



Inhibition of MEK5 suppresses TDP-43 toxicity via the mTOR-independent activation of the autophagy-lysosome pathway

Myungjin Jo ^a, Shinrye Lee ^a, Kiyoun Kim ^{b, c}, Seongsoo Lee ^d, Sang Ryong Kim ^{e, f},
Hyung-Jun Kim ^{a, *}

^a Korea Brain Research Institute, Department of Neural Development and Disease, 61, Cheomdan-ro, Dong-gu, Daegu, 41062, Republic of Korea

^b Soonchunhyang Institute of Medi-bio Science, Soonchunhyang University, Cheonan, 31151, South Korea

^c Department of Medical Biotechnology, Soonchunhyang University, Asan, 31538, South Korea

^d Gwangju Center, Korea Basic Science Institute (KBSI), Gwangju, 61186, South Korea

^e School of Life Sciences, BK21 Plus KNU Creative BioResearch Group, Institute of Life Science & Biotechnology, Kyungpook National University, Daegu, 41566, South Korea

^f Brain Science and Engineering Institute, Kyungpook National University, Daegu, 41944, South Korea

ARTICLE INFO

Article history:

Received 9 April 2019

Accepted 12 April 2019

Available online 17 April 2019

Keywords:

MEK5

Autophagy-lysosome pathway

TDP-43

ALS

ABSTRACT

The most prominent hallmarks of many neurodegenerative diseases are the accumulation of misfolded protein aggregates and the death of certain neuronal populations. Autophagy is the major intracellular mechanism that degrades protein aggregates and damaged cellular components. Many studies have reported that the dysfunction of autophagy is associated with several neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and Parkinson's disease. Here, we identified a novel mechanism of autophagy regulation. Inhibition of MEK5 reduced the level of p62 and increased the ratio of LC3-II to LC3-I, which is a marker for the activation of the autophagy-lysosome pathway (ALP). One of the most well-known regulators of the ALP is mTOR, and previous studies have reported that the major substrate of MEK5 is ERK5. However, we found that MEK5 modulates the autophagy-lysosome pathway in an mTOR- and ERK5-independent manner. Moreover, MEK5 inhibition alleviated the mislocalization of TDP-43 (an ALS-associated protein) and cell death in TDP-43-GFP-expressing neuronal cells. Taken together, these findings suggest that MEK5 is a novel autophagy modulator and that this kinase could be a therapeutic target for neurodegenerative diseases such as amyotrophic lateral sclerosis.

© 2019 Elsevier Inc. All rights reserved.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease characterized by the progressive degeneration of motor neurons in the brain and spinal cord, leading to muscle weakness and eventually to death [1,2]. Transactive response DNA-binding protein 43 kDa (TDP-43) is known as a major disease-related protein in sporadic and familial ALS [3–6]. The cytoplasmic aggregates of TDP-43 in affected neurons are a key feature of ALS and of frontotemporal lobar dementia with TDP-43 inclusions (FTLD-TDP). Neurotoxicity due to increased TDP-43 levels is currently well-accepted as a contributing factor to the neurodegeneration of ALS and of other neurodegenerative diseases that

are associated with the accumulation of TDP-43 inclusions, known as TDP-43 proteinopathies [3–6]. Several lines of evidence suggest that the induction of the autophagy-lysosome pathway (ALP) enhances the TDP-43 turnover rate and reduces the TDP-43-induced neurotoxicity [7–11]. These findings raise the possibility that ALP may play a critical role in TDP-43 proteinopathies.

The ALP is a major degradation pathway involved in the removal of dysfunctional organelles and aggregated proteins [12]. Nutrients, growth factors, and stress signal-induced activation of the ALP are negatively regulated by the mammalian target of rapamycin (mTOR) pathway [13–15]. The dysregulation of mTOR signaling is associated with several neurodegenerative diseases, such as ALS [16,17], Alzheimer's disease [18], Huntington disease [19], and Parkinson's disease [20]. Moreover, rapamycin (mTOR inhibitor)-induced autophagy attenuated TDP-43-induced motor dysfunction [8,17]. Notably, iPSC-derived neurons and astrocytes from human ALS patients showed decreased neurotoxicity and

* Corresponding author.

E-mail address: kijang1@kbrri.re.kr (H.-J. Kim).

neurodegeneration in response to an autophagy inducer [16]. However, mTOR inhibitors produce many adverse events that can be serious and debilitating [21]. Therefore, mTOR-independent ALP modulation may contribute to the development of therapeutic strategies for neurodegenerative diseases.

Mitogen-activated protein kinase 5 (MEK5) is a member of the mitogen-activated protein kinase (MAPK) kinase family. MEK5 activates extracellular-signal-regulated kinase 5 (ERK5) and subsequently activates its downstream signaling pathway and is associated with cell survival, apoptosis, angiogenesis, cell migration, and cell proliferation [22–26]. However, it has never been determined whether MEK5 is associated with ALP.

In the present study, we found that MEK5 inhibition activates ALP in a mTOR- and ERK5-independent manner. Moreover, we showed that MEK5 inhibition attenuated TDP-43-induced neurotoxicity and mislocalization. Therefore, MEK5 is a novel autophagy modulator and could be a promising therapeutic target for TDP-43 proteinopathies.

2. Materials and methods

2.1. Reagents and antibodies

The following reagents were purchased from the indicated providers: BIX02189 (Selleckchem), rapamycin (InvivoGEN), Torin1 (Tocris) and DMSO (Sigma). The following antibodies were used for immunoblotting: rabbit anti-p62 (Sigma; P0067), rabbit anti-LC3 (MBL; PM036), HRP-conjugated anti- α -tubulin (Cell Signaling; 9099), rabbit anti-phospho-mTOR (S2448) (Cell Signaling; 5536), rabbit anti-phospho-mTOR (S2481) (Cell Signaling; 2974), rabbit anti-mTOR (Cell Signaling; 2983), rabbit anti-phospho-ULK1 (S757) (Cell Signaling; 6888), rabbit anti-ULK1 (Abcam; ab128859), mouse anti-MEK5 (Santa Cruz; sc-135986), rabbit anti-ERK5 antibody (Cell Signaling; 3372), HRP-conjugated anti-rabbit IgG (Santa Cruz; sc-2004), and HRP-conjugated anti-mouse IgG (Santa Cruz; sc-2005). The following antibodies were used for immunocytochemistry (ICC): rabbit anti-cleaved caspase-3 antibody (Cell Signaling; 9664) and Alexa 594-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories; 111-585-144).

