Research Communication

SecinH3 Attenuates TDP-43 p.Q331K-Induced Neuronal Toxicity by Suppressing Endoplasmic Reticulum Stress and Enhancing Autophagic Flux

Wentao Hu[1]
Xi Liu[1,2]
Shang Wang[1]
Guifang Sun[1]
Ran Zhao[1]
Hong Lu[1]*

[1]Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan Province, China
[2]Department of Anesthesiology/Critical Care Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal, adult-onset, neurodegenerative disease. The transactivating response region DNA binding protein 43 (TDP-43) p.Q331K mutation (TDP-43 Q331K) has previously been identified in ALS as a disease-causing mutation with neurotoxicity. SecinH3, a cytohesin inhibitor, has neuroprotective effects against mutant superoxide dismutase 1 (SOD1) toxicity. However, whether SecinH3 protects against mutant TDP-43 p.Q331K protein toxicity and its potential molecular mechanisms have not yet been investigated. To determine whether TDP-43 Q331K induces neuronal toxicity, SH-SY5Y, a human derived neuronal cell line were selected as an *in vitro* model of neuronal function. SH-SY5Y cells were transiently transfected with TDP-43 wild-type or TDP-43 Q331K. Remarkably, TDP-43 Q331K induced neuronal damage via endoplasmic reticulum (ER) stress-mediated apoptosis and the impairment of the autophagic flux. SecinH3 was demonstrated to successfully attenuate the TDP-43 Q331K-induced neuronal toxicity by suppressing ER stress-mediated apoptosis and enhancing the autophagic flux. Taken together, our *in vitro* study provided evidence that SecinH3 exerts neuroprotective effects against TDP-43 Q331K-mediated neuronal toxicity and was able to elucidate its mode of action. SecinH3 could, therefore, be considered a promising candidate as a therapeutic agent of ALS. © 2018 IUBMB Life, 9999(9999):1–8, 2018

Keywords: *amyotrophic lateral sclerosis; TDP-43; apoptosis; endoplasmic reticulum stress.*

Abbreviations: *ALS, amyotrophic lateral sclerosis; TDP-43, transactivating response region DNA binding protein 43; TDP-43 Q331K, TDP-43 p.Q331K mutation; WT, wild-type; ER, endoplasmic reticulum; UPR^{ER}, ER unfolded protein response; SOD1, superoxide dismutase 1; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; HRP, horseradish peroxidase; Bcl-2, B-cell lymphoma 2; GRP-78, 78 kDa glucose-regulated protein; PDI, protein disulfide isomerase; ATF4, activating transcription factor 4; CHOP, cell death regulator C/EBP homologous protein; LC3, microtubule-associated protein 1A/1B-light chain 3; PERK, protein kinase R-like endoplasmic reticulum kinase; eIF2α, eukaryotic initiation factor-2α*

Volume 9999, Number 9999, , Pages 1–8

*Address correspondence to: Hong Lu, Department of Neurology, The First Affiliated Hospital of Zhengzhou University, 1 Jianshe East Road, Zhengzhou, Henan Province 450052, China. Tel: +86–0371-66862092. E-mail: zzu_luhong@163.com

Received 16 June 2018; accepted 3 September 2018

DOI 10.1002/iub.1951

Published online 00 Month 2018 in Wiley Online Library (wileyonlinelibrary.com)

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal and incurable disease that is characterized by progressive neurodegeneration of motor neurons in the brain stem and spinal cord (1). The underlying mechanisms of motor neuron degeneration in ALS are still elusive. Despite numerous attempts to find effective treatments, Riluzole is still the only therapeutic option available for ALS, but it only modestly slows the disease progression (2). Therefore, there is a need to find new and effective therapies for ALS.

The transactivating response region DNA binding protein 43 (TDP-43) has been identified as a major protein constituent of inclusions in most patients with ALS (3). TDP-43 is predominantly located in the nucleus where it is involved in regulating RNA processing, including transcriptional regulation, splicing and microRNA processing (4). A previous study showed that overexpression of the wild-type (WT) TDP-43 affects the lethality and pathology and that

these effects can be enhanced by the pathogenic mutation (5, 6). Among them, the TDP-43 p.Q331K mutation (TDP-43 Q331K) is a very common gene mutation, which initiates the onset of ALS. When expressed in mice, the TDP-43 Q331K has been shown to provoke an age-dependent lower motor neuron disease and the associated progressive decline in motor performance and motor neuron death (7). Mice overexpressing the human TDP-43 WT developed no overt physical or pathological phenotype, while those overexpressing the human TDP-43 Q331K developed an age-dependent mild motor and pathological phenotype (8). Moreover, expression of the TDP-43 Q331K in NSC-34 cells resulted in abnormities of action potential initiation and voltage-gated sodium channel (9). Although a link between TDP-43 Q331K and neurodegenerative diseases has been evidenced by numerous studies, there is still no consensus on the definitive pathological role of TDP-43 Q331K in ALS.

