). (A) Co-immunoprecipitation (Co-IP) results showed that Drosha protein associated with wtFUS and mutated TDP-43 (S379E and S403/404E) but did not bind to wtFUS and TDP-43 (S409/410E). (B) Western blot showed that TDP-43 (S409/410E) destabilized Drosha and a non-phosphorylatable mutation of TDP-43 (S409/410A) was not decreased Drosha protein. (C) qRT-PCR analysis demonstrated that TDP-43, TDP-43 (S409/410E) and TDP-43 (S409/410A) did not change the mRNA levels of Drosha. Significantly different at *, $p < 0.05$; **, $p < 0.005$. (D) Co-IP analysis showed that Drosha and wtFUS were associated with wtTDP-43 and TDP-43 (S409/410A). Interaction of Drosha and wtFUS was prevented by TDP-43 (S409/410E). An arrow represents wtFUS. All data represent the average ± SEM of five separate experiments.

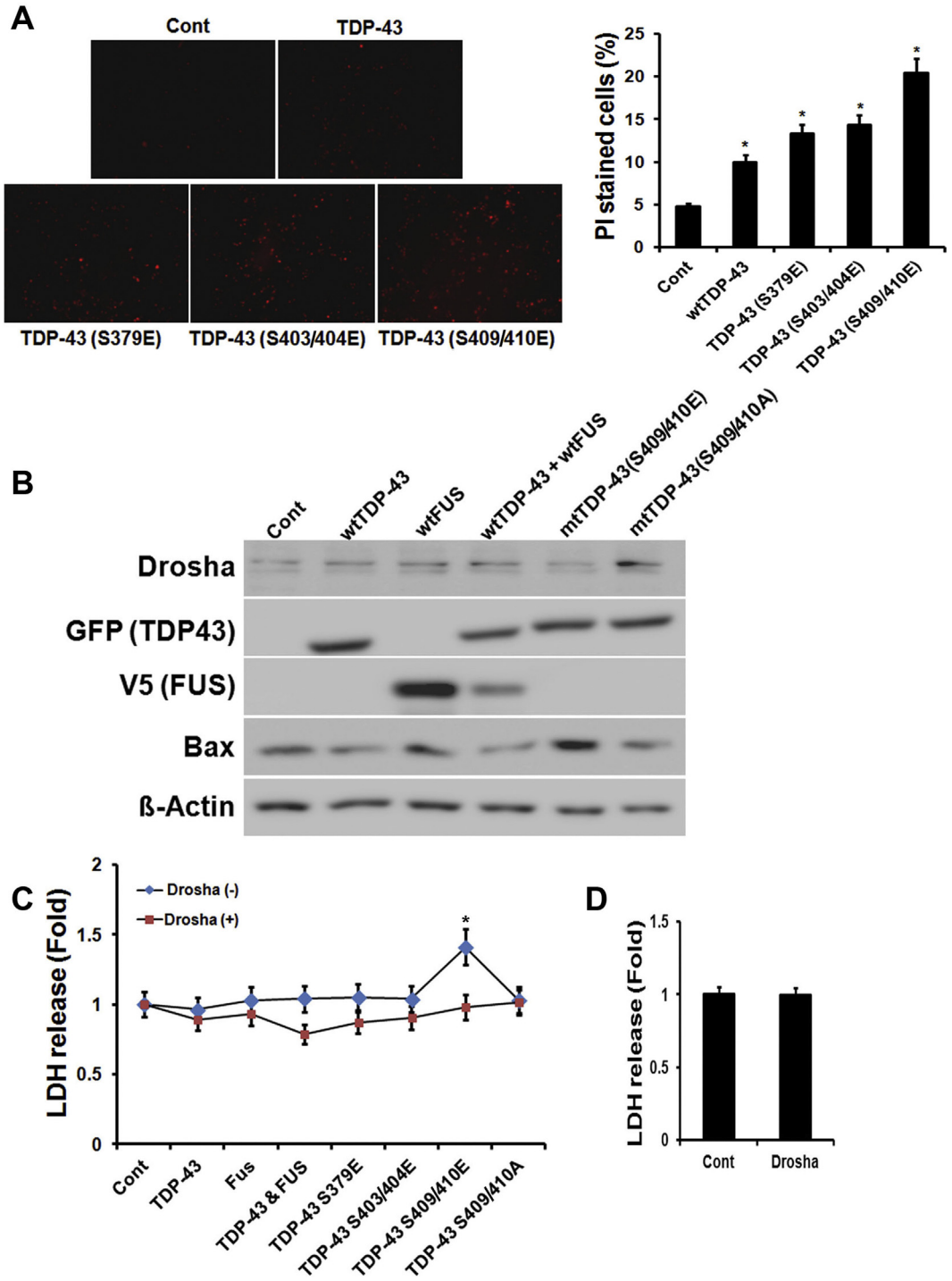

Fig. 4. TDP-43 (S409/410E) generates cytotoxicity and down regulates Drosha protein in Neuro 2A cells. (A) Propidium iodide (PI) staining showed that transfected TDP-43 (S409/410E) most strongly induced cytotoxicity. (B) Western blot data showed that Bax was increased by TDP-43 (S409/410E), but other constructs (Controls, wtTDP-43, wtFUS and TDP-43 (S409/410A)) did not show a significant change. (C) Lactate dehydrogenase (LDH) release analysis showed that wtTDP-43, wtFUS and mutated TDP-43 (S379E, S404/404E, and S409/410A) did not induce cytotoxicity, but TDP-43 (S409/410E) induced cytotoxicity. Simultaneously increased Drosha and TDP-43 mutants rescued the induction of TDP-43 (S409/410E) cytotoxicity. (D) Overexpressed Drosha did not show any cytotoxicity. Significantly different at *, $p < 0.05$; **, $p < 0.005$. These data represent the average ± SEM of three separate experiments.

determine whether the phosphorylated TDP-43 (S409/410E) is responsible for the interaction with Drosha, we developed a TDP-43 mutant (S409/410A) that produces protein in a non-phosphorylatable state (Supplementary Fig. 2). The ectopic over-expression of TDP-43 (S409/410A) did not show any variation in Drosha protein and transcripts (Fig. 3B, C). Co-immunoprecipitation results showed that only TDP-43 (S409/410E) did not associate with Drosha and wtFUS, while wtTDP-43 and TDP-43 (S409410A) still interacted with Drosha and wtFUS (Fig. 3D). This result explained the mechanism underlying Drosha stability, as the results are consistent with the results of the double knock down of wtTDP-43 and wtFUS, which resulted in a decreased level of Drosha protein

