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Inhibition of p53 transactivation functionally interacts with microtubule stabilization to suppress excitotoxicity-induced axon degeneration

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ABSTRACT

Axon degeneration is a hallmark of many neurological disorders, including Alzheimer's disease, motor neuron disease, and nerve trauma. Multiple factors trigger axon degeneration, and glutamate excitotoxicity is one of them. We have recently found that stabilization of microtubules and components of the dynein–dynactin complex modulate the process of excitotoxicity-induced axon degeneration. However, the molecular mechanisms involving these microtubule-based functions remain poorly understood. Here, we used hippocampal cultures and find that inhibition of p53 transactivation and microtubule stabilization function cooperatively to suppress excitotoxicity-induced mitochondrial dysfunction. Inhibition of p53 association with mitochondria has no effect on excitotoxicity-induced mitochondrial dysfunction, however, induces axon degeneration in normal condition. Association of p150Glued with mitochondria is significantly increased by simultaneously inhibiting p53 transactivation and microtubule stabilization under excitotoxic condition. Importantly, we find that inhibition of p53 transactivation and microtubule stabilization function cooperatively to suppress excitotoxicity-induced axon degeneration. Overexpression of p150Glued does not improve the effect by inhibition of p53 transactivation on axon degeneration suggesting that p150Glued and p53 function in a linear pathway in the process of axon degeneration.

1. Introduction

Axon degeneration is an active, controlled, and versatile process of the axonal compartment competent for self-destruction. It can be observed in conditions such as neurodegenerative disease, nerve trauma, and pruning during normal neuronal development [1]. The process of axon degeneration is shared with sequence of events, including microtubule disassembly, axonal swelling/beading, axon fragmentation, and removal of the remnants by activated glia [2]. In most neurodegenerative disease, axonal loss precedes the appearance of symptoms and the loss of neuronal cell bodies [1]. In a mouse model of progressive motor neuropathy and superoxide dismutase 1 transgenic mouse, axon degeneration correlates with the onset and progression of the disease as well as the time of death irrespective of the genetic inhibition of motor neuron cell body death [3,4]. Thus, axon degeneration is likely to be distinct from cell body death and the leading cause of many neurodegenerative diseases.

Context dependent factors, including insults such as excessive glutamate exposure, could trigger axon degeneration. Glutamate is an excitatory neurotransmitter in the CNS that plays a pivotal role in long-term potentiation and cognitive functions such as learning and memory. However, exposure to excessive glutamate overactivates glutamate receptors and triggers neurodegenerative processes known as excitotoxicity and induces morphological changes in the axon and neuronal death [5]. Excitotoxicity is linked to chronic neurological disorders, such as Alzheimer's disease and amyotrophic lateral sclerosis, and acute central nervous system insults [6]. The mechanism underlying excitotoxicity is complex. Overactivated NMDA receptors trigger calcium influx, and lead to numerous events that are detrimental to normal neuronal function, including acute mitochondrial dysfunction and free radical production [6]. Therefore, identifying converging mechanisms triggered by excitotoxicity is important for the better understanding of the degenerative processes.

Recently, we identified the dynein–dynactin complex, a microtubule-based retrograde transport protein complex, as novel modulators of excitotoxicity-induced axon degeneration [5]. Overexpression of p150Glued, a major component of the dynactin complex, and dynein intermediate chain (DIC), a major component

Abbreviations: DIC, dynein intermediate chain; DIV, days *in vitro*; DMSO, dimethyl sulfoxide; GFP, green fluorescent protein; PFT, Pifithrin; VDAC, voltage-dependent anion channel; WT, wild type.

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of the dynein complex that interacts with p150Glued and links dynein and dynactin complexes, significantly suppressed excitotoxicity-induced axon degeneration. In addition, suppression of axon degeneration by p150Glued overexpression was further achieved by microtubule stabilization [7]. Thus, components of the dynein–dynactin complex and microtubule stability are important factors to protect axons from degeneration. Among known cargos of the dynein–dynactin complex, a tumor suppressor protein p53 associates with dynein in the cytoplasm prior to being transported and accumulated in the nucleus [8]. p53 is a transcription factor that transactivates genes with a variety of functions, including cell cycle arrest and apoptosis, and triggers apoptosis by translocating to the mitochondria [9,10]. In neurons, p53 localize in the axon and growth cones and could function to assist proper axonal development and wiring [11].

