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Brief communication

Genetic activation of parkin rescues TAF15-induced neurotoxicity in a *Drosophila* model of amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that is characterized pathologically by the loss of motor neurons. Mutations in the *TAF15* gene have been implicated in the pathogenesis of ALS. TATA-binding protein associated factor 15 (TAF15) accumulates as cytoplasmic aggregates in neuronal cells, the clearance of which may be a therapeutic strategy for ALS. However, the identification of a novel regulator for protection against a TAF15-induced proteinopathy and the exact pathogenic mechanism of TAF15-induced neurodegeneration remain to be elucidated. Here, we show that parkin directly binds to TAF15 and that parkin overexpression can suppress the defective phenotypes, including the life span and locomotive activity of a TAF15-induced proteinopathy. We also found that overexpression of parkin in neuronal cells leads to a reduction in TAF15 levels, because of the E3 ubiquitin ligase activity of parkin. Our study provides in vivo evidence supporting the use of parkin for neuroprotection in a TAF15-induced proteinopathy and offers new insights into the pathogenic mechanisms underlying TAF15-induced ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that is characterized pathologically by the loss of upper and lower motor neurons (Cleveland and Rothstein, 2001). Although most cases of ALS occur in a sporadic manner, 10% of ALS cases are hereditary and can be attributed to mutations in specific genes. Pathogenic mutations in several genes, including *SOD1*, *TDP-43*, *FUS*, *UBQLN2*, *OPTN*, *VCP*, and *C9ORF72*, have been linked to the familial form of ALS (DeJesus-Hernandez et al., 2011; Deng et al., 2011; Fecto and Siddique, 2011; Ince et al., 2011; Kapeli et al., 2017). Recently, dominant mutations in genes coding for different DNA/RNA-binding proteins with prion-like domains, including TAR DNA-binding protein 43 (TDP-43), fused in sarcoma (FUS), Ewing sarcoma protein, and TATA-binding protein associated factor 15 (TAF15), have been found in familial and sporadic cases of ALS (Gitler and Shorter, 2011; Lagier-Tourenne and Cleveland, 2009; Ticozzi et al., 2011). TAF15, FUS, and Ewing sarcoma protein constitute the ten-eleven translocation family of RNA-binding proteins. Similar to TDP-43 and FUS properties in vitro, TAF15 also forms aggregates in the cytoplasm, leading to increased neuronal toxicity (Neumann et al., 2011). Recently, mutations in TAF15 have been implicated in the pathogenesis of familial and sporadic ALS (Couthouis et al., 2011; Ticozzi et al., 2011). Similar to TDP-43–induced and FUS-induced proteinopathies, the sporadic ALS-linked variants of TAF15 caused cytoplasmic aggregate formation in the spinal cord neurons of rat embryos and neurodegeneration in *Drosophila* (Couthouis et al., 2011). Although the aggregation of TAF15 proteins is accompanied by the loss of their nuclear localization, it remains unclear whether TAF15 aggregation or mislocalization to the cytoplasm is the pathogenic event that initiates ALS (Schwartz et al., 2015). Furthermore, genetic variants of TAF15 in ALS patients and a role for TAF15 in frontotemporal lobar degeneration have been demonstrated (Svetoni et al., 2016). These findings highlight an important role of TAF15 in the pathogenesis of ALS. Therefore, clearance of cytoplasmic TAF15 aggregates may be a therapeutic strategy for ALS. However, the identification of a novel regulator for protection against TAF15-induced neurotoxicity and the exact pathogenic mechanism of TAF15-induced neurodegenerative disease have not yet been elucidated.

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Recent studies have shown that protein quality control mechanisms are critical factors in the pathogenesis of various neurodegenerative diseases. To investigate the molecular mechanism of TAF15-induced ALS pathogenesis, we used the *Drosophila* model system to study the underlying pathogenic mechanisms of neurotoxicity by overexpressing human TAF15. We found that parkin is a novel interacting partner of TAF15 in *Drosophila*. Parkin is an E3 ubiquitin ligase involved in the ubiquitin-proteasome system (Dawson and Dawson, 2003), which has a protective function against a number of toxic insults, including several molecules that are substrates for ubiquitination (Lo Bianco et al., 2004; Yang et al., 2003). Here, we show that overexpression of parkin in a TAF15-expressing background partially rescues the defective phenotypes, including the life span and locomotive activity, which are TAF15 induced. Furthermore, our results show that the level of TAF15 expression dramatically decreases when coexpressed with parkin in fly heads. In addition, downregulation of parkin enhanced the expression level of TAF15 in TAF15-expressing fly heads. These data suggest that TAF15 may be a novel substrate of parkin. Thus, our results provide new insights into the pathogenic mechanisms underlying TAF15-induced ALS and suggest drug targets for developing therapeutic approaches to TAF15-associated neurodegenerative diseases.