2.2. Cell lines

Neuro-2a (N2a) mouse neuroblastoma cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco), 100 U/ml penicillin, and 100 μ g/ml streptomycin (Gibco). Cells were grown at 37 °C in a humidified atmosphere containing 5% CO₂.

2.3. Primary cortical neuron cell culture

Primary cultures of dissociated cerebral cortical neurons were prepared from 16-day-old embryonic mice as described previously [27]. Briefly, mouse embryos were decapitated, and the brains were rapidly removed and placed in a culture dish containing HBSS (Gibco). Cortices were isolated, transferred to a conical tube and washed. Cortical tissues were enzymatically digested with pre-warmed papain (20 units/ml) (Worthington Biochemical Corporation) and DNase I (0.005%) for 10 min at 37 °C. The cortical cells were centrifuged at 800 rpm for 10 min at room temperature, and the dissociated cells that were obtained were seeded onto plates coated with poly-D-lysine (Sigma-Aldrich) in neurobasal media containing 2 mM glutamine (Gibco), N2 supplement (Gibco), B27 supplement (Gibco), and penicillin-streptomycin (Gibco). The culture media were changed initially after 5 days and every 3 days thereafter, and the cells were used after being cultured for 10 days.

2.4. Cytotoxicity assay

N2a cells (4×10^4 cells/well) were grown in 96 well plates and treated with MG132, rotenone or BIX02189, as indicated, for 24 h. DMSO was used as a vehicle control. To measure cytotoxicity, a Cell Counting Kit-8 (CCK-8; Enzo Life Science) was used according to the manufacturer's instructions. Briefly, 10 μ l of CCK-8 reagent was added into each well, and the plate was incubated at 37 °C for 2 h. The absorbance at 450 nm was measured by using a microplate reader (Tecan). Cell viability is expressed as a percentage of the control. All experiments were performed in triplicate.

2.5. Transfection

N2a cells were transfected with *Gfp* (*pCMV-AC-Gfp*) or human *TDP-43* (*pCMV-AC-TDP-43-Gfp*) using Lipofectamine 3000 reagent (Invitrogen) according to the manufacturer's instructions. For siRNA transfection, cells were transfected with control siRNA (Santa Cruz; sc-37007), mouse *Mek5* siRNA (Santa Cruz; sc-35912) or mouse *Erk5* siRNA (Santa Cruz; sc-35340) by using Lipofectamine 3000 reagent. After 48 h of transfection, the knockdown of target proteins was confirmed by immunoblot analysis.

2.6. Immunostaining

Cells were fixed in 4% paraformaldehyde (PFA) in PBS for 30 min. Then, cells were incubated with the rabbit anti-cleaved caspase-3 antibody (1:500 dilution) at 4 °C overnight, followed by incubation for 1 h at room temperature with secondary antibody (Alexa-594 conjugated goat anti-rabbit IgG; 1:500 dilution). All antibodies were diluted in PBS with 0.3% Triton X-100 and 1% BSA. The samples were mounted and counterstained with gelatin containing 4',6-diamidino-2-phenylindole (DAPI).

2.7. Immunoblot analysis

Cells were lysed in ice-cold RIPA lysis buffer (Cell Signaling Technology) containing protease and phosphatase inhibitor cocktails (Roche). The protein concentration of the cell lysates was determined using a BCA protein assay kit (Thermo Scientific). Next, the protein extracts were mixed with LDS sample buffer containing reducing agent and then boiled at 95 °C for 10 min. An equal amount of protein was separated using 4–12% Bis-Tris gels or 3–8% Tris-Acetate gels (Invitrogen) and transferred to polyvinylidene fluoride filter membranes (PVDF; Novex). The membranes were blocked using 5% skim milk in TBS with 0.025% Tween 20 and incubated sequentially with antibodies as indicated. Blots were detected with an ECL prime kit (GE Healthcare). Samples from three independent experiments were used, and the relative expression levels were determined using a Fusion-FX (Viber Lourmat).

2.8. Cyto-ID autophagy analysis

To detect autophagic vesicles, a Cyto-ID[®] Green Autophagy Kit (Enzo Life Science) was used according to the manufacturer's instructions. Briefly, cells were incubated with Cyto-ID dye at 37 °C for 30 min and fixed in 4% PFA in PBS for 30 min. All reagents were diluted in 1 $\times$ assay buffer with 2% FBS. The samples were mounted with DAPI and observed with a fluorescence microscope or confocal microscope.

2.9. Statistical analysis

Data were analyzed with a Student's *t*-test or one-way ANOVA test, depending on the comparison variables, with post hoc analysis