Here, we identify p53 that functions cooperatively with microtubule stability in the process of excitotoxicity-induced axon degeneration. Using primary hippocampal cultures, we find that inhibition of p53 transactivation by Pifithrin-α (PFT-α) and microtubule stabilization by taxol function cooperatively to suppress excitotoxicity-induced mitochondrial dysfunction. Inhibition of mitochondrial association of p53 by PFT-μ has no effect on excitotoxicity-induced mitochondrial dysfunction, however, induces axon degeneration in normal condition. Importantly, we find that simultaneous PFT-α and taxol treatments suppress excitotoxicity-induced axon degeneration. Overexpression of p150Glued does not improve the suppression of axon degeneration by inhibition of p53 transactivation suggesting that p150Glued and p53 function in a linear pathway in the process of axon degeneration.

2. Materials and methods

2.1. cDNA cloning and expression vectors

Methods for cloning rat p150Glued cDNA and the generation of GFP-p150Glued WT in pEGFP-C1 vector (Clontech) are previously described [5].

2.2. Antibodies and compounds

Primary antibodies used are rat monoclonal anti-GFP (Nacalai Tesque), mouse monoclonal anti-p150Glued (BD Biosciences, N-terminal recognition), mouse monoclonal anti-Dynein intermediate chains cytoplasmic (Millipore), mouse monoclonal anti-βIII-tubulin (Covance), and rabbit polyclonal anti-VDAC (Cell Signaling) antibodies. Secondary antibodies are horseradish peroxidase (HRP)-conjugated anti-mouse or anti-rabbit IgG (Cell Signaling), and Alexa Fluor® 488, 568, or 647 fluorescents (Invitrogen). Compounds used are Pifithrin-α (PFT-α, Calbiochem), Pifithrin-μ (PFT-μ, Calbiochem), and Paclitaxel (taxol, Sigma).

2.3. Neuron culture, immunofluorescence, and transfection

Preparation of primary Wistar rat hippocampal neurons and culture conditions for indirect immunofluorescence labeling of transfected neurons were performed as previously described [5]. Procedures were approved by the Osaka University Institutional Guidelines for the Care and Use of Laboratory Animals. Transfection was performed using AMAXA Nucleofector transfection system (Lonza) with 4 μg of plasmid DNA and 3×10^6 of cells. Images were captured by confocal laser microscopy FV1000 system (Olympus) with 40× and 60× oil-immersion objective lenses, with 2× or 3× zoom.

2.4. Neurite beading analysis

Hippocampal cultures subjected for neurite beading analysis were incubated for 8 or 14 days *in vitro* (DIV) and treated with 50 μM glutamate (Sigma), 10 μM PFT-α, 10 μM PFT-μ, 100 nM taxol, DMSO (Sigma), or vehicle. By observing visually-isolated transfected neurons, a bead was defined as follows: Using FV10-ASW1.7 software (Olympus), images were captured with the same exposure and the signal intensity of a dot labeled with GFP and βIII-tubulin was "more than 1500". When the signal intensity of the neurite shaft adjacent to the dot was "less than 200", the dot was defined as "a bead". Neurite of 100 μm was regarded as one segment and two segments were analyzed for each neuron and when the total sum was more than 10 beads, the neuron contained "bead-containing neurites".

2.5. Formazan dye assay

Mitochondrial dehydrogenase activity of hippocampal cultures treated with 50 μM glutamate, 10 μM PFT-α, 10 μM PFT-μ, 100 nM taxol, DMSO, and vehicle for 3 h, was measured using tetrazolium salt, WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] (Cell Count Reagent SF, Nacalai Tesque). Cells of 7×10^4 were seeded on 24-well plates (Nunc) pre-coated with 0.05 mg/ml poly-L-lysine (Sigma) and incubated for 8 DIV at 37 °C, 5% CO_2. Assay was performed mainly under manufacture's instruction and O.D. 450 nm was measured for formazan dye production.