2. Materials and methods

2.1. Drosophila *stocks*

All stock flies were raised at 25 °C on standard food. Crosses were performed using a standard procedure, and all progeny were raised at 29 °C. The *UAS-TAF15* lines were a gift from Dr N. Bonini. The *UAS-parkin*, *UAS-parkin*^{K71P}, *UAS-parkin*$^{\Delta N}$, and *UAS-PINK1* lines were obtained from Dr J. Chung. The *UAS-parkin RNAi* (BL31259) and *UAS-ubiquitin* (BL32055) lines were obtained from the Bloomington *Drosophila* Stock Center. The pan-neuronal driver, *elav-Gal4*, and the eye-specific driver, *GMR-Gal4* lines, were also obtained from the Bloomington *Drosophila* Stock Center. W^{1118} flies were used as a control. Fly lines are listed in Table S1.

2.2. *Immunoprecipitation assay*

Two hundred male fly heads were dissected from 7-day-old flies and extracted with an immunoprecipitation (IP) lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 5 mM ethylenediaminetetraacetic acid, 5 mM ethylene glycol tetraacetic acid, 0.1% NP-40, and 1× protease inhibitor cocktail, pH 8.0). The supernatant was collected and mixed with anti-TAF15 antibody (4 μg; GeneTex) or anti-*Drosophila* parkin antibody (1:100, a gift from Leo Pallack, University of Washington) at 4 °C for 4 hours. The samples were mixed with protein G sepharose (Sigma-Aldrich) at 4 °C for 12 hours. After 8 washes with the IP lysis buffer, samples were denatured in 2× SDS sample buffer, separated using a 4%–12% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, transferred to polyvinylidene difluoride membranes (Millipore), and analyzed using Western blotting.

2.3. *Life span and climbing assay*

Twenty male flies of each genotype were placed into different vials and maintained at 29 °C. On each of the subsequent days, all groups were transferred to fresh vials. The number of dead flies was recorded.

Climbing performance was assessed using a negative geotaxis assay. Ten male flies of each age group were anesthetized with CO_2 and placed in a column vial. After a 30-min recovery, flies were tapped to the bottom of the column. Flies that climbed to the top of the column were counted after 1 minute. The assay was repeated 4 times for each sample of flies at 5-minute intervals.

2.4. *Immunohistochemistry*

Adult fly brains were dissected from 14-day-old flies and fixed with 4% formaldehyde in a fixative buffer (100 mM PIPES, 1 mM ethylene glycol tetraacetic acid, 1% Triton X-100, and 2 mM $MgSO_4$, pH 6.9). The brains were then stained overnight at 4 °C with rabbit anti-TAF15 antibody (1:200; GeneTex). The samples were then incubated with Cy3-conjugated secondary antibodies (1:200; Jackson ImmunoResearch) and DAPI (1:500; Sigma-Aldrich). All images were analyzed using a DE/LSM510 NLO Carl Zeiss confocal microscope.

2.5. *Western blot analysis*

Protein extracts for Western blot analysis were prepared by homogenizing ten 14-day-old male fly heads. The total protein extracts (10 μg) were separated using a 4%–12% gradient SDS-PAGE gel and transferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with tris-buffered saline with 4% nonfat dry milk or 4% bovine serum albumin for 1 hour. The following primary antibodies were used: rabbit anti-TAF15 (1:1000; GeneTex), rabbit anti-*Drosophila* parkin (1:1000; a gift from Leo Pallanck, University of Washington), mouse anti-ubiquitin (1:1000; Enzo Life Sciences), and rabbit anti-β-actin (1:4000; Cell Signaling). The primary antibodies were detected with the following horseradish peroxidase (HRP)–conjugated secondary antibodies: goat anti-rabbit IgG HRP conjugate and goat anti-mouse IgG HRP conjugate (1:2000; Millipore). Detection was carried out using an ECL-Plus kit (Amersham).

2.6. *In vivo ubiquitination assay*

Two hundred male fly heads were dissected from 7-day-old flies and homogenized in the IP lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 10% glycerol, 5 mM ethylenediaminetetraacetic acid, 5 mM ethylene glycol tetraacetic acid, 0.1% NP-40, and 1× protease inhibitor cocktail, pH 8.0) with 50 μM MG132 (Millipore) and centrifuged at 12,000 × *g* for 30 minutes at 4 °C. The supernatant was incubated with an anti-TAF15 antibody (3 μg; GeneTex) and then with protein G sepharose (GE Healthcare) for 5 hours at 4 °C. Beads were washed 5 times with phosphate-buffered saline and boiled in the SDS sample buffer (Invitrogen).