ALS is mediated by aberrant protein homeostasis (i.e., ER stress and autophagy) and/or changes in RNA processing (10). The ER unfolded protein response (UPR^{ER}), whose activation triggered by ER stress, alleviates stress through increasing the protein folding ability, inhibiting general protein translation and promoting the degradation of misfolded proteins. However, if this response is inadequate, then cell death becomes inevitable (11). TDP-43 is inherently aggregation-prone and has a tendency to form toxic aggregates, especially in the presence of ALS-related mutations (12). As an ER stress inhibitor, methylene blue is considered to have a neuroprotective role for its potent suppression of TDP-43 toxicity. Similarly, three additional compounds, salubrinal, guanabenz, and a novel compound phenazine, have now also been shown to protect against mutant human TDP-43 toxicity by reducing the ER stress response (2). Several studies have demonstrated that impaired scavenging function of autophagosomes may be observed in Alzheimer's, Parkinson's, and Huntington's diseases (13). Autophagic changes occur mainly in the motor neurons in the spinal cords of ALS mice at a relatively early stage. Selective motor neuron degeneration of ALS may be attributed to the impairment of the autophagic pathway (14). SecinH3 is a cytohesin inhibitor that has neuroprotective effects against mutant superoxide dismutase 1 (SOD1) toxicity (15). However, whether SecinH3 protects against mutant TDP-43 p.Q331K protein toxicity and its potential molecular mechanisms have not yet been explored.

In this study, we demonstrated that TDP-43 Q331K induced neuronal damage via ER stress-mediated apoptosis and the impairment of the autophagic flux in SH-SY5Y cells. Furthermore, we also demonstrated that SecinH3, a cytohesin inhibitor, has neuroprotective effects on mutant TDP-43 p.Q331K-mediated neuronal toxicity. Our findings indicate that SecinH3 attenuated TDP-43 Q331K-induced neuronal toxicity by suppressing ER stress-mediated apoptosis and enhancing autophagic flux. Therefore, SecinH3 could be served as one of a promising therapeutic strategy of ALS.

MATERIALS AND METHODS

Cell Culture, Transfection, and Drug Treatment

SH-SY5Y cells were cultured in Dulbecco's modified Eagle's medium (GIBCO, Carlsbad, CA) supplemented with 10% fetal bovine serum (PAN Biotech, Adenbach, GER) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA). Cells were incubated at 37 °C with 5% CO_2. The mammalian expression plasmids pEGFP-TDP43 WT and pEGFP-TDP43 p.Q331K were constructed. cDNA sequences were based on the accession number NM_007375.3 for human TARDBP. The WT and mutant gene constructs were inserted into the pEGFP-C1 vector for expression of human WT or mutant TDP-43 protein by cloning. Custom mutagenesis to generate the Q331K (point mutation c.991C > A) TDP-43 amino acid substitutions were carried out (16). SecinH3 was purchased from Selleck (Houston, TX) and was dissolved in vehicle (DMSO). Plasmids were transiently transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA) at a cell density of 40% confluency in opti-DMEM medium. To determine the transfection rate, 48 h later, the transfected cell were visualized by an epifluorescence microscope (iX 83 Olympus, Japan). Sixteen hours after transfection, the medium was replaced with fresh complete DMEM medium. After transient transfection, the cells were conditioned with different dosages of SecinH3 at a final concentration of 10, 20, 50, 150 μM or with vehicle for 48 h before harvest (15). Cells only transfected with empty vector and only received equal volume of vehicle treatment served as a control.

MTT and LDH Assays

The cell viability was estimated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay as previously described (17). In brief, after condition with SecinH3, the treated and untreated transfected cells were added with 0.5 mg mL^{-1} MTT (Sigma-Aldrich, St. Louis, MO) and incubated at 37 °C for another 4 h. After aspirating the MTT-containing media, DMSO was added into the well and the optical density was measured at 568 nm with a colorimetric. Lactate dehydrogenase (LDH), as a marker for cytotoxicity, was analyzed by a LDH cytotoxicity assay kit (Beyotime, China) according to the manufacturer's instructions. Three independent experiments from three batch of transfected cell/well were measured.

Flow Cytometry Assay

Both the SecinH3 treated and untreated transfected cells were washed twice with 1 × PBS on ice. Cell apoptosis was detected using the Annexin V-APC/7-AAD apoptosis detection kit (keyGEN BioTECH, China) according to the manufacturer's instructions. Samples were analyzed with a BD Calibur flow cytometry (BD Biosciences).

Western Blotting

Cells were first washed with ice cold PBS twice before lysed in RIPA buffer (Beyotime Biotechnology, China). The cells were homogenized with a sonicator system (Qsonica, Newtown, CT) on ice. Cell lysate was then centrifuged at 3,000 rcf for 5 min

and subjected to estimate the protein content with a bicinchoninic acid assay. The proteins were subsequently denatured in loading buffer by boiling 5 min and equal amounts of total protein were loaded and separated by SDS-PAGE. After transferring the protein to nitrocellulose membranes, the blots were first blocked with 5% nonfat dry milk in TBST (0.25% Triton X-100 in PBS, pH 7.4) for 2 h and then incubated with the designated primary antibodies for overnight at 4 °C. After several washes in TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary bodies, for example, anti-rabbit IgG (Boster, China) or antimouse IgG (Boster, China) at 1:1,000 dilution for another 2 h at room temperature. Membranes were washed three times in PBS and the specific protein bands were visualized by ECL treatment (Thermo, Waltham, MA). The primary antibodies used were: mouse polyclonal anti-B-cell lymphoma 2 (Bcl-2) antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal anti-Bcl-xL antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal cleaved-caspase-3 antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal anti-78 kDa glucose-regulated protein (GRP-78) (1:500 dilution, Abcam, UK), rabbit polyclonal antiprotein disulfide isomerase (PDI) antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal antiactivating transcription factor 4 (ATF4) antibody (1:1,000 dilution, Cell Signaling Technology), mouse polyclonal anticell death regulator C/EBP homologous protein (CHOP) antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal anti-caspase-12 antibody(1:300 dilution, Abcam, UK), rabbit polyclonal anti-Beclin1 antibody (1:1,000 dilution, Cell Signaling Technology), rabbit polyclonal anti-microtubule-associated protein 1A/1B-light chain 3 (LC3) antibody (1:1,000 dilution, Cell Signaling Technology), mouse monoclonal anti-p62 antibody (1:1,000 dilution, Cell Signaling Technology) or mouse monoclonal anti-β-actin antibody (1:200 dilution, Boster, China). Band intensity was quantified with image J software (NIH, Bestheda). Protein expression levels were normalized by their bands intensity to that of internal control.