2.6. Western blot detection

Hippocampal cultures of 4×10^6 were treated with 50 μM glutamate, 10 μM PFT-α, 100 nM taxol, and vehicle. Cells were harvested and mitochondrial fractions were obtained by using Mitochondria Isolation Kit for Cell Cultures (Pierce) under manufacture's instruction. Post-nuclear supernatants were quantified and approximately 11 μg of mitochondrial and cytosolic fractions in sum was subjected for Western blot analysis. Samples were prepared in sample buffer, boiled, and loaded onto 5–20% polyacrylamide gradient gel (Gellex International), transferred to nitrocellulose membranes by iblot TM gel transfer system (Invitrogen), further blotted and detected as previously described [5]. Blotted membranes were subjected to new rounds of probing using WB Stripping Solution (Nacalai Tesque). Densitometry was performed on Western blots for quantification.

2.7. Statistics

Statistical analyses were done by one-way ANOVA with Tukey–Kramer post test. In all instances, a value of $p < 0.05$ was considered significant.

3. Results

3.1. Simultaneous PFT-α and taxol treatment suppress mitochondrial dysfunction

We have previously shown that overexpresssion of p150Glued and DIC, and stabilization of axonal microtubules by taxol suppress excitotoxicity-induced axon degeneration [5,7]. p53 is a known cargo of the microtubule-based retrograde transport protein complex as it associates with dynein [8,12]. Genetic loss of p53 or inhibition of p53 transactivation by a compound, PFT-α, is known to suppress mitochondrial membrane depolarization induced by excitotoxic and oxidative insults [13]. To elucidate

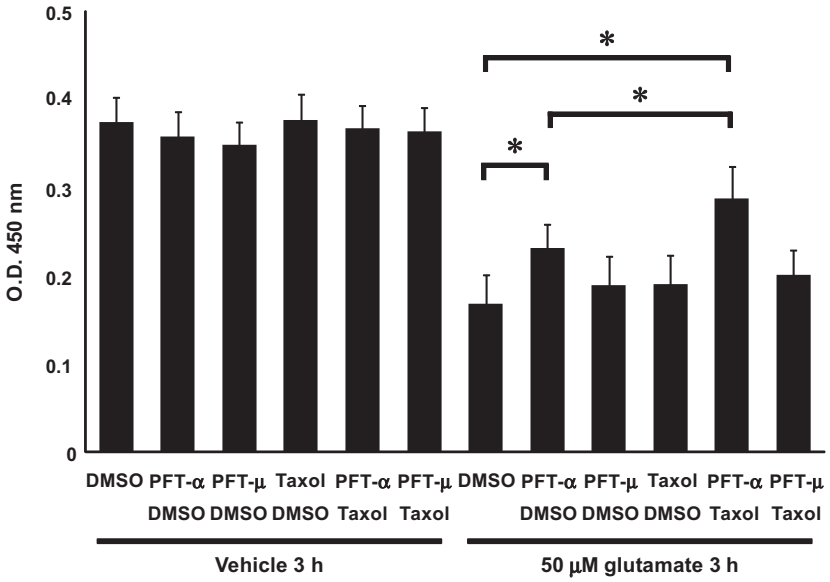

Fig. 1. PFT-α and taxol cooperatively suppress mitochondrial dysfunction. Mitochondrial dehydrogenase activity was analyzed using tetrazolium salt, WST-8, and formazan dye production was quantified by measuring O.D. 450 nm. In 3 h treatment of vehicle added with DMSO, 10 μM PFT-α, 10 μM PFT-μ, 100 nM taxol, 10 μM PFT-α + 100 nM taxol, and 10 μM PFT-μ + 100 nM taxol, showed 0.38, 0.36, 0.35, 0.38, 0.37, and 0.36, respectively. In 3 h treatment of 50 μM glutamate added with DMSO, 10 μM PFT-α, 10 μM PFT-μ, 100 nM taxol, 10 μM PFT-α + 100 nM taxol, and 10 μM PFT-μ + 100 nM taxol, showed 0.17, 0.23, 0.19, 0.19, 0.29, and 0.20, respectively ($^{*}p < 0.01$). Statistics represent mean ± SEM of 3 independent experiments and 20 independent sets of samples.