3. Results

To find novel regulators of TAF15-induced neurodegenerative disease, we sought to identify novel binding partners of TAF15 that are related to protein quality control. We first performed a gene ontology analysis using the GeneMANIA prediction server (Warde-Farley et al., 2010) to generate networks of interaction between TAF15 and parkin (Fig. S1). Because the results showed that TAF15 exist in tight networks with parkin and various other proteins, we speculate that TAF15 binds to parkin. We subsequently examined this physical interaction by coimmunoprecipitation using TAF15-expressing *Drosophila* head extracts. We found that parkin reciprocally coimmunoprecipitated with TAF15 (Fig. 1A and B, arrowhead). This result showed that parkin is a novel interacting partner of TAF15 in *Drosophila*. Notably, parkin is an E3 ubiquitin ligase involved in the degradation of misfolded or aggregated proteins (Shimura et al., 2000). Furthermore, previous studies have indicated that parkin increases proteasome activity, leading to the degradation of ubiquitinated proteins (Burns et al.,

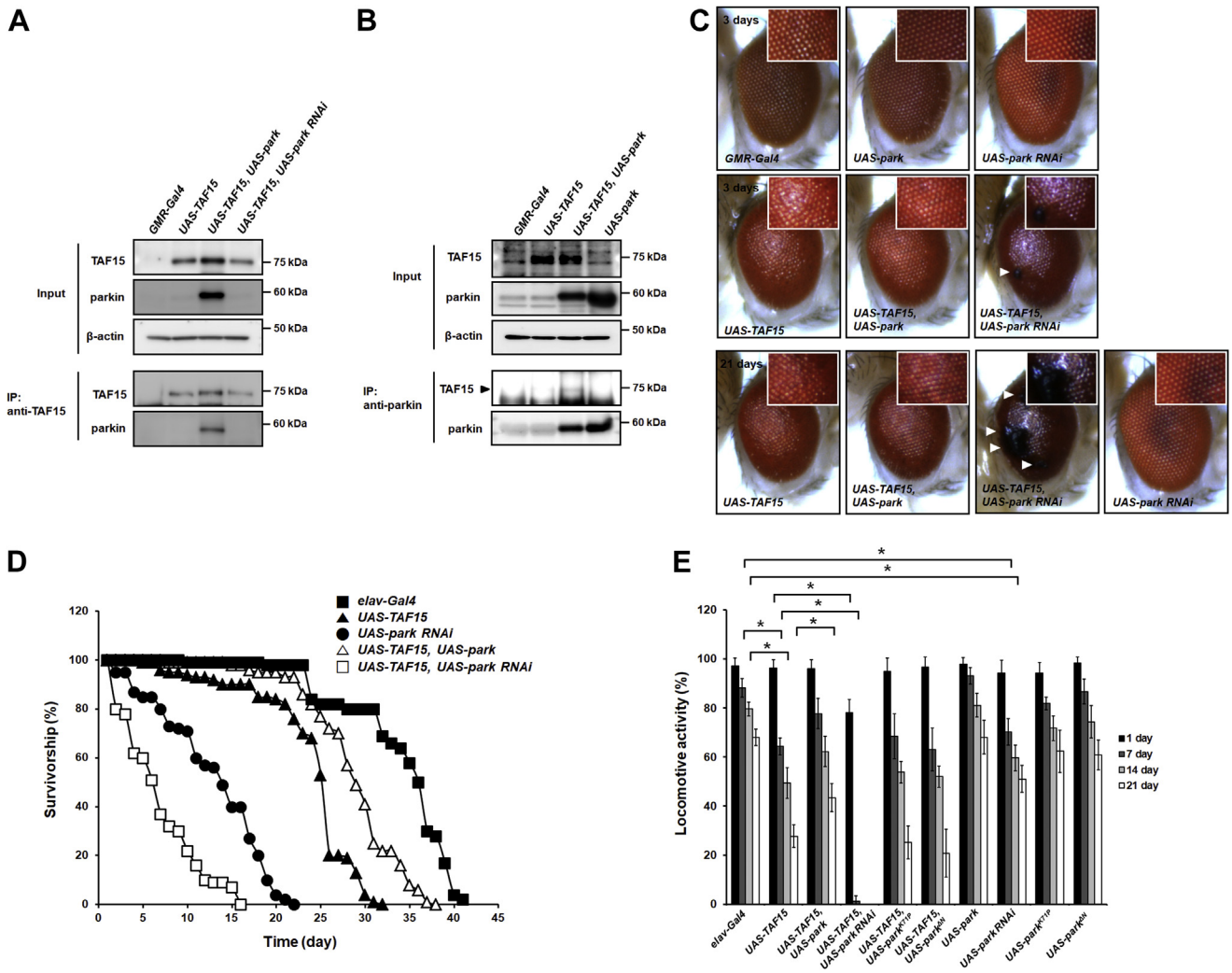

Fig. 1. Parkin is a novel binding partner and regulator for a TAF15-induced proteinopathy in *Drosophila*. (A and B) Overexpression of TAF15 together with parkin in fly heads allowed the reciprocal coimmunoprecipitation of TAF15 with parkin using anti-TAF15 and anti-parkin as capture antibodies and anti-*Drosophila* parkin and anti-TAF15 antibodies for Western blotting (arrowhead). TAF15 bound to the parkin in fly head extracts. β-actin and total protein input were used as protein loading controls. (C) Genetic modulation of parkin altered the TAF15-induced retinal toxicity. A normal eye phenotype was observed in *GMR-Gal4* control flies and flies expressing parkin or *parkin* RNAi alone in the eye (top panels, 3-day-old flies). Overexpression of parkin by the *GMR-Gal4* eye-specific driver suppressed the rough eye phenotypes of TAF15-overexpressing flies. The TAF15-induced rough eye phenotype was enhanced in transgenic flies coexpressing TAF15 with *parkin* RNAi (middle panels, 3-day-old flies, arrowhead: necrotic plaque). The necrotic plaques in *parkin* RNAi and TAF15 coexpressing flies were severely increased in an age-dependent manner (bottom panels, 21-day-old flies, arrowheads). (D) Life span of adult male flies. Overexpression of parkin in neuronal cells partially improved the reduced life span seen in TAF15-induced flies. Overexpression of *parkin* RNAi with TAF15 severely reduced the life span of these flies ($n \geq 150$). Flies from each genotype were subjected to survival assays at 29 °C. (E) Percentage of locomotive activity. Locomotive activity of control and TAF15-expressing flies in the presence of parkin overexpression or RNAi. Overexpression of parkin in neuronal cells suppressed the reduced locomotive activity in TAF15-induced flies. Flies coexpressing TAF15 with *parkin* RNAi displayed severely defective locomotive activity. Error bars represent mean ± standard deviation of 3 independent experiments. The significance was determined using two-way ANOVA with a Tukey's multiple-comparison test (*$p < 0.01$). Abbreviations: ANOVA, analysis of variance; TAF15, TATA-binding protein associated factor 15.