Statistical Analysis

All data were expressed as the mean ± standard deviation (SD) and was analyzed by the SPSS statistical software (SPSS version 16.0). The statistical significance of differences between experimental groups was evaluated by one-way analysis of variance (ANOVA) and Tukey post hoc test. A P value <0.05 was considered statistically significant.

RESULTS

SecinH3 Attenuated the TDP-43 Q331K Overexpression Induced Neuronal Toxicity

To determine whether TDP-43 Q331K causes neuronal toxicity, the SH-SY5Y cells were transiently transfected with TDP-43 WT or TDP-43 Q331K plasmid. EGFP signal can be visualized in SH-SY5Y cells 48 h after transfected with TDP-43 WT and TDP-43 Q331K which label tagged with EGFP at the N-terminus (Fig. 1). MTT assays were used to compare the neuronal toxicity of the wild type and mutant, with and without SecinH3 treatment. As shown in Fig. 2A, compared to cells expressing TDP-43 WT and the controls, the cell viability of the SH-SY5Y cells was significantly reduced when TDP-43 Q331K was overexpressed. However, the cell viability of the TDP-43 Q331K transfected cells was rescued in the presence of different doses of SecinH3 than that of vehicle treated group. Among the different dosages, 50 μM shown to be the optimal one. In addition, to confirm our findings, we investigated the neuronal death by measuring the release of LDH. Our data shown that LDH levels were higher in the cells expressing TDP-43 Q331K compared to the cells expressing wildtype TDP-43, which was rescued by SecinH3 with different doses. 50 μM SecinH3 was the optimal dose (Fig. 2B). Because cell apoptosis is an important factor that affects cell function, we further investigated the apoptosis

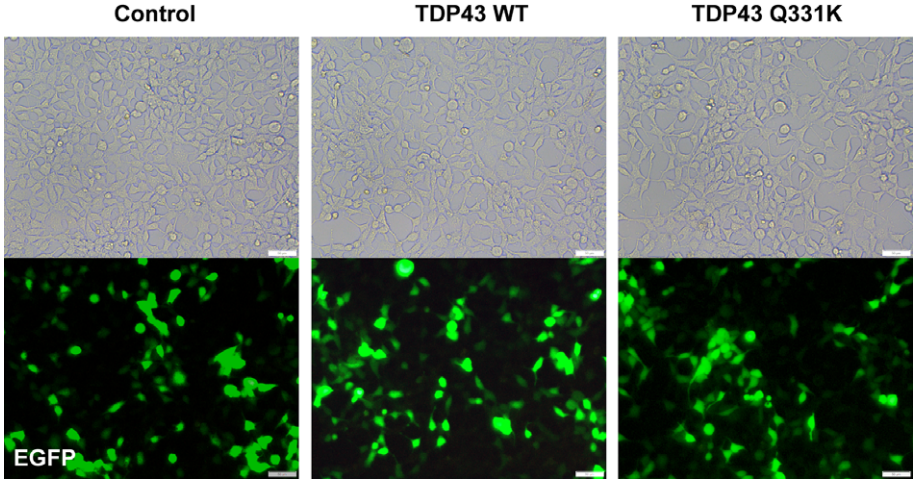

FIG 1 *Representitive image of SH-SY5Y cells after transfections. Upper panel shows phrase contrast image of SH-SY5Y cells. Lower panel shows fluorescence photography of EGFP to confirm the successive transfections, at the same position as the bright field pictures. Scale bar = 50 μm.*