the functional relevance of p53 and microtubule-based functions in modulating excitotoxicity-induced defects, we took a pharmacological approach to inhibit p53 function. We used PFT-α that inhibits p53-dependent transactivation, and PFT-μ that specifically inhibits p53 association with mitochondria and apoptosis [14,15]. Previous studies have used PFT-α at 0.5–10 μM and PFT-μ at 10–50 μM concentrations to treat neurons [11,15], thus, we selected the concentration of 10 μM for our study. In 8 DIV hippocampal cultures, simultaneous treatment with glutamate and PFT-α, but not PFT-μ, for 3 h suppressed excitotoxicity-induced mitochondrial dysfunction by approximately 35% compared with that of glutamate treatment alone (Fig. 1). Simultaneous treatment with glutamate, PFT-α, and taxol further suppressed excitotoxicity-induced mitochondrial dysfunction by approximately 70% and 24% compared with that of glutamate alone and glutamate with PFT-α treatments, respectively (Fig. 1). Subtle effect was observed by simultaneous treatment with glutamate, PFT-μ, and taxol, or with glutamate and taxol compared with glutamate treatment (Fig. 1). No notable alteration was observed among treatments in vehicle treated conditions (Fig. 1). This result indicates that inhibition of p53 transactivation and microtubule stabilization function cooperatively to suppress excitotoxicity-induced mitochondrial dysfunction.

3.2. PFT-μ induces axon degeneration

A focal bead-like swelling phenotype in neurites is an early neurodegenerative feature in acute and chronic neurological disorders [16]. In 8 DIV hippocampal neurons, we observed GFP-overexpressing visually-isolated neurons containing beading neurites at basal levels when treated with either PFT-α or PFT-μ for 3 h compared with that treated with DMSO for 10 h (Fig. 2B). However, treatment with PFT-μ for 6 and 10 h showed approximately 17% and 44% the number of neurons with beading neurites, respectively, an increase of degenerating axons more than 17-fold with 6 h and more than 40-fold with 10 h treatments, compared with that of DMSO control (Fig. 2A and B). This phenotype with PFT-μ was also observed in mature 14 DIV hippocampal neurons as 10 h treatment with PFT-μ showed approximately 40% the number of neurons with beading neurites, a more than 10-fold increase compared with that of DMSO control (Fig. 2C). This result indicates that inhibition of mitochondrial association of p53, but not the transactivation function of p53, induces axon degeneration.

3.3. PFT-α and taxol cooperatively increase p150Glued association to mitochondria

The dynein–dynactin complex is known to associate with mitochondria possibly through DIC and dynactin components, including p150Glued, and drives retrograde mitochondria movements [17]. As simultaneous PFT-α and taxol treatment suppresses excitotoxicity-induced mitochondrial dysfunction (Fig. 1), we addressed whether mitochondrial association of major components of the dynein–dynactin complex is altered by PFT-α and taxol treatments. We obtained mitochondrial fractions from indicated conditions and assessed if there are quantitative alterations in p150Glued and DIC associated with mitochondria (Fig. 3A and B). Excitotoxicity generates a C-terminal truncated form of p150Glued that contributes to the process of axon degeneration and the alteration of cargo localization within neurites [5,7]. In excitotoxic condition, mitochondrial association of both normal and truncated forms of p150Glued was moderately increased when treated with PFT-α or taxol compared with that of DMSO control (Fig. 3A and B). By contrast, more than 70% increase was observed by simultaneous PFT-α and taxol treatment compared with that of DMSO control (Fig. 3A and B). Mitochondrial association of DIC was moderately altered within 20% for each condition compared with that of DMSO control in excitotoxic condition (Fig. 3A and B). This result indicates that mitochondria association of p150Glued is increased by simultaneous inhibition of p53 transactivation and microtubule stabilization and suggests a contribution of p150Glued in modulating mitochondrial function under excitotoxic condition.