2009; Khandelwal et al., 2010). Therefore, these data led us to investigate the importance of the *parkin* gene on protein quality control by modifying a neurodegenerative phenotype in TAF15-expressing flies.

We next examined the genetic interactions between *parkin* and *TAF15* in *Drosophila* eye. Taking advantage of the retinal degeneration phenotype induced by TAF15 overexpression using the *GMR-Gal4* driver, we performed genetic interaction experiments. As shown in Fig. 1C, expression of parkin and *parkin* RNAi did not result in obvious defects in the eye (Fig. 1C and Fig. S2). However, expression of TAF15 led to retinal degeneration, consistent with previous research (Couthouis et al., 2011). Overexpression of the *parkin* gene in the *Drosophila* eye suppressed the retinal degenerative phenotype induced by TAF15. Furthermore, specific down-regulation of the *parkin* gene severely enhanced the rough eye phenotype in TAF15-expressing flies. These flies often had necrotic plaques (Fig. 1C, arrowheads), indicative of considerable cell death. The necrotic plaques in *parkin* RNAi and TAF15 coexpressing flies were severely increased in an age-dependent manner. Furthermore, we examined the effects of parkin coexpression on life span. Previous research showed that TAF15 induced a dramatically reduced life span when expressed in the neuronal system (Couthouis et al., 2011). TAF15 has activity sufficient to confer neurodegeneration, in a manner similar to that of other ALS-causing proteins, including TDP-43 and FUS (Couthouis et al., 2011). When driven by the neuron-specific *elav-Gal4* driver, the overexpression of TAF15 significantly affected the viability of the flies in our experiments. Consistent with their phenotypes in the eye, TAF15-expressing flies displayed a decreased life span. The expression of parkin increased the life span of the TAF15-expressing

flies, while the downregulation of parkin in TAF15-expressing flies severely decreased their life span when compared with flies that expressed either TAF15 or *parkin* RNAi (Fig. 1D). We also found that the locomotive activity of TAF15-expressing flies was markedly improved in the presence of parkin overexpression. However, parkin downregulation dramatically promoted an age-dependent impairment of locomotive activity in TAF15-expressing flies (Fig. 1E). We next expressed membrane-tethered red fluorescent proteins (mCD8-RFP) using the motor neuron-specific *D42-Gal4* driver to visualize motor neuronal morphology in adult fly legs. TAF15-expressing flies displayed increased axonal degeneration in the motor neurons of their legs. The expression of parkin rescued the axonal degeneration in TAF15-expressing flies (Fig. S3). Taken together, these results indicate that parkin is a novel modifier of TAF15-induced neurodegeneration and that the neuron-specific expression of parkin protects against TAF15-induced neurotoxicity in *Drosophila*.