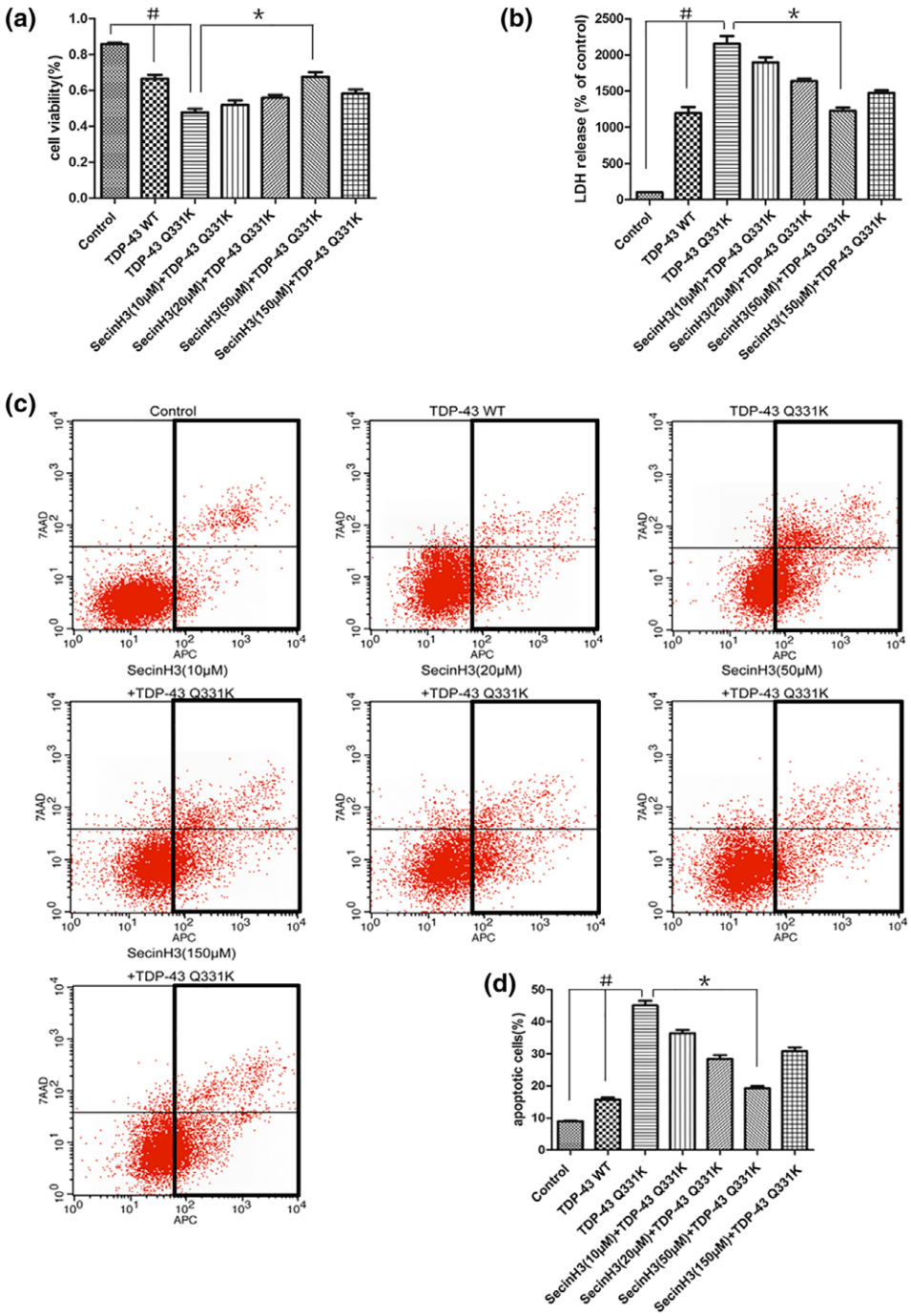

FIG 2

SecinH3 attenuated the TDP-43 Q331K over expression induced neuronal toxicity. A, Cell viability was measured with a MTT assay, quantification showed a decreased cells viability in cells transfected with TDP-43 Q331K, which was minimized by SecinH3. Different doses of SecinH3 were tested, 50 μM was the optimal dose. Data were collected from three independent experiments. (#*P < 0.05* vs.*TDP-43 WT and control group; ***P < 0.05* vs. *TDP-43 Q331K group). B, The release of LDH into the media was used as an indicator of neuronal toxicity. Quantitative results showed more death in cells transfected with TDP-43 Q331K, which was rescued by SecinH3 with different doses. 50 μM SecinH3 was the optimal dose. Data were collected from three independent experiments. (*#*P < 0.05* vs.*TDP-43 WT and control group; ***P < 0.05* vs. *TDP-43 Q331K group). C, Neuronal apoptosis was detected with an annexin V-FITC/PI kit according to the manufacturer's instruction. The lower left quadrant contained intact cells, early-apoptotic cells are localized in the lower right quadrant, while necrotic or late-apoptotic cells are found in the upper right quadrant. D, The percentage of apoptosis was counted including early apoptosis and late apoptosis (located in the bold rectangle). Quantitative results showed an increase in cell apoptosis when transfected with TDP-43 Q331K, which was reduced by SecinH3 with different doses. 50 μM SecinH3 was the optimal dose. Data were collected from three independent experiments. (*#*P < 0.05* vs.*TDP-43 WT and control group; ***P < 0.05* vs. *TDP-43 Q331K group).*

levels of the cells with different treatment. The apoptotic cell count was higher in cells expressing TDP-43 Q331K compared with cells expressing TDP-43 WT or the control cells. SecinH3 treatment exhibited a decreased trend of apoptosis rate compare with vehicle treated group, among the other dosages, 50 μM of SecinH3 manifested a significant decrease of apoptosis rate than that of vehicle-treat group (Fig. 2C,D).

In addition, the neuronal toxicity of TDP-43 Q331K was further confirmed by comparing the expression levels of antiapoptotic and proapoptotic proteins by immunoblotting. Our data indicated that, compared with TDP-43 WT and controls, the cells overexpressing TDP-43 Q331K markedly suppressed the levels of the antiapoptotic proteins as Bcl-2 and Bcl-xL and upregulated proapoptotic protein levels of cleaved caspase-3. Interestingly, cells transfected with TDP-43 Q331K exposed with 50 μM of SecinH3 also significantly increased the expression of antiapoptotic and decreased the proapoptotic proteins to protect the SH-SY5Y cells against apoptosis compared with vehicle-treated group (Fig. 3A,B). Our results, suggested that SecinH3 can attenuated the TDP-43 Q331K over expression induced neuronal toxicity.