(Fig. 2A). Altogether, these results indicate that the phosphomimetic mutant of TDP-43 (S409/410E) not only decrease the Drosha protein level, but also interfered with the protein–protein interaction between itself and wtFUS and Drosha.

3.4. TDP-43 (S409/410E) destabilizes Drosha protein and generates cytotoxicity in Neuro 2A cells

Previous studies have suggested that the hyperphosphorylated TDP-43 shows abnormal cytoplasmic aggregations and causes cytotoxicity in ALS and FTLD-U [17,18]. To test whether expression of TDP-43 mutants was also toxic to neuronal cells, we transfected Neuro 2A cells with TDP-43 (S379E, S403/404E and S409/410E) and control constructs. We employed propidium iodide (PI) staining to validate cell death. We found that rate of cell death with the hyperphosphorylated TDP-43 (S409/410E) was higher than that in others (Fig. 4A). To confirm that TDP-43 (S409/410E) enhanced cytotoxicity, we analyzed the protein levels of Bax, one of the apoptosis-inducing proteins, under conditions in which mutated TDP-43 (S409/410E) is overexpressed. Interestingly, Bax was significantly increased in TDP-43 (S409/410E) (Fig. 4B). Because Drosha protein level was reduced by TDP-43 (S409/410E), reduction in Drosha protein level by TDP-43 (S409/410E) might be related with cell death. To further confirm whether Drosha is involved in cell death induced by TDP-43 (S409/410E), we measured lactate dehydrogenase (LDH) release in Neuro 2A cells with overexpressed TDP-43 mutants with or without Drosha. As expected, TDP-43 (S409/410E) stimulated cell death but overexpression of Drosha remarkably rescued the cytotoxicity of TDP-43 (S409/410E) in Neuro 2A cells (Fig. 4C). The ectopic overexpression of Drosha did not show significant the cytotoxicity (Fig. 4D). These results indicate that destabilized Drosha protein by TDP-43 (S409/410E) is associated with increased cell death.