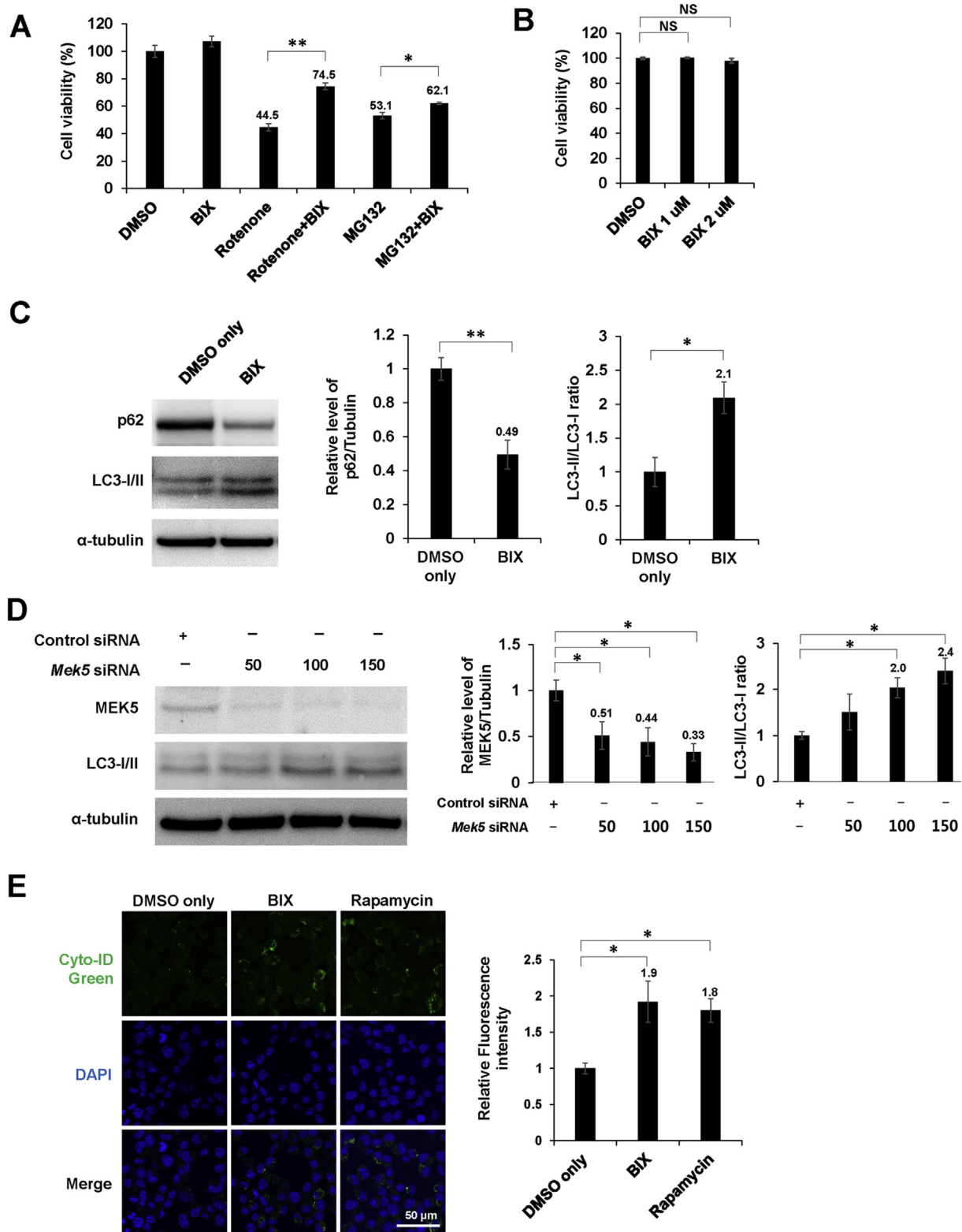
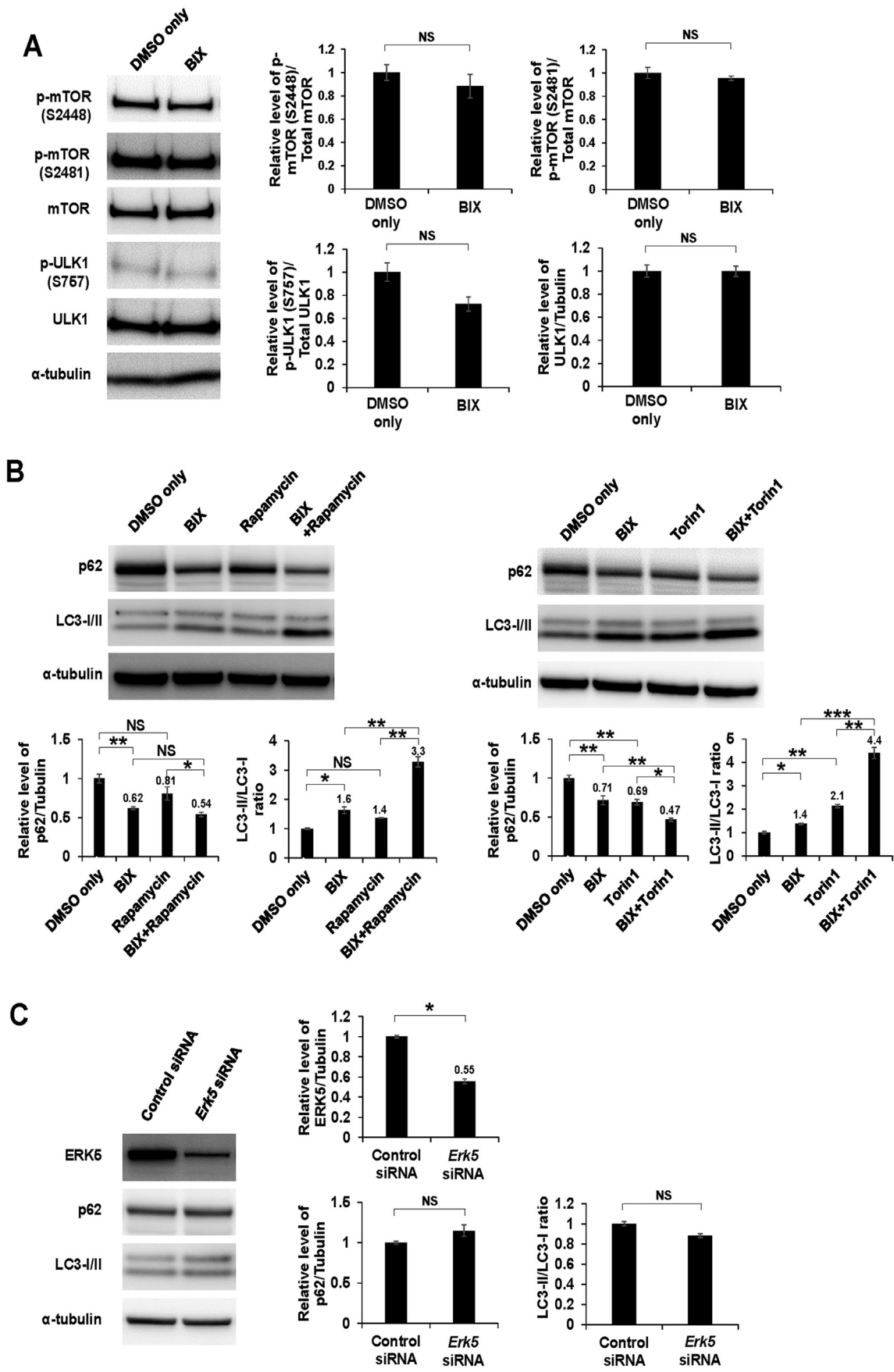