SecinH3 Relieves TDP-43 Q331K Induces ER Stress-Associated Apoptosis in Neuronal Cells

Mislocalization of TDP-43 from the nucleus to the cytoplasm is associated with cellular toxicity and an increase in cytoplasmic TDP-43 may initiate ER stress (18, 19). To investigate if the mutant form of TDP-43 have effect on ER stress, the expression levels of several key mediators of ER stress, including ER chaperone GRP-78, ATF4, CHOP, PDI, and the proapoptotic caspase-12 were determined in SH-SY5Y cells transfected with TDP-43 WT or TDP-43 Q331K. Our observations suggest that the expression of all of the above ER stress-mediated proteins were significantly upregulated when TDP-43 Q331K was overexpressed as compared to TDP-43 WT. Their expression levels was downregulated by a treatment with SecinH3 (50 μM), thereby relieving the TDP-43 Q331K induced ER stress-associated apoptosis (Fig. 4A,B). Taken together, the data clearly suggested that TDP-43 Q331K can induce neuronal cell death through the activation of ER stress-associated apoptosis. Also, SecinH3 (50 μM) was shown to be able to protect the cells from TDP-43 Q331K induced cell death by suppressing ER stress.

SecinH3 Rescued TDP-43 Q331K Induced Autophagic Flux Dysfunctions in Neuronal Cells

Autophagy is important for the scavenging of aggregated toxic proteins and the degradation of damaged organelles (20). Cellular levels of p62 inversely correlate with the autophagic flux and thus often be adopted to monitoring the autophagic flux changes (14). Moreover, p62 is a protein with dual-binding sites for LC3. To enable a comprehensive analysis of the autophagic flux, the protein levels of LC3-I, LC3-II, Beclin1, and p62 were analyzed. We found that compared to TDP-43 WT overexpression, overexpression of TDP-43 Q331K triggered the expression of Beclin1 and p62, as well as converted the expression levels of LC3-I and LC3-II in SH-SY5Y cells. Furthermore, by treating the TDP-43 Q331K transfected cells with SecinH3, the p62 protein levels decreased significantly (Fig. 5A,B). Our data, taken together, suggested that overexpressing TDP-43 Q331K impaired cell autophagic flux function and SecinH3 treatment reversed the TDP-43 Q331K induced autophagic flux dysfunctions in neuronal cells.

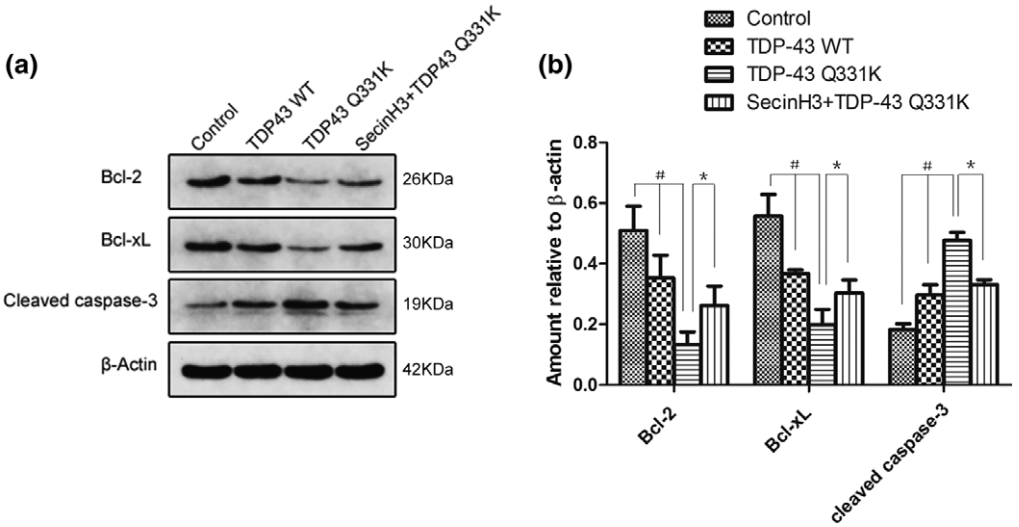

FIG 3 *SecinH3 relieves TDP-43 Q331K induces ER stress-associated apoptosis in neuronal cells. A, Expression levels of apoptosis associated protein by Western blotting after plasmid transfection and drug treatment. Bcl-2, Bcl-xl, and cleaved caspase-3 were detected by their antibodies and β-actin served as an internal control. B, Quantification showed that TDP-43 Q331K transfected cells had significantly higher cleaved caspase-3 levels and decreased Bcl-2 and Bcl-xL expression levels compared to TDP-43 WT transfected cells and control cells. 50 μM SecinH3 rescued the TDP-43 Q331K phenotype. Values are shown as the mean ± SD from three independent experiments. (#P < 0.05 vs. TDP-43 WT and control group; *P < 0.05 vs. TDP-43 Q331K group).*