4. Discussion

WtTDP-43 and wtFUS have many functional and structural similarities, as they are both DNA/RNA binding proteins involved in RNA metabolism [1,19]. Mutations TDP-43 and FUS stimulate aggregate formation and cause neurodegeneration in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) [1,19]. Nonetheless, the mechanisms underlying abnormal function of TDP-43 and FUS are currently unknown. In the present study, we found that the level of Drosha protein, one of the most important miRNA processing molecules [20], is slightly increased as a result of wtTDP-43 and wtFUS activation. Among our other finding, we also identified that Drosha protein level was dramatically decreased by simultaneous loss of wtTDP-43 and wtFUS in Neuro 2A cells. The mRNA levels of Drosha did not significantly change under gain- and loss of wtTDP-43 and wtFUS. Our CHX experiment results showed that Drosha protein stability mediated by wtTDP-43 and wtFUS is stronger than that observed in controls and that simultaneously overexpressed wtTDP-43 and wtFUS mediate high stability of Drosha. These results led us to hypothesize that TDP-43 mutation in ALS and FTLD-U may affect the stability of Drosha.

Mutations in TDP-43 and FUS are among the most critical causes of neurodegenerative diseases [1]. Recently hyperphosphorylated TDP-43 (S379, S403/404, and S409/410) was detected in ALS patients [17,21]. FUS mutation in ALS has also been reported; it abnormally increases interaction with SMN [22,23]. Overexpression of phosphomimetic TDP-43 (S409/410E) significantly downregulated Drosha in Neuro 2A cells without variation in Drosha transcripts. Our study indicates that the protein–protein interaction of Drosha and wtFUS is reduced by TDP-43 (S409/410E). It suggests that mutant TDP-43 (S409/410E) not only disturbs association of Drosha and wtFUS, but also destabilizes Drosha protein.

Most TDP-43 mutants stimulate cytotoxicity which induces cell death and regulate miRNA processing in ALS [24–30]. In our experiments, the levels of Bax, which is an apoptosis-inducing protein, were highly increased by overexpressed TDP-43 (S409/410E) in Neuro 2A cells. LDH release assay also showed that mutant TDP-43 (S409/410E) significantly induced apoptotic cell death. Importantly, cells damage caused by mutant TDP-43 (S409/410E) was rescued by overexpressed Drosha. These results suggested that the phosphomimetic mutant TDP-43 (S409410E) induces Drosha protein destabilization and cell death in Neuro 2A cells.

In summary, our findings provide several potential mechanistic evidences. First, Drosha protein stability was regulated by TDP-43 and FUS. Second, the interaction of Drosha and FUS was disturbed by a phosphomimetic mutant TDP-43 (S409/410E). Last, apoptotic cell death in Neuro 2A cells was induced by mutant TDP-43 (S409/410E). The mechanism(s) by which the phosphomimetic TDP-43 (S409/410E) dysregulates Drosha stability and induces cell death needs to be further examined. Identifying the abnormally processed miRNAs will explain additional roles of mutant TDP-43 (S409/410E) in cytotoxicity in different and yet undescribed cellular processes.

Acknowledgements

We thank Dr. V. Narry Kim for providing Drosha plasmid. This study was supported by grants from the Korea Healthcare Technology R&D project, Ministry of Health and Welfare, Republic of Korea (HI13C1334 and HI14C3347)

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.06.125.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.06.125.