Previous studies have indicated that parkin has an E3 ubiquitin ligase activity (Cha et al., 2005; Um et al., 2010). To determine the molecular mechanism of parkin-mediated protection against TAF15-induced neurodegeneration, we measured the protective effect on locomotive activity in mutants coexpressing parkin and TAF15. Interestingly, the expression of 2 mutants of parkin, *parkin*$^{\Delta N}$, a ubiquitin-like domain deletion mutant, and *parkin*^{K71P} that contains a point mutation similar to the R42P mutation in autosomal recessive juvenile parkinsonism patients, did not suppress the decreased locomotive activity in TAF15-induced flies (Fig. 1E). These results indicate that parkin suppressed the defective phenotype of the TAF15-induced flies in an E3 ubiquitin ligase activity-dependent manner, potentially further affecting the protein levels of TAF15. To investigate this possibility, we first analyzed the levels of TAF15 in *Drosophila* head extracts. Total proteins were extracted from fly heads, and Western blot analysis was carried out to quantify the amount of TAF15 in a parkin and TAF15 coexpressing mutant in comparison with a TAF15-expressing mutant. As expected, we found that the amount of TAF15 was dramatically decreased in the parkin and TAF15 coexpressing flies (Fig. 2A). Furthermore, the amount of TAF15 in TAF15-expressing flies was strongly increased by the knockdown of parkin (Fig. 2B). Moreover, consistent with our data on locomotive activity, parkin^{K71P} expression did not decrease the TAF15 protein level in TAF15-expressing flies (Fig. 2C). In vivo immunostaining analysis of *Drosophila* whole-brain tissues from TAF15-expressing flies was conducted using TAF15 antibodies. Previous studies have indicated that TAF15 is mislocalized and accumulates in the cytoplasm (Couthouis et al., 2011; Marko et al., 2012). Our results reveal that TAF15 appears to get mislocated and accumulates in the cytoplasm in the brain cells of TAF15-inducing flies. Interestingly, the density of TAF15 signals in the brain were significantly suppressed when coexpressed with parkin (Fig. 2D, magnified view). In addition, we examined the effects of phosphatase and tensin homolog-induced putative kinase 1 (PINK1) overexpression in TAF15-expressing