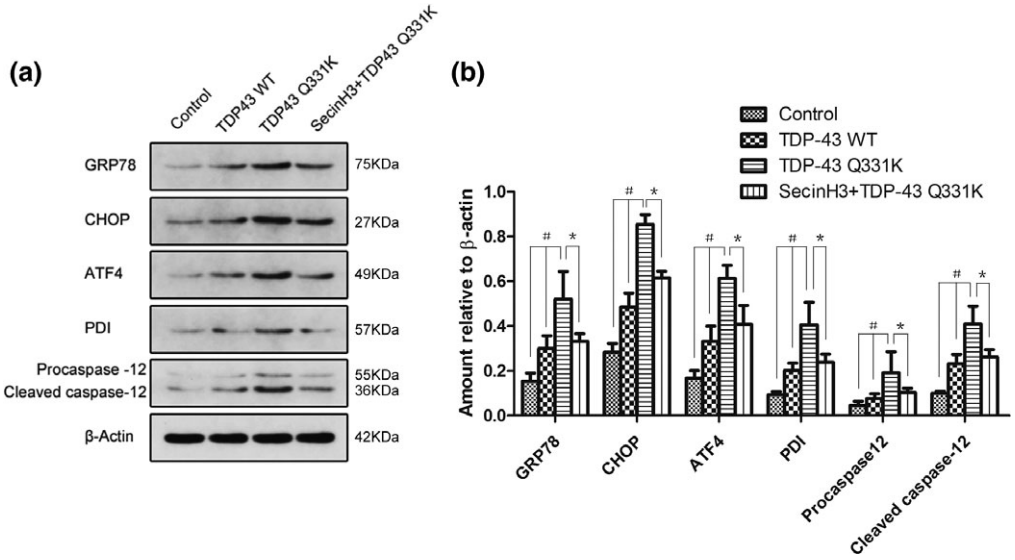

FIG 4 *TDP-43 Q331K evokes ER stress-associated apoptosis and this is relieved by SecinH3. A, Expression levels of apoptosis associated protein by Western blotting after plasmid transfection and drug treatment. GRP78, PDI, AFT4, CHOP, and caspase-12 were detected by their antibodies and β-actin served as an internal control. B, Quantification showed that TDP-43 Q331K transfected cells displayed significantly higher GRP78, PDI, AFT4, CHOP, and caspase-12 levels in comparison to TDP-43 WT transfected cells and control cells. 50 μM SecinH3 rescued the TDP-43 Q331K phenotype. Values are shown as the mean ± SD from three independent experiments. (#P < 0.05 vs. TDP-43 WT and control group; *P < 0.05 vs. TDP-43 Q331K group).*

DISCUSSION

To date, ALS is an incurable devastating disease. The only approved drug for ALS treatment is Riluzole, but it has little effect on symptom relief or even on extending patient survival time (21). TDP-43 is considered to be the major component of the protein aggregates that are characteristic of most forms of ALS (22). Mutations in the TDP-43 gene are linked to sporadic and non-SOD1 familial ALS. These mutations induce the cytoplasmic mislocalization of TDP-43 protein and thus lead to cytoplasmic TDP toxicity (16, 18, 23–26). In this study, the

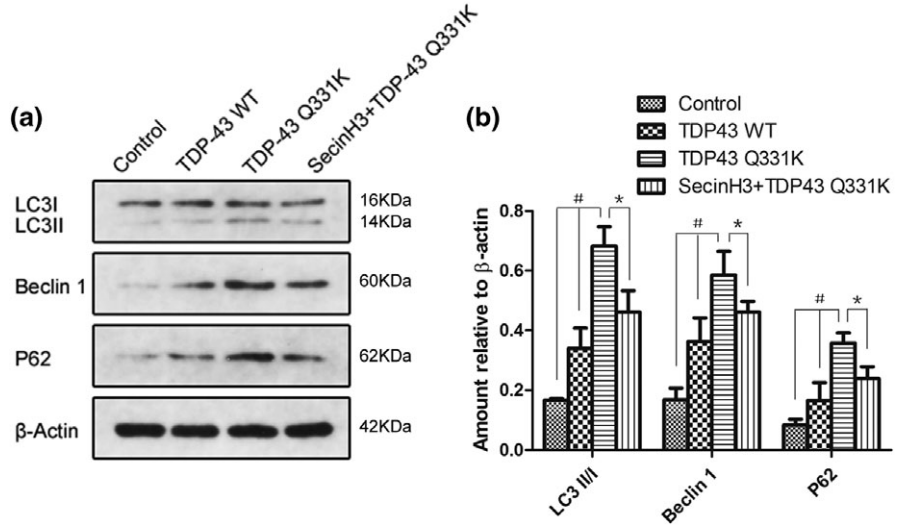

FIG 5 *SecinH3 rescued TDP-43 Q331K induced autophagic flux dysfunctions. A, Western blotting was performed using lysates of SH-SY5Y cells transfected with TDP-43 WT or TDP-43 Q331K for 16 h, followed by a treatment with 50 μM SecinH3 for 48 h. Expression levels of apoptosis associated protein by Western blotting after plasmid transfection and drug treatment. LC3II/LC3I, Beclin1, and p62 were detected by their antibodies and β-actin served as an internal control. B, Quantification showed that TDP-43 Q331K transfected cells had significantly higher p62 levels and a significantly lower LC3II/LC3I ratio, impairing the autophagic flux in comparison to TDP-43 WT transfected cells and control cells. 50 μM SecinH3 rescued the TDP-43 Q331K phenotype. Values are shown as the mean ± SD from three independent experiments. (#P < 0.05 vs. TDP-43 WT and control group; *P < 0.05 vs. TDP-43 Q331K group).*

overexpression of TDP-43 Q331K was shown to suppress the cell viability of SH-SY5Y cells as compared to cells transfected with TDP-43 WT and controls. We also found that, compared to TDP-43 WT transfected and control cells, this mutation induced the proapoptotic protein and decreased the antiapoptotic protein levels. More importantly, a treatment with SecinH3, a cytohesin inhibitor, ameliorated the TDP-43 Q331K-mediated neuronal toxicity and displayed neuroprotective effects in our ALS cell model. These observations suggest that cytohesin inhibitors could be a promising therapeutic strategy for ALS.