3.4. Simultaneous PFT-α and taxol treatment suppress axon degeneration

As inhibition of p53 transactivation by PFT-α and microtubule stabilization by taxol function cooperatively to suppress mitochondrial dysfunction, we assessed whether simultaneous PFT-α and

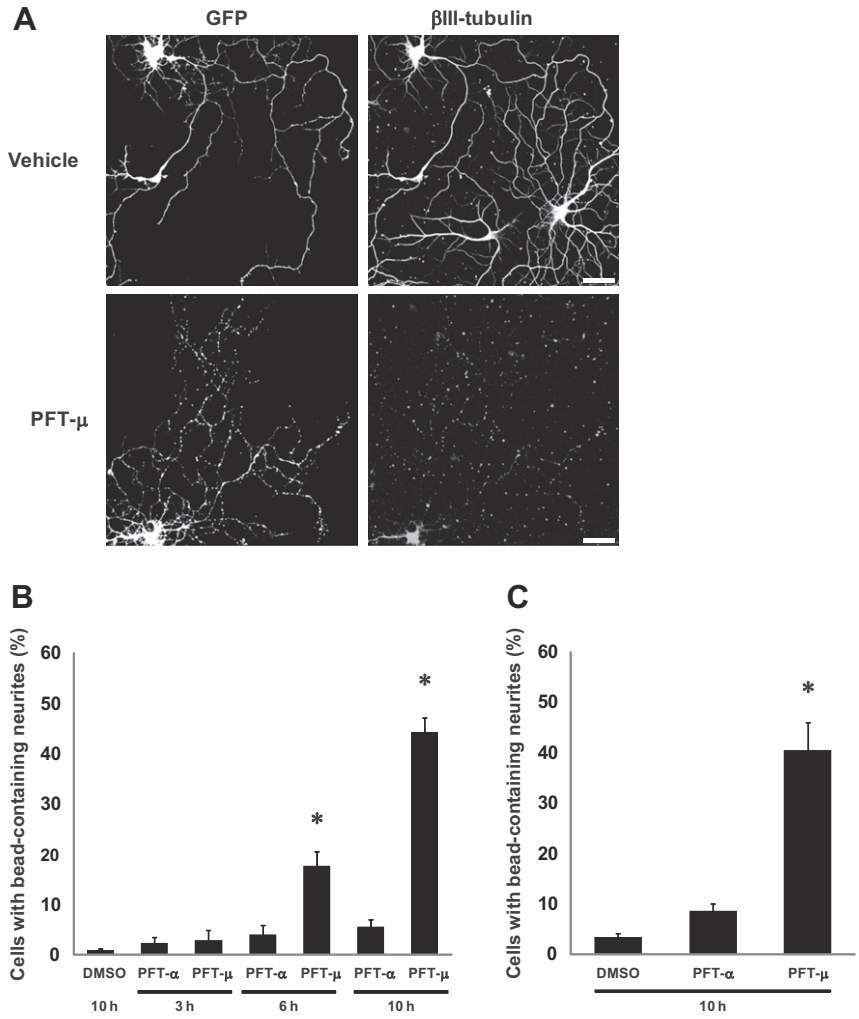

Fig. 2. PFT-μ induces axon degeneration. (A) Representative images of GFP vector-transfected 8 DIV neurons treated with 10 μM PFT-μ or DMSO for 6 h and labeled for GFP and βIII-tubulin. Bars: 40 μm. (B) Quantification of neurons containing beading neurites (>187 cells counted for each condition). Neurons treated with DMSO for 10 h showed 1.0%. Neurons treated with 10 μM PFT-α and 10 μM PFT-μ for 3 h showed 2.3% and 3.0%, respectively. Neurons treated with 10 μM PFT-α and 10 μM PFT-μ for 6 h showed 4.0% and 17.7%, respectively. Neurons treated with 10 μM PFT-α and 10 μM PFT-μ for 10 h showed 5.7% and 44.3%, respectively. $^{*}p < 0.01$, compared with 10 h DMSO control. (C) Quantification of 14 DIV neurons containing beading neurites (>312 cells counted for each condition). In 10 h treatment, neurons treated with DMSO, 10 μM PFT-α, and 10 μM PFT-μ showed 3.4%, 8.6%, and 40.4%, respectively. $^{*}p < 0.01$, compared with DMSO control. All statistics represent mean ± SEM of 3 independent experiments.