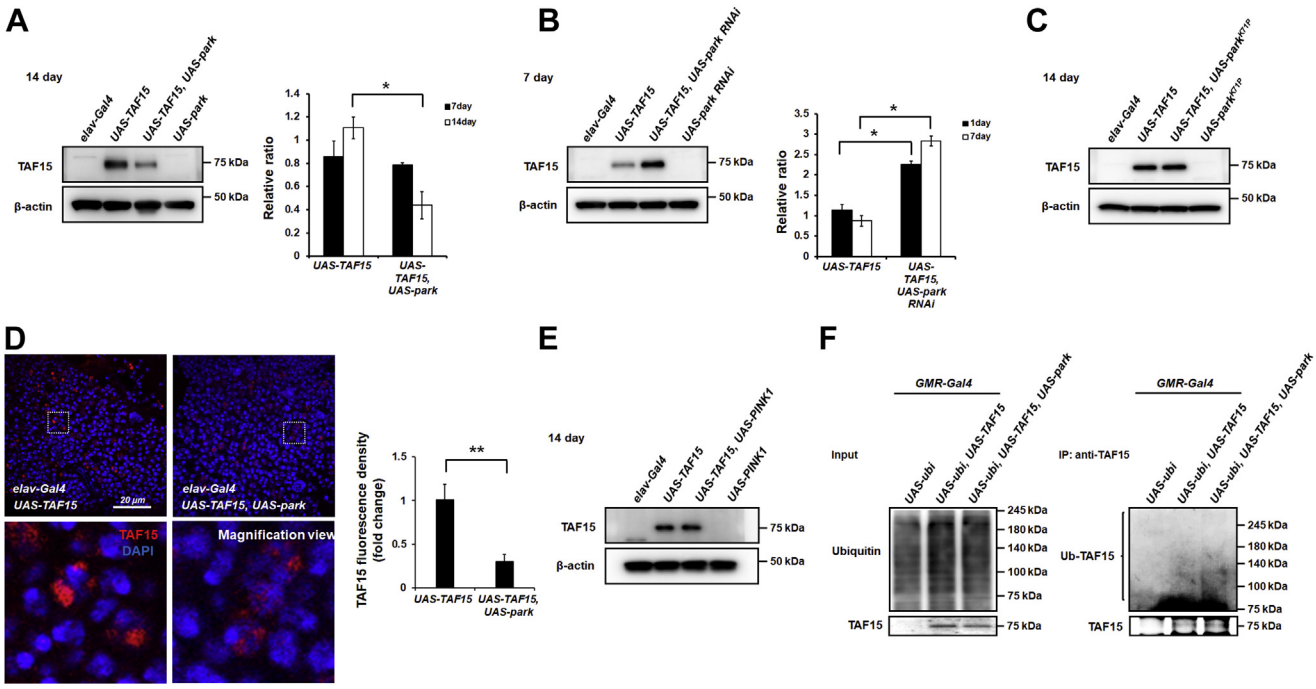

Fig. 2. Parkin regulates the protein level of TAF15 by ubiquitination. (A) Protein levels of TAF15 in 14-day-old parkin coexpressing fly heads. TAF15 and parkin coexpression in neuronal cells resulted in a significant decrease in TAF15 levels. β-actin was used as a loading control. Error bars represent mean ± standard deviation of 3 independent experiments. The experimental significance was determined using a one-way ANOVA (*$p < 0.01$). (B) Protein level of TAF15 in 7-day-old *parkin* RNAi coexpressing fly heads. *Parkin* RNAi led to enhanced TAF15 levels. β-actin was used as a loading control. Error bars represent mean ± standard deviation of 3 independent experiments. The experimental significance was determined using a one-way ANOVA (*$p < 0.01$). (C) Protein level of TAF15 in 14-day-old parkin^{K71P} coexpressing fly heads. The mutant form of parkin, parkin^{K71P}, expression did not decrease the amount of protein in TAF15-expressing flies. β-actin was used as a loading control. (D) TAF15 aggregates in the fly brain. Brains dissected from 14-day-old TAF15-expressing or parkin and TAF15 coexpressing flies were stained with anti-TAF15 antibody (red) and DAPI (blue). Cytoplasmic aggregates were observed in the brain of TAF15-expressing flies. The density of TAF15 signals in the brain was significantly suppressed when coexpressed with parkin (dotted boxes, magnified images; n ≥ 6 brains per genotypes). Statistical significance was determined using the Student t-test (**$p < 0.001$). (E) Overexpression of PINK1 does not have any effect on TAF15 protein levels. β-actin was used as a loading control. (F) Western blot analysis showing in vivo ubiquitination of TAF15 in flies coexpressing parkin and ubiquitin. Ubiquitination of TAF15 is regulated by parkin. Overexpression of TAF15 together with parkin in fly heads showed the coimmunoprecipitation of ubiquitin using anti-TAF15 as a capture antibody and anti-ubiquitin and anti-TAF15 antibodies for Western blotting. Ubiquitinated TAF15 was increased in parkin coexpressing fly heads. All flies were raised at 29 °C. Abbreviations: ANOVA, analysis of variance; PINK1, phosphatase and tensin homolog-induced putative kinase 1; TAF15, TATA-binding protein associated factor 15. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

flies. However, the overexpression of PINK1 did not have any effect on TAF15 protein levels (Fig. 2E). Thus, our results suggest that parkin regulates the amount of TAF15 protein and that the suppressive effect on the TAF15 protein level by parkin is dependent on E3 ubiquitin ligase activity.

It is unclear whether TAF15 can be ubiquitinated by parkin. Therefore, the ability of parkin to ubiquitinate TAF15 was examined. We coexpressed TAF15 and ubiquitin in the eye and performed IP with anti-TAF15 antibody to show ubiquitinated TAF15 in the presence of parkin expression. Parkin overexpression markedly promoted the ubiquitination of TAF15. This observation indicates that parkin drives the ubiquitination of TAF15 (Fig. 2F). Previous studies have suggested that parkin increases proteasome activity, leading to the degradation of ubiquitinated proteins (Hong et al., 2014; Um et al., 2010). Therefore, a lack of parkin activity and proteasomal inhibition may have led to the accumulation of TAF15 and its decreased solubility. Next, to investigate whether autophagy is involved in the degradation of TAF15 by parkin expression, we assessed autophagy using an Atg8-GFP reporter under the control of *elav-Gal4*, which permits the monitoring of autophagy induction, as Atg8 is rapidly degraded in the vacuole but GFP is not (Klionsky et al., 2007). There were no detectable changes in the formation of free GFP in the head extracts of TAF15-expressing flies. Furthermore, we found that there was no considerable change in the autophagic flux between TAF15 expression and TAF15 coexpression with parkin (Fig. S4). Together, these data support the hypothesis that parkin contributes to TAF15-induced neurodegeneration and proteinopathy.

4. Discussion

The purpose of our study was to identify a novel regulator for protection from neurotoxicity and to further understand the protective mechanism against a TAF15-induced proteinopathy, using genetic and molecular experimental approaches in *Drosophila*. Several studies suggest that the ubiquitination of aggregated proteins may offer neuroprotection against various neurodegenerative diseases. This is an attractive proposition considering that the parkin E3 ubiquitin ligase can degrade toxic proteins or aggregates in the cytoplasm. Several studies have examined the role of parkin in neurodegenerative diseases, which are caused by aberrant protein aggregation. Overexpression of parkin has been shown to protect against the neurotoxicity of human A30P α-synuclein in a rat model of Parkinson's disease (Lo Bianco et al., 2004). The overexpression of parkin in amyloid β transgenic mice restores synaptic plasticity and behavioral dysfunction (Hong et al., 2014). Furthermore, parkin overexpression during aging has been shown to decrease proteotoxicity (Rana et al., 2013).