Fig. 1. Inhibition of MEK5 activates the autophagy-lysosome pathway. (A, B) N2a cells were pretreated with BIX02189 (2 μ M) for 30 min and subsequently treated with MG132 (5 μ M) or rotenone (10 μ M) for 24 h. CCK-8 analysis was then performed. (C) N2a cells were incubated with BIX02189 (2 μ M). At 24 h after treatment, the cells were harvested for total protein extraction. Western blot analysis for p62 or LC3-II/I protein expression. (D) N2a cells were transfected with control siRNA (50 nM) or MEK5-specific siRNA (50, 100 and 150 nM) for 2 days. Western blot analysis for MEK5 or LC3-II/I protein expression. Quantification of the immunoblots was performed from 3 independent experiments. (E) To visualize the autophagy flux, N2a cells were incubated with a BIX02189 (2 μ M) for 4 h. Rapamycin (200 nM) was used as a positive control. Immunocytochemistry was used to detect the Cyto-ID autophagy dye (green). Quantification of the intensity of Cyto-ID-green was performed using ImageJ. Data are presented as the mean $\pm$ S.E.M. * p < 0.05, ** p < 0.005 (Student's t -test).



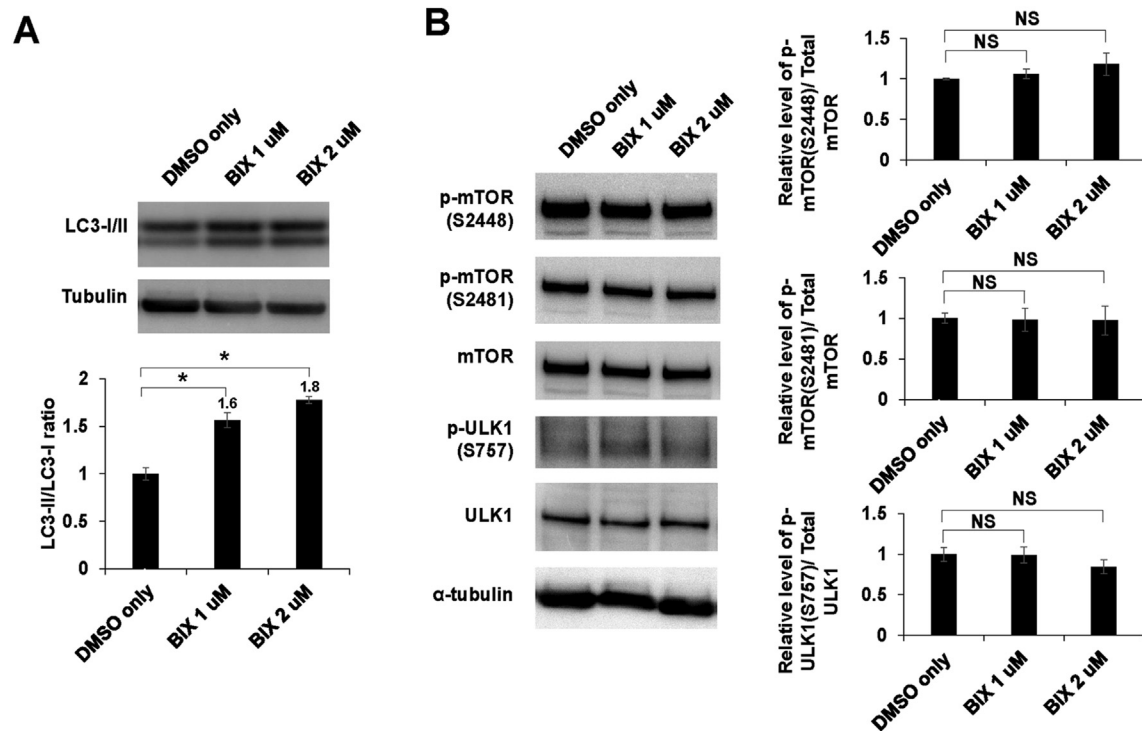


Fig. 3. MEK5 inhibition induces autophagy activation in primary cortical neurons. (A, B) Primary cortical neurons were incubated with BIX02189 (1 or 2 μ M) for 24 h. Western blot analysis for LC3-II/I, phospho-mTOR (S2448 or S2481), total mTOR, phospho-ULK1 (S757) or total ULK1 protein expression was performed. Quantification of the immunoblots from 3 independent experiments was performed. Data are presented as the mean $\pm$ S.E.M. * p < 0.05 (Student's t -test), NS, not significant.

as indicated (GraphPad Prism Software). Differences were considered significant when p < 0.05 and are indicated as follows: * p < 0.05; ** p < 0.005; and N.S., not significant.