References

[1] C. Lagier-Tourenne, D.W. Cleveland, Rethinking ALS: the FUS about TDP-43, Cell 136 (2009) 1001–1004.
[2] D.R. Rosen, Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis, Nature 363 (1993) 59–62.
[3] N. Ticozzi, C. Vance, A.L. Leclerc, P. Keagle, J.D. Glass, D. McKenna-Yasek, P.C. Sapp, V. Silani, D.A. Bosco, C.E. Shaw, R.H. Brown Jr., J.E. Landers, Mutational analysis reveals the FUS homolog TAF15 as a candidate gene for familial amyotrophic lateral sclerosis, Am. J. Med. Genet. B Neuropsychiatr. Genet. 3 (2011) 285–290.
[4] J. Couthouis, M.P. Hart, R. Erion, O.D. King, Z. Diaz, T. Nakaya, F. Ibrahim, H.J. Kim, J. Mojsilovic-Petrovic, S. Panossian, C.E. Kim, E.C. Frackelton, J.A. Solski, K.L. Williams, D. Clay-Falcone, L. Elman, L. McCluskey, R. Greene, H. Hakonarson, R.G. Kalb, V.M. Lee, J.Q. Trojanowski, G.A. Nicholson, I.P. Blair, N.M. Bonini, V.M. Van Deerlin, Z. Mourelatos, J. Shorter, A.D. Gitler, Evaluating the role of the FUS/TLS-related gene EWSR1 in amyotrophic lateral sclerosis, Hum. Mol. Genet. 13 (2012) 2899.
[5] T.J. Kwiatkowski Jr., D.A. Bosco, A.L. Leclerc, E. Tamrazian, C.R. Vanderburg, C. Russ, A. Davis, J. Gilchrist, E.J. Kasarskis, T. Munsat, Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis, Science 323 (2009) 1205–1208.
[6] Yuki Inukai, Takashi Nonaka, Tetsuaki Arai, M. Yoshida, Y. Hashizume, T.G. Beach, E. Buratti, F.E. Baralle, H. Akiyama, S. Hisanaga, M. Hasegawa, Abnormal phosphorylation of Ser409/410 of TDP-43 in FTLD-U and ALS, FEBS Lett. 582 (2008) 2899–2904.
[7] J. Sreedharan, I.P. Blair, V.B. Tripathi, X. Hu, C. Vance, B. Rogelj, S. Ackerley, J.C. Durnall, K.L. Williams, E. Buratti, F. Baralle, J. de Belleroche, J.D. Mitchell, P.N. Leigh, A. Al-Chalabi, C.C. Miller, G. Nicholson, C.E. Shaw, TDP-43