Mutations in TAF15 have been implicated in familial ALS (Couthouis et al., 2011; Ticozzi et al., 2011). TAF15 is predominantly found in the cell nucleus, and it is also present in the cytoplasm of the cell (Marko et al., 2012; Neumann et al., 2011). Furthermore, in familial ALS, TAF15 is mislocalized to the cytoplasm and then found in cytoplasmic aggregates of neuronal cells (Couthouis et al., 2011). However, the exact pathogenic mechanism of TAF15-induced proteinopathy and the molecular mechanisms for removing TAF15 aggregates in the cytoplasm have not been fully elucidated. Here, we showed a critical relationship between parkin E3 ubiquitin ligase and TAF15. Our results showed that parkin dysfunction may play pathogenic roles in TAF15-induced neurodegenerative disease and that overexpression of parkin suppressed the defective phenotypes, including the life span and locomotive activity of TAF15-induced proteinopathy. Notably, Western blotting and immunostaining revealed that overexpression of parkin in neuronal cells led to the reduction of TAF15 levels dependent on parkin-mediated E3 ubiquitin ligase activity. Parkin directly bound to TAF15 and mediated its degradation in response to ubiquitination. Previous studies have suggested that parkin increases proteasome activity, leading to the degradation of ubiquitinated proteins. Therefore, a lack of parkin activity and proteasomal inhibition may have led to TAF15 accumulation and its decreased solubility. The activation of parkin led to the ubiquitination of TAF15, indicating the involvement of parkin in TAF15 cellular metabolism, including its cytosolic accumulation and aggregation. Furthermore, our data showed that PINK1 failed to rescue TAF15-expressing flies. Thus, *TAF15* either functions downstream of *parkin* or acts independently of *parkin* in a parallel pathway downstream of *PINK1*.

In conclusion, because the pathogenesis of TAF15-induced proteinopathy involves cytosolic aggregation of toxic proteins, it is important to have a mechanism that mediates protein degradation and prevents aggregate formation. Our study provides in vivo evidence supporting the use of parkin for neuroprotection in a TAF15-induced proteinopathy.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.023.

References

Burns, M.P., Zhang, L., Rebeck, G.W., Querfurth, H.W., Moussa, C.E., 2009. Parkin promotes intracellular Abeta1-42 clearance. Hum. Mol. Genet. 18, 3206–3216.
Cha, G.H., Kim, S., Park, J., Lee, E., Kim, M., Lee, S.B., Kim, J.M., Chung, J., Cho, K.S., 2005. Parkin negatively regulates JNK pathway in the dopaminergic neurons of Drosophila. Proc. Natl. Acad. Sci. U S A 102, 10345–10350.
Cleveland, D.W., Rothstein, J.D., 2001. From charcot to lou gehrig: deciphering selective motor neuron death in ALS. Nat. Rev. Neurosci. 2, 806–819.
Couthouis, J., Hart, M.P., Shorter, J., DeJesus-Hernandez, M., Erion, R., Oristano, R., Liu, A.X., Ramos, D., Jethava, N., Hosangadi, D., Epstein, J., Chiang, A., Diaz, Z., Nakaya, T., Ibrahim, F., Kim, H.J., Solski, J.A., Williams, K.L., Mojsilovic-Petrovic, J., Ingre, C., Boylan, K., Graff-Radford, N.R., Dickson, D.W., Clay-Falcone, D., Elman, L., McCluskey, L., Greene, R., Kalb, R.G., Lee, V.M., Trojanowski, J.Q., Ludolph, A., Robberecht, W., Andersen, P.M., Nicholson, G.A., Blair, I.P., King, O.D., Bonini, N.M., Van Deerlin, V., Rademakers, R., Mourelatos, Z., Gitler, A.D., 2011. A yeast functional screen predicts new candidate ALS disease genes. Proc. Natl. Acad. Sci. U S A 108, 20881–20890.
Dawson, T.M., Dawson, V.L., 2003. Molecular pathways of neurodegeneration in Parkinson's disease. Science 302, 819–822.
DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.
Deng, H.X., Chen, W., Hong, S.T., Boycott, K.M., Gorrie, G.H., Siddique, N., Yang, Y., Fecto, F., Shi, Y., Zhai, H., Jiang, H., Hirano, M., Rampersaud, E., Jansen, G.H.,