taxol treatment could further protect axons from degeneration. The neurite beading phenotype of GFP-overexpressing neurons treated with glutamate, PFT-α, taxol, DMSO, and vehicle for 3 h was analyzed (Fig. 4A). Treatments with PFT-α and taxol in combination with glutamate suppressed approximately 30% and 24%, respectively, the number of neurons with beading neurites compared with that of glutamate treatment alone (Fig. 4B). Simultaneous PFT-α and taxol treatment in combination with glutamate suppressed approximately 45% the number of neurons with beading neurites compared with that of glutamate treatment alone (Fig. 4B). This result indicates that inhibition of p53 transactivation and microtubule stabilization functions cooperatively to protect axons from excitotoxicity-induced degeneration. Previously, we have shown that microtubule stabilization by taxol and overexpression of p150Glued function cooperatively to suppress excitotoxicity-induced axon degeneration [7]. Thus, we next addressed whether p150Glued overexpression shows a cooperative effect with the inhibition of p53 transactivation. By analyzing neurons overexpressing GFP-p150Glued WT, treatment with PFT-α in combination with glutamate moderately suppressed approximately 11% the number of neurons with beading neurites compared with that of glutamate treatment alone (Fig. 4B). Simultaneous PFT-α and taxol treatment in combination with glutamate suppressed approximately 30% the number of neurons with beading neurites compared with that of glutamate treatment alone (Fig. 4B). As treatment with taxol in combination with glutamate suppressed approximately 30% the number of neurons with beading neurites compared with that of glutamate treatment alone, additional effect by inhibition of p53 transactivation was not observed (Fig. 4B). This result indicates that either inhibition of p53 transactivation or overexpression of p150Glued and microtubule stabilization function cooperatively to protect axons from excitotoxicity-induced degeneration.

4. Discussion

Our results demonstrate for the first time that inhibition of p53 transactivation functionally interacts with microtubule stabilization to protect axons from excitotoxicity-induced degeneration and suppress mitochondrial dysfunction. Importantly, the inhibited function of p53 that influences the morphological defect of