3. Results

3.1. MEK5 downregulation activates the autophagy-lysosome pathway in neuronal cells

Previous studies have suggested that the proteotoxic stress induced by oxidative stress or the impairment of the ubiquitin-proteasome system (UPS) can be attenuated by activation of the ALP activation [28–30]. Therefore, small molecules that mitigate rotenone (inducer of reactive oxygen species)-induced toxicity and/or MG132 (UPS inhibitor)-induced toxicity may modulate the ALP. Thus, we screened compounds that suppress MG132- or rotenone-induced cellular toxicity using a kinase inhibitor library in mouse neuronal N2a cells. BIX02189, a specific inhibitor of MEK5, significantly rescued the cytotoxicity of MG132 and rotenone treatment (Fig. 1A). We also showed that 2 μ M of BIX02189 did not affect cell viability in N2a cells (Fig. 1B) compared to that in the control. To determine whether MEK5 regulates ALP, we examined the protein levels of p62/SQSTM1 and LC3 in N2a cells following treatment with BIX02189. The levels of the p62 protein were decreased by BIX02189 treatment compared to those in the control. In addition, a noticeable increase in the ratio of LC3-II/LC3-I was observed after

treatment with BIX02189 (Fig. 1C) compared to that in the control. The downregulation of p62 and the increase of LC3-II/LC3-I are typical markers for ALP activation. To rule out the possibility of off-target effects of BIX02189, we confirmed these results using *Mek5* siRNA. Consistently, the knockdown of *Mek5* also increased the LC3-II/LC3-I ratio (Fig. 1D) compared to that in the control. In addition, we visualized the autophagic vesicles after treatment with BIX02189 or rapamycin using a Cyto-ID green immunofluorescence autophagy kit. Rapamycin is a well-known activator of the ALP [31]. The number of Cyto-ID-positive cells was significantly increased by the inhibition of MEK5 and by rapamycin treatment compared to that in the control (Fig. 1E). These results indicate that MEK5 inhibition activates the autophagy-lysosome pathway in neuronal cells.

3.2. MEK5 modulates the autophagy-lysosome pathway via mTOR-independent and noncanonical MEK5 signaling pathways

The most well-known regulatory mechanism of ALP is the mTOR pathway. To determine whether the inhibition of MEK5 occurs through an mTOR-dependent pathway, we first measured the phosphorylation of mTOR in BIX02189-treated N2a cells. The phosphorylation levels of mTOR (S2448 and S2481, active phosphorylation sites) were not affected by BIX02189 treatment compared to those in the control. Additionally, treatment with BIX02189 did not have a significant effect on the phosphorylation at

Fig. 2. Inhibition of MEK5 induces autophagy activation via an ERK5- and mTOR-independent pathway. (A) N2a cells were incubated with BIX02189 (2 μ M) for 24 h. Western blot analysis for the phospho-mTOR (S2481 or S2488), total mTOR, phospho-ULK1 (S757) and total ULK1 protein expression was performed. (B) N2a cells were incubated with either 200 nM Rapamycin, 2 μ M BIX02189, or both and were incubated with either 20 nM Torin1, 2 μ M BIX02189, or both. At 24 h after treatment, the cells were harvested for total protein extraction. The LC3-II/I ratio or the autophagic degradation of p62 were evaluated by Western blotting. Quantification of the immunoblots from 3 independent experiments was performed. Data are presented as the mean $\pm$ S.E.M. * p < 0.05, ** p < 0.005, *** p < 0.001 (one-way ANOVA with Tukey's multiple comparison test). (C) N2a cells were transfected with control siRNA (50 nM) or ERK5-specific siRNA (50 nM) for 2 days. Western blot analysis for ERK5, p62 or LC3-II/I protein expression was performed. Quantification of the immunoblots from 3 independent experiments was performed. Data are presented as the mean $\pm$ S.E.M. * p < 0.05 (Student's t -test), NS, not significant.