Donkervoort, S., Bigio, E.H., Brooks, B.R., Ajroud, K., Sufit, R.L., Haines, J.L., Mugnaini, E., Pericak-Vance, M.A., Siddique, T., 2011. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477, 211–215.
Fecto, F., Siddique, T., 2011. Making connections: pathology and genetics link amyotrophic lateral sclerosis with frontotemporal lobe dementia. J. Mol. Neurosci. 45, 663–675.
Gitler, A.D., Shorter, J., 2011. RNA-binding proteins with prion-like domains in ALS and FTLD-U. Prion 5, 179–187.
Hong, X., Liu, J., Zhu, G., Zhuang, Y., Suo, H., Wang, P., Huang, D., Xu, J., Huang, Y., Yu, M., Bian, M., Sheng, Z., Fei, J., Song, H., Behnisch, T., Huang, F., 2014. Parkin overexpression ameliorates hippocampal long-term potentiation and beta-amyloid load in an Alzheimer's disease mouse model. Hum. Mol. Genet. 23, 1056–1072.
Ince, P.G., Highley, J.R., Kirby, J., Wharton, S.B., Takahashi, H., Strong, M.J., Shaw, P.J., 2011. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. Acta Neuropathol. 122, 657–671.
Kapeli, K., Martinez, F.J., Yeo, G.W., 2017. Genetic mutations in RNA-binding proteins and their roles in ALS. Hum. Genet. 136, 1193–1214.
Khandelwal, P.J., Dumanis, S.B., Feng, L.R., Maguire-Zeiss, K., Rebeck, G., Lashuel, H.A., Moussa, C.E., 2010. Parkinson-related parkin reduces alpha-Synuclein phosphorylation in a gene transfer model. Mol. Neurodegener. 5, 47.
Klionsky, D.J., Cuervo, A.M., Seglen, P.O., 2007. Methods for monitoring autophagy from yeast to human. Autophagy 3, 181–206.
Lagier-Tourenne, C., Cleveland, D.W., 2009. Rethinking ALS: the FUS about TDP-43. Cell 136, 1001–1004.
Lo Bianco, C., Schneider, B.L., Bauer, M., Sajadi, A., Brice, A., Iwatsubo, T., Aebischer, P., 2004. Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an alpha-synuclein rat model of Parkinson's disease. Proc. Natl. Acad. Sci. U S A 101, 17510–17515.
Marko, M., Vlassis, A., Guialis, A., Leichter, M., 2012. Domains involved in TAF15 subcellular localisation: dependence on cell type and ongoing transcription. Gene 506, 331–338.
Neumann, M., Bentmann, E., Dormann, D., Jawaid, A., DeJesus-Hernandez, M., Ansorge, O., Roeber, S., Kretzschmar, H.A., Munoz, D.G., Kusaka, H., Yokota, O., Ang, L.C., Bilbao, J., Rademakers, R., Haass, C., Mackenzie, I.R., 2011. FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain 134, 2595–2609.
Rana, A., Rera, M., Walker, D.W., 2013. Parkin overexpression during aging reduces proteotoxicity, alters mitochondrial dynamics, and extends lifespan. Proc. Natl. Acad. Sci. U S A 110, 8638–8643.
Schwartz, J.C., Cech, T.R., Parker, R.R., 2015. Biochemical properties and biological functions of FET proteins. Annu. Rev. Biochem. 84, 355–379.
Shimura, H., Hattori, N., Kubo, S., Mizuno, Y., Asakawa, S., Minoshima, S., Shimizu, N., Iwai, K., Chiba, T., Tanaka, K., Suzuki, T., 2000. Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat. Genet. 25, 302–305.
Svetoni, F., Frisone, P., Paronetto, M.P., 2016. Role of FET proteins in neurodegenerative disorders. RNA Biol. 13, 1089–1102.
Ticozzi, N., Vance, C., Leclerc, A.L., Keagle, P., Glass, J.D., McKenna-Yasek, D., Sapp, P.C., Silani, V., Bosco, D.A., Shaw, C.E., Brown Jr., R.H., Landers, J.E., 2011. Mutational analysis reveals the FUS homolog TAF15 as a candidate gene for familial amyotrophic lateral sclerosis. Am. J. Med. Genet. B Neuropsychiatr. Genet. 156B, 285–290.
Um, J.W., Im, E., Lee, H.J., Min, B., Yoo, L., Yoo, J., Lubbert, H., Stichel-Gunkel, C., Cho, H.S., Yoon, J.B., Chung, K.C., 2010. Parkin directly modulates 26S proteasome activity. J. Neurosci. 30, 11805–11814.
Warde-Farley, D., Donaldson, S.L., Comes, O., Zuberi, K., Badrawi, R., Chao, P., Franz, M., Grouios, C., Kazi, F., Lopes, C.T., Maitland, A., Mostafavi, S., Montojo, J., Shao, Q., Wright, G., Bader, G.D., Morris, Q., 2010. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic Acids Res. 38, W214–W220.
Yang, Y., Nishimura, I., Imai, Y., Takahashi, R., Lu, B., 2003. Parkin suppresses dopaminergic neuron-selective neurotoxicity induced by Pael-R in Drosophila. Neuron 37, 911–924.