ER stress is mainly associated with intracellular inclusion bodies in Alzheimer's disease, Parkinson's disease and ALS (27). ER stress triggers the UPR that acts to alleviate the stress. Upon ER stress, GRP-78 is separated from protein kinase R-like endoplasmic reticulum kinase (PERK), and ATF6 activates them. PERK phosphorylates eukaryotic initiation factor-2α (eIF2α), which in turn leads to the activation of the ATF4 transcription factor as well as the ATF4 target gene CHOP. ATF6, on the other hand, is transported to the Golgi body where it is to be cleaved. The cytosolic domain of ATF6 is then translocated to the nucleus to initiate PDI expression. However, if this response to the ER stress is unable to rescue the cell, then apoptosis will be induced via the activation of the cell death protein transcription factor CHOP and caspase 12 (28). Our data show that the TDP-43 Q331K evoked the activation of two arms of the UPR. The expression levels of GRP-78, ATF4, CHOP, and PDI, as well as cleaved caspase 12, were higher in the TDP-43 Q331K expressing cells compared to TDP-43 WT. Moreover, treatment of the mutant TDP-43 p.Q331K expressing cells with 50 μM SecinH3 reversed these changes in protein expression levels. These results indicated that the expression of mutant TDP-43 stimulated the UPR and that the cytohesin inhibitor SecinH3 could attenuated this response. These observations suggest that SecinH3 protects cells from TDP-43 Q331K induced cell death by suppressing ER stress.

Autophagy is a highly regulated, multistep process and its dysfunction has long been linked to a variety of neurodegenerative diseases (29, 30). As mentioned earlier, when TDP-43 is excluded from the nucleus, cytoplasmic aggregates are formed in brain and spinal cord of ALS patients. These aggregated TDP-43 can be phosphorylated, ubiquitinated, and degraded by autophagy (31, 32). It is the failure of removing these aggregates that is linked to human ALS (20). In addition, previous work had demonstrated that impaired autophagy existed in the cells expressing mutant TDP-43 (33). In this study, we found that the LC3-II/ LC3-I ratio and Beclin 1 were significantly increased in cells expressing TDP-43 Q331K compared to TDP-43 WT. However, the autophagy substrate p62 was also significantly accumulated in cells expressing TDP-43 Q331K. Interestingly, treatment of these cells with SecinH3 reversed the observed increase in p62 levels. These results suggested that TDP-43 Q331K causes a defect in the autophagic flux and that SecinH3 may exert a protective effect by enhancing the autophagic flux.

In summary, we report for the first time that overexpression of TDP-43 Q331K, a common mutation form of TDP-43 in ALS induced severe neuronal toxicity in SH-SY5Y cells and that SecinH3 was able to reduce this toxicity. Our result further confirmed that TDP-43 Q331K exerts its neurotoxicity by inducing ER stress-associated apoptosis and impairing autophagic flux. SecinH3 treatment protects against ER stress-associated apoptosis and enhance autophagic flux even though at a high expression level of neuronal toxic mutant TDP-43 p.Q331K. Our study confirmed the rational that reducing ER stress and maintaining active autophagy should be taken into consideration in ALS treatment. Definitely, more *in vitro* and *in vivo* translational studies focused on the safety and efficiency of SecinH3 in neuron or in animal model are needed in order to provide a promising therapeutic strategy in treating the ALS disease.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 81501111) and the Chinese Postdoctoral Scientific Research Fund (No. 2016 M590687) as well as the Youth Innovation Fund of The First Affiliated Hospital of Zhengzhou University.

REFERENCES

[1] Wang, W., Li, L., Lin, W. L., Dickson, D. W., Petrucelli, L., et al. (2013) The ALS disease-associated mutant TDP-43 impairs mitochondrial dynamics and function in motor neurons. Hum. Mol. Genet. 22, 4706–4719.

[2] Vaccaro, A., Patten, S. A., Aggad, D., Julien, C., Maios, C., et al. (2013) Pharmacological reduction of ER stress protects against TDP-43 neuronal toxicity in vivo. Neurobiol. Dis. 55, 64–75.

[3] Neumann, M., Sampathu, D. M., Kwong, L. K., Truax, A. C., Micsenyi, M. C., et al. (2006) Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.

[4] Buratti, E., and Baralle, F. E. (2010) The multiple roles of TDP-43 in pre-mRNA processing and gene expression regulation. RNA Biol. 7, 420–429.

[5] Wils, H., Kleinberger, G., Janssens, J., Pereson, S., Joris, G., et al. (2010) TDP-43 transgenic mice develop spastic paralysis and neuronal inclusions characteristic of ALS and frontotemporal lobar degeneration. Proc. Natl. Acad. Sci. USA 107, 3858–3863.

[6] Zhou, H., Huang, C., Chen, H., Wang, D., Landel, C. P., et al. (2010) Transgenic rat model of neurodegeneration caused by mutation in the TDP gene. PLoS Genet. 6, e1000887.