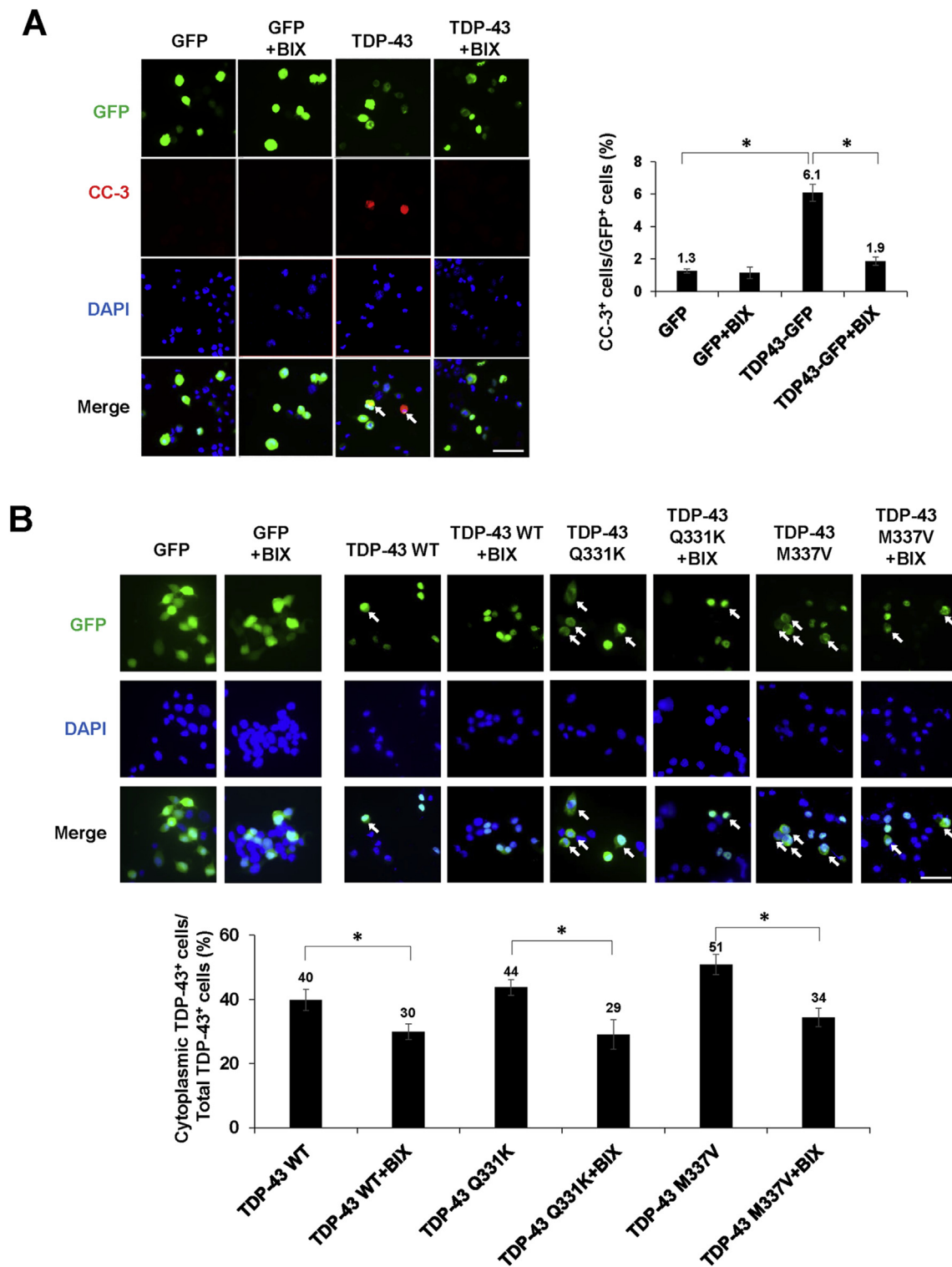


Fig. 4. MEK5 inhibition attenuates the TDP-43-induced neuronal toxicity and mislocalization of TDP-43. (A) N2a cells were transiently transfected with the control vector (*pCMV6-AC-GFP*) or TDP-43 expression construct (*pCMV6-AC-TDP-43-GFP*). Cells overexpressing GFP or TDP-43-GFP were treated with a BIX02189 (2 μ M) for 24 h. Immunocytochemistry was subsequently performed to detect the cleaved caspase-3 (CC-3; red) or DAPI (nuclei; blue). Arrowheads indicate the colocalization of CC-3 with TDP-43-GFP-positive cells. (B) N2a cells were transiently transfected with control vector (*pCMV6-AC-GFP*) or TDP-43 WT and mutant (Q331K and M337V) expression vectors. Cells overexpressing GFP or TDP-43 (WT, Q331K and M337V)-GFP were treated with BIX02189 (2 μ M) for 24 h. Arrows indicate TDP-43 cytoplasmic mislocalization in cells. Data are presented as the mean $\pm$ S.E.M. of 3 independent experiments. * $p < 0.05$ (one-way ANOVA with Tukey's multiple comparison test). Scale bars, 50 μ m.

Ser757 of ULK1, which is phosphorylated by mTOR, compared to that in the control (Fig. 2A). These data suggest that MEK5 inhibition does not affect the mTOR pathway. To confirm these results, we used mTOR inhibitors such as rapamycin and torin1. Cotreatment with BIX02189 and rapamycin or torin1 resulted in a higher LC3-II/LC3-I ratio than that of single treatment with each inhibitor. Moreover, the p62 protein level was more greatly decreased by cotreatment with BIX02189 and an mTOR inhibitor compared to that in other conditions (Fig. 2B). This result demonstrates that MEK5 can activate the ALP in mTOR-inhibited conditions. Thus, we concluded that MEK5 modulates the autophagy-lysosome pathway via mTOR-independent pathway.

Most of the known functions of MEK5 are mediated by ERK5 activation [32,33]. Therefore, we tested whether the MEK5-ERK5 axis regulates the activation of autophagy. Although the ERK5 protein level was effectively downregulated by *Erk5* siRNA compared to that in the control, the expression levels of p62 and the LC3-II/LC3-I ratio were not significantly altered compared to those following transfection with the control siRNA (Fig. 2C). Taken together, our results indicate that MEK5 modulates the ALP through mTOR- and ERK5-independent pathway. In addition, we confirmed the effects of the MEK5 inhibitor on autophagy flux in primary cortical neurons. The ratio of LC3-II/LC3-I was noticeably increased by BIX02189 treatment (Fig. 3A) compared to that in the control. Moreover, the phosphorylation levels of mTOR and ULK1 were not significantly altered by BIX02189 treatment in primary cortical neurons compared to those in the control (Fig. 3B). All of these results suggest that the inhibition of MEK5 activates the ALP in an mTOR- and an ERK5-independent manner in neurons.

3.3. MEK5 inhibition mitigates the TDP-43-induced neuronal toxicity and cytoplasmic accumulation of TDP-43

The Mislocalization and aggregation of the TDP-43 protein is a hallmark of ALS. Previous studies showed that the overexpression of TDP-43 causes neuronal toxicity [3,34,35]. Furthermore, TDP-43-induced neurotoxicity is greatly attenuated by autophagy stimulators in rat cortical neurons [16]. Thus, we hypothesized that MEK5 inhibition may reduce TDP-43-induced neurotoxicity through the ALP. To determine whether MEK5 modulates the neurotoxicity induced by TDP-43 overexpression, we monitored the levels of cleaved caspase-3 (CC3) by immunostaining after inhibiting MEK5 in N2a cells expressing TDP-43. CC3 is a typical marker for apoptotic cell death. We found that BIX02189 treatment significantly rescued the cell toxicity induced by TDP-43 overexpression (Fig. 4A). Next, we assessed the effect of MEK5 on the mislocalization of the TDP-43 wild type protein and of TDP-43 ALS-associated mutants, such as M337V and Q331K. In normal cells, TDP-43 mainly localizes to the nucleus. However, in pathological conditions, the TDP-43 protein mislocalizes to the cytoplasm. To evaluate the mislocalization of the TDP-43 protein, N2a cells were transfected with a control GFP construct, a GFP-tagged TDP-43 wild-type construct or GFP-tagged mutant isoform constructs (Q331K and M337V). The subcellular localization of TDP-43 was analyzed by confocal microscopy using GFP (green) and DAPI (blue, nuclear marker). MEK5 inhibition effectively reduced the cytoplasmic TDP-43 levels in both the mutant- and wild-type-TDP-43-expressing cells (Fig. 4B). Taken together, these findings suggest that MEK5 could mitigate TDP-43-induced neuronal toxicity and prevent the cytoplasmic accumulation of TDP-43.