mutations in familial and sporadic amyotrophic lateral sclerosis, Science 319 (2008) 1668–1672.
[8] C. Colombrita, E. Onesto, F. Megiorni, A. Pizzuti, F.E. Baralle, E. Buratti, V. Silani, A. Ratti, TDP-43 FUS RNA binding proteins bind distinct sets of cytoplasmic messenger RNAs and differently regulate their post-transcriptional fate in motoneuron-like cells, J. Biol. Chem. 287 (2012) 15635–15647.
[9] Clotilde Lagier-Tourenne, Magdalini Polymenidou, Don W. Cleveland, TDP-43 and FUS/TLS. Emerging roles in RNA processing and neurodegeneration, Hum. Mol. Genet. 19 (2010) R46–R64.
[10] Manuela Neumann, Linda K. Kwong, Edward B. Lee, E. Kremmer, A. Flatley, Y. Xu, M.S. Forman, D. Troost, H.A. Kretzschmar, J.Q. Trojanowski, V.M. Lee, Phosphorylation of S409410 of TDP-43 is a consistent feature in all sporadic and familial forms of TDP-43 proteinopathies, Acta Neuropathol. 117 (2009) 137–149.
[11] A. Lujambio, S.W. Lowe, The microcosmos of cancer, Nature 482 (2012) 347–355.
[12] H.I. Im, P.J. Kenny, MicroRNAs in neuronal function and dysfunction, Trends Neurosci. 35 (2012) 325–334.
[13] J. Han, Y. Lee, KH. Yeom, JW. Nam, I. Heo, JK. Rhee, SY. Sohn, Y. Cho, BT. Zhang, VN. Kim, Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex, Cell. 125(206) 887–901.
[14] J. Han, J.S. Pedersen, S.C. Kwon, C.D. Belair, Y.K. Kim, K.H. Yeom, W.Y. Yang, D. Haussler, R. Blelloch, V.N. Kim, Posttranscriptional crossregulation between Drosha and DGCR8, Cell 136 (2009) 75–84.
[15] R.I. Gregory, K.P. Yan, G. Amuthan, T. Chendrimada, B. Doratotaj, N. Cooch, R. Shiekhattar, The microprocessor complex mediates the genesis of microRNAs, Nature 432 (2004) 235–240.
[16] V. Di Carlo, E. Grossi, P. Laneve, M. Morlando, S. Dini Modigliani, M. Ballarino, I. Bozzoni, E. Caffarelli, TDP-43 regulates the microprocessor complex activity during in vitro neuronal differentiation, Mol. Neurobiol. 48 (2013) 952–963.
[17] M. Hasegawa, T. Arai, T. Nonaka, F. Kametani, M. Yoshida, Y. Hashizume, T.G. Beach, E. Buratti, F. Baralle, M. Morita, I. Nakano, T. Oda, K. Tsuchiya, H. Akiyama, Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis, Ann. Neurol. 64 (2008) 60–70.
[18] T. Nonaka, T. Arai, E. Buratti, F.E. Baralle, H. Akiyama, M. Hasegawa, Phosphorylated and ubiquitinated TDP-43 pathological inclusions in ALS and FTLD-U are recapitulated in SH-SY5Y cells, FEBS Lett. 583 (2009) 394–400.
[19] I.R. Mackenzie, R. Rademakers, M. Neumann, TDP-43 and FUS in amyotrophic lateralsclerosis and frontotemporal dementia, Lancet Neurol. 10 (2010) 995–1007.
[20] Y. Lee, C. Ahn, J. Han, H. Choi, J. Kim, J. Yim, J. Lee, P. Provost, O. Rådmark, S. Kim, V.N. Kim, The nuclear RNase III Drosha initiates microRNA processing, Nature 425 (2003) 415–419.
[21] T. Arai, I.R. Mackenzie, M. Hasegawa, T. Nonoka, K. Niizato, K. Tsuchiya, S. Iritani, M. Onaya, H. Akiyama, Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies, Acta Neuropathol. 117 (2009) 125–136.
[22] S. Sun, S.C. Ling, J. Qiu, C.P. Albuquerque, Y. Zhou, S. Tokunaga, H. Li, H. Qiu, A. Bui, G.W. Yeo, E.J. Huang, K. Eggan, H. Zhou, X.D. Fu, C. Lagier-Tourenne, D.W. Cleveland, ALS-causative mutations in FUS/TLS confer gain and loss of function by altered association with SMN and U1-snRNP, Nat. Commun. 27 (2015) 6171.
[23] A. Chari, E. Paknia, U. Fischer, The role of RNP biogenesis in spinal muscular atrophy, Curr. Opin. Cell. Biol. 21 (2009) 387–393.
[24] S. Herdewyn, C. Cirillo, L. Van Den Bosch, W. Robberecht, P. Vanden Berghe, P. Van Damme, Prevention of intestinal obstruction reveals progressive neurodegeneration in mutant TDP-43 (A315T) mice, Mol. Neurodegener. 17 (2014) 9–24.
[25] H. Dong, L. Xu, L. Wu, X. Wang, W. Duan, H. Li, C. Li, Curcumin abolishes mutant TDP-43 induced excitability in a motoneuron-like cellular model of ALS, Neuroscience 11 (2014) 141–153.
[26] S. Yan, C.E. Wang, W. Wei, M.A. Gaertig, L. Lai, S. Li, X.J. Li, TDP-43 causes differential pathology in neuronal versus glial cells in the mouse brain, Hum. Mol. Genet. 23 (2014) 2678–2693.
[27] Y. Kawahara, A. Mieda-Sato, TDP-43 promotes microRNA biogenesis as a component of the Drosha and Dicer complexes, Proc. Natl. Acad. Sci. U S A 109 (2012) 3347–3352.
[28] S.T. Warraich, S. Yang, G.A. Nicholson, I.P. Blair, TDP-43: a DNA and RNA binding protein with roles in neurodegenerative diseases, Int. J. Biochem. Cell. Biol. 42 (2010) 1606–1609.
[29] M. Abe, N.M. Bonini, MicroRNAs and neurodegeneration: role and impact, Trends Cell. Biol. 23 (2013) 30–36.
[30] N. Liu, M. Landreh, K. Cao, M. Abe, G.J. Hendriks, J.R. Kennerdell, Y. Zhu, L.S. Wang, N.M. Bonini, The microRNA miR-34 modulates ageing and neurodegeneration in Drosophila,, Nature 15 (2012) 519–523.