[7] Ditsworth, D., Maldonado, M., McAlonis-Downes, M., Sun, S., Seelman, A., et al. (2017) Mutant TDP-43 within motor neurons drives disease onset but not progression in amyotrophic lateral sclerosis. Acta Neuropathol. 133, 907–922.

[8] Mitchell, J. C., Constable, R., So, E., Vance, C., Scotter, E., et al. (2015) Wild type human TDP-43 potentiates ALS-linked mutant TDP-43 driven progressive motor and cortical neuron degeneration with pathological features of ALS. Acta Neuropathol. Commun. 3, 36.

[9] Dong, H., Xu, L., Wu, L., Wang, X., Duan, W., et al. (2014) Curcumin abolishes mutant TDP-43 induced excitability in a motoneuron-like cellular model of ALS. Neuroscience 272, 141–153.

[10] Philips, T., and Rothstein, J. D. (2015) Rodent models of amyotrophic lateral sclerosis. Curr. Protoc. Pharmacol. 69, 1–21.

[11] Ilieva, E. V., Ayala, V., Jove, M., Dalfo, E., Cacabelos, D., et al. (2007) Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain 130, 3111–3123.

[12] Johnson, B. S., Snead, D., Lee, J. J., McCaffery, J. M., Shorter, J., et al. (2009) TDP-43 is intrinsically aggregation-prone, and amyotrophic lateral sclerosis-linked mutations accelerate aggregation and increase toxicity. J. Biol. Chem. 284, 20329–20339.
[13] Wong, E., and Cuervo, A. M. (2010) Autophagy gone awry in neurodegenerative diseases. Nat. Neurosci. 13, 805–811.
[14] Zhang, X., Li, L., Chen, S., Yang, D., Wang, Y., et al. (2011) Rapamycin treatment augments motor neuron degeneration in SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Autophagy 7, 412–425.
[15] Zhai, J., Zhang, L., Mojsilovic-Petrovic, J., Jian, X., Thomas, J., et al. (2015) Inhibition of cytohesins protects against genetic models of motor neuron disease. J. Neurosci. 35, 9088–9105.
[16] Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., et al. (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.
[17] Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.
[18] Barmada, S. J., Skibinski, G., Korb, E., Rao, E. J., Wu, J. Y., et al. (2010) Cytoplasmic mislocalization of TDP-43 is toxic to neurons and enhanced by a mutation associated with familial amyotrophic lateral sclerosis. J. Neurosci. 30, 639–649.
[19] Hall, C. E., Yao, Z., Choi, M., Tyzack, G. E., Serio, A., et al. (2017) Progressive motor neuron pathology and the role of astrocytes in a human stem cell model of VCP-related ALS. Cell Rep. 19, 1739–1749.
[20] Cai, Y., Arikkath, J., Yang, L., Guo, M. L., Periyasamy, P., et al. (2016) Interplay of endoplasmic reticulum stress and autophagy in neurodegenerative disorders. Autophagy 12, 225–244.
[21] Castillo, K., Nassif, M., Valenzuela, V., Rojas, F., Matus, S., et al. (2013) Trehalose delays the progression of amyotrophic lateral sclerosis by enhancing autophagy in motoneurons. Autophagy 9, 1308–1320.
[22] Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., et al. (2006) TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem. Biophys. Res. Commun. 351, 602–611.
[23] Van Deerlin, V. M., Leverenz, J. B., Bekris, L. M., Bird, T. D., Yuan, W., et al. (2008) TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol. 7, 409–416.
[24] Rutherford, N. J., Zhang, Y. J., Baker, M., Gass, J. M., Finch, N. A., et al. (2008) Novel mutations in TARDBP (TDP-43) in patients with familial amyotrophic lateral sclerosis. PLoS Genet 4, e1000193.
[25] Yokoseki, A., Shiga, A., Tan, C. F., Tagawa, A., Kaneko, H., et al. (2008) TDP-43 mutation in familial amyotrophic lateral sclerosis. Ann. Neurol. 63, 538–542.
[26] Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., et al. (2008) TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat. Genet. 40, 572–574.
[27] Kanekura, K., Suzuki, H., Aiso, S., and Matsuoka, M. (2009) ER stress and unfolded protein response in amyotrophic lateral sclerosis. Mol. Neurobiol. 39, 81–89.
[28] Tadic, V., Prell, T., Lautenschlaeger, J., and Grosskreutz, J. (2014) The ER mitochondria calcium cycle and ER stress response as therapeutic targets in amyotrophic lateral sclerosis. Front. Cell Neurosci. 8, 147.
[29] Mizushima, N., and Komatsu, M. (2011) Autophagy: renovation of cells and tissues. Cell 147, 728–741.
[30] Vidal, R. L., Matus, S., Bargsted, L., and Hetz, C. (2014) Targeting autophagy in neurodegenerative diseases. Trends Pharmacol. Sci. 35, 583–591.
[31] Majcher, V., Goode, A., James, V., and Layfield, R. (2015) Autophagy receptor defects and ALS-FTLD. Mol. Cell Neurosci. 66, 43–52.
[32] Bose, J. K., Huang, C. C., and Shen, C. K. (2011) Regulation of autophagy by neuropathological protein TDP-43. J. Biol. Chem. 286, 44441–44448.
[33] Perera, N. D., Sheean, R. K., Lau, C. L., Shin, Y. S., Beart, P. M., et al. (2018) Rilmenidine promotes MTOR- independent autophagy in the mutant SOD1 mouse model of amyotrophic lateral scierosis without slowing disease progression. Autophagy 14, 534–551.