4. Discussion

In this study, we showed that MEK5 inhibition mitigates TDP-43-induced neuronal toxicity through the mTOR-independent

activation of ALP. Numerous studies revealed that the ALP is regulated by the mTOR signaling pathway. During starvation or pathological conditions, autophagy flux is induced by suppressing mTOR activity. Many mTOR inhibitors have been tested for the treatment of neurodegenerative diseases, such as AD, PD, and ALS [36–38]. Although the inhibition of mTOR activity effectively induces the ALP, mTOR is a multifunctional protein that plays a role in cell growth and proliferation, protein synthesis, the activation of metabolism, and cell motility [39]. Thus, the inhibition of mTOR signaling could lead to fatal adverse events. Because of these limitations, it is necessary to find a novel autophagy activator that regulates the ALP via an mTOR-independent pathway.

MEK5 belongs to the MAPK family protein. The best and one of the only known targets of MEK5 is ERK5. The MEK5-ERK5 pathway is associated with many biological functions, such as cell proliferation, survival and apoptosis [33]. Many studies have focused on the role of the MEK5-ERK5 pathway in cancer [32,40]. However, we found that the knockdown of *Erk5* did not affect the ALP. This means that MEK5 regulates the ALP via an unknown MEK5 substrate. Therefore, further studies are warranted to determine how MEK5 modulates the ALP.

TDP-43-induced neurotoxicity is a well-known contributor to the pathology of ALS and other neurodegenerative diseases that are associated with the accumulation of TDP-43, which are known as TDP-43 proteinopathies, such as FTLD and AD [3–6]. The most common pathological feature of TDP-43 proteinopathies is the accumulation of intracellular ubiquitin-positive inclusions in degenerating neurons [41,42]. Interestingly, several genes associated with ALS, such as *Sequestosome-1* (p62), *Valosin-containing protein*, *Optineurin*, *TANK-binding kinase 1* and *Ubiquilin-2*, are linked to the protein quality-control system, such as the UPS and ALP [43,44]. These findings raise the possibility that the proteotoxicity induced by the accumulation of proteinaceous inclusions in neurons may be a common pathological contributor in TDP-43 proteinopathies. In summary, we demonstrated that MEK5 is a novel mTOR-independent autophagy regulator. We also found that MEK5 inhibition attenuated the cytoplasmic accumulation and toxicity of TDP-43 in TDP-43-overexpressing neuronal cells. Therefore, our data suggest that targeting the MEK5-mediated ALP may represent a novel therapeutic intervention for neurodegenerative diseases with TDP-43 proteinopathy.

Acknowledgements

This work was supported by the KBRI research program of the Ministry of Science, ICT & Future Planning (19-BR-02-03); the Soonchunhyang University Research Fund; the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (NRF-2017R1C1B2007941, NRF-2017R1C1B1008825); and the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health and Welfare, South Korea (grant number: HI14C1135). Confocal microscopy data were acquired at the Advanced Neural Imaging Center of KBRI.

Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.bbrc.2019.04.088>.

References

- [1] A. Al-Chalabi, A. Jones, C. Troakes, et al., The genetics and neuropathology of amyotrophic lateral sclerosis, *Acta Neuropathol.* 124 (2012) 339–352.

- [2] D.W. Cleveland, J.D. Rothstein, From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS, *Nat. Rev. Neurosci.* 2 (2001) 806–819.
- [3] E.L. Scotter, H.J. Chen, C.E. Shaw, TDP-43 proteinopathy and ALS: insights into disease mechanisms and therapeutic targets, *Neurotherapeutics* 12 (2015) 352–363.
- [4] R.M. Liscic, L.T. Grinberg, J. Zidar, et al., ALS and FTLD: two faces of TDP-43 proteinopathy, *Eur. J. Neurol.* 15 (2008) 772–780.
- [5] B.D. James, R.S. Wilson, P.A. Boyle, et al., TDP-43 stage, mixed pathologies, and clinical Alzheimer's-type dementia, *Brain* 139 (2016) 2983–2993.
- [6] K.A. Josephs, D.W. Dickson, N. Tosakulwong, et al., Rates of hippocampal atrophy and presence of post-mortem TDP-43 in patients with Alzheimer's disease: a longitudinal retrospective study, *Lancet Neurol.* 16 (2017) 917–924.
- [7] Zeroing in on Amyotrophic lateral sclerosis. A look at a new era of ALS research, in: *Health after 50 with Scientific American Consumer Health*, vol. 27, 2015, pp. 4–5.
- [8] I.F. Wang, B.S. Guo, Y.C. Liu, et al., Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 15024–15029.
- [9] K.W. Cheng, M.J. Lin, C.K. Shen, Rapamycin alleviates pathogenesis of a new *Drosophila* model of ALS-TDP, *J. Neurogenet.* 29 (2015) 59–68.
- [10] C. Leibiger, J. Deisel, A. Aufschnaiter, et al., TDP-43 controls lysosomal pathways thereby determining its own clearance and cytotoxicity, *Hum. Mol. Genet.* 27 (2018) 1593–1607.
- [11] D.K.H. Nguyen, R. Thombre, J. Wang, Autophagy as a common pathway in amyotrophic lateral sclerosis, *Neurosci. Lett.* 697 (2019) 34–48.
- [12] N. Mizushima, B. Levine, A.M. Cuervo, et al., Autophagy fights disease through cellular self-digestion, *Nature* 451 (2008) 1069–1075.
- [13] S. Diaz-Troya, M.E. Perez-Perez, F.J. Florencio, et al., The role of TOR in autophagy regulation from yeast to plants and mammals, *Autophagy* 4 (2008) 851–865.
- [14] C.H. Jung, S.H. Ro, J. Cao, et al., mTOR regulation of autophagy, *FEBS Lett.* 584 (2010) 1287–1295.
- [15] Y.Y. Chang, G. Juhasz, P. Goraksha-Hicks, et al., Nutrient-dependent regulation of autophagy through the target of rapamycin pathway, *Biochem. Soc. Trans.* 37 (2009) 232–236.
- [16] S.J. Barmada, A. Serio, A. Arjun, et al., Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models, *Nat. Chem. Biol.* 10 (2014) 677–685.
- [17] K.W. Hsueh, T.W. Chiou, S.F. Chiang, et al., Autophagic down-regulation in motor neurons remarkably prolongs the survival of ALS mice, *Neuropharmacology* 108 (2016) 152–160.
- [18] P. Spilman, N. Podlutska, M.J. Hart, et al., Inhibition of mTOR by rapamycin abolishes cognitive deficits and reduces amyloid-beta levels in a mouse model of Alzheimer's disease, *PLoS One* 5 (2010) e9979.
- [19] B. Ravikumar, C. Vacher, Z. Berger, et al., Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease, *Nat. Genet.* 36 (2004) 585–595.
- [20] L. Crews, B. Spencer, P. Desplats, et al., Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of alpha-synucleinopathy, *PLoS One* 5 (2010) e9313.
- [21] B. Kaplan, Y. Qazi, J.R. Wellen, Strategies for the management of adverse events associated with mTOR inhibitors, *Transplant. Rev.* 28 (2014) 126–133.
- [22] C.L. Chen, S. Sangiah, J.D. Roder, et al., Pharmacokinetics and plasma protein binding of the new potent class III antiarrhythmic agent 3-[4-(1H-imidazol-1-yl)benzoyl]-7-isopropyl-3, 7-diazabicyclo[3.3.1]nonane dihydroperchlorate, *Arzneimittelforschung* 45 (1995) 670–675.
- [23] N. Mody, J. Leitch, C. Armstrong, et al., Effects of MAP kinase cascade inhibitors on the MKK5/ERK5 pathway, *FEBS Lett.* 502 (2001) 21–24.
- [24] M. Hayashi, J.D. Lee, Role of the BMK1/ERK5 signaling pathway: lessons from knockout mice, *J. Mol. Med. (Berl.)* 82 (2004) 800–808.
- [25] M. Hayashi, C. Fearn, B. Eliceiri, et al., Big mitogen-activated protein kinase 1/extracellular signal-regulated kinase 5 signaling pathway is essential for tumor-associated angiogenesis, *Cancer Res.* 65 (2005) 7699–7706.
- [26] X. Wang, C. Tournier, Regulation of cellular functions by the ERK5 signalling pathway, *Cell. Signal.* 18 (2006) 753–760.
- [27] W. Araki, K. Yuasa, S. Takeda, et al., Overexpression of presenilin-2 enhances apoptotic death of cultured cortical neurons, *Ann. N. Y. Acad. Sci.* 920 (2000) 241–244.
- [28] C. Geronimo-Olvera, L. Massieu, Autophagy as a homeostatic mechanism in response to stress conditions in the central nervous system, *Mol. Neurobiol.* (2019), <https://doi.org/10.1007/s12035-019-1546-x>.
- [29] J. Lee, D.G. Jo, D. Park, et al., Adaptive cellular stress pathways as therapeutic targets of dietary phytochemicals: focus on the nervous system, *Pharmacol. Rev.* 66 (2014) 815–868.
- [30] M. Redmann, V. Darley-Usmar, J. Zhang, The role of autophagy, mitophagy and lysosomal functions in modulating bioenergetics and survival in the context of redox and proteotoxic damage: implications for neurodegenerative diseases, *Aging and disease* 7 (2016) 150–162.
- [31] S. Thellung, A. Corsaro, M. Nizzari, et al., Autophagy activator drugs: a new opportunity in neuroprotection from misfolded protein toxicity, *Int. J. Mol. Sci.* 20 (2019).
- [32] V.T. Hoang, T.J. Yan, J.E. Cavanaugh, et al., Oncogenic signaling of MEK5-ERK5, *Cancer Lett.* 392 (2017) 51–59.
- [33] B.A. Drew, M.E. Burrow, B.S. Beckman, MEK5/ERK5 pathway: the first fifteen years, *Biochim. Biophys. Acta* 1825 (2012) 37–48.
- [34] M. Shenouda, A.B. Zhang, A. Weichert, et al., Mechanisms associated with TDP-43 neurotoxicity in ALS/FTLD, *Adv Neurobiol* 20 (2018) 239–263.
- [35] H.J. Kim, A.R. Raphael, E.S. LaDow, et al., Therapeutic modulation of eIF2alpha phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models, *Nat. Genet.* 46 (2014) 152–160.
- [36] S. Sarkar, Regulation of autophagy by mTOR-dependent and mTOR-independent pathways: autophagy dysfunction in neurodegenerative diseases and therapeutic application of autophagy enhancers, *Biochem. Soc. Trans.* 41 (2013) 1103–1130.
- [37] M.A. Rahman, H. Rhim, Therapeutic implication of autophagy in neurodegenerative diseases, *BMB Rep* 50 (2017) 345–354.
- [38] N. Fujikake, M. Shin, S. Shimizu, Association between autophagy and neurodegenerative diseases, *Front. Neurosci.* 12 (2018) 255.
- [39] M.M. Lerch, D.L. Conwell, J. Mayerle, The anti-inflammasome effect of lactate and the lactate GPR81-receptor in pancreatic and liver inflammation, *Gastroenterology* 146 (2014) 1602–1605.
- [40] D.M. Pereira, A.E. Simoes, S.E. Gomes, et al., MEK5/ERK5 signaling inhibition increases colon cancer cell sensitivity to 5-fluorouracil through a p53-dependent mechanism, *Oncotarget* 7 (2016) 34322–34340.
- [41] C. Bendotti, M. Marino, C. Cheroni, et al., Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response, *Prog. Neurobiol.* 97 (2012) 101–126.
- [42] M. Neumann, D.M. Sampathu, L.K. Kwong, et al., Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis, *Science* 314 (2006) 130–133.
- [43] A.E. Renton, A. Chio, B.J. Traynor, State of play in amyotrophic lateral sclerosis genetics, *Nat. Neurosci.* 17 (2014) 17–23.
- [44] J.K. Lee, J.H. Shin, J.E. Lee, et al., Role of autophagy in the pathogenesis of amyotrophic lateral sclerosis, *Biochim. Biophys. Acta* 1852 (2015) 2517–2524.