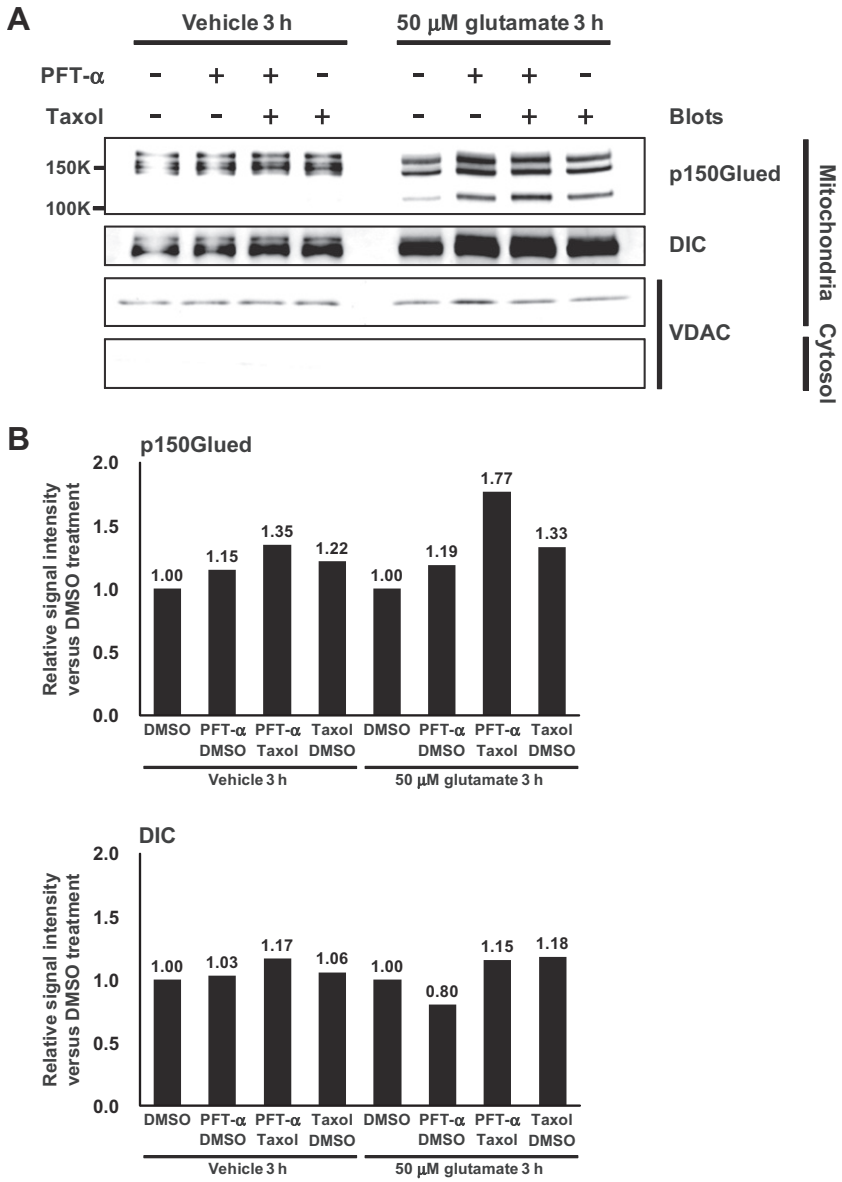

Fig. 3. PFT-α and taxol cooperatively increase p150Glued association with mitochondria. (A) Western blot detections of p150Glued, DIC, and VDAC were performed on a single sheet using mitochondrial and cytosolic fractions prepared from 8 DIV hippocampal cultures treated as indicated for 3 h. Detection of p150Glued was performed with the N-terminal-recognizing antibody. VDAC is used as a mitochondrial fraction marker. (B) Quantified relative signal intensity of p150Glued and DIC blots for each treatment shown in (A) versus DMSO control. Signal intensity of blots of total p150Glued and DIC was normalized by VDAC signal intensity.

axon and mitochondrial dysfunction is the transactivation function. Mitochondrial association of p53, that could induce apoptosis, does not have notable impact in excitotoxic condition. However, in normal condition, inhibition of p53 association with mitochondria induces deleterious effects on axons. Thus our results indicate that the stability of microtubules in axons is compromised and that the destructive mechanisms are initiated through p53 transactivation in excitotoxic condition, generating the idea that both microtubule stability and p53 function are critical for the maintenance of axonal integrity and mitochondrial function.

Transport of mitochondria along axons over substantial distances is crucial for the maintenance of neuronal structure and function [17]. Mitochondria supplies metabolites and energy in the form of ATP to where they are consumed in axons, however, excitotoxicity triggers excessive calcium influx and mitochondrial dysfunction with events, including mitochondrial outer membrane permeabilization [6]. A significant improvement of mitochondrial function in excitotoxic condition was observed by simultaneously inhibiting p53 transactivation and stabilizing microtubules that was mainly due to the inhibition of p53 transactivation, as taxol treatment alone showed subtle effect on defective mitochondrial function. p53 transactivates a number of genes with variety of functions, including cell cycle arrest and apoptosis [9,10]. Among p53-transactivated gene products engaged in cell cycle arrest, p21 has previously been shown to protect cortical neurons from excitotoxicity-induced degeneration [18]. This suggests that p53-transactivated gene products that function in cell cycle arrest could protect mitochondrial function. In this case, inhibition of p53 transactivation would not be beneficial for neurons. On the other hand, p53 transactivates gene products Bax, PUMA, and Noxa, all of which exert pro-apoptotic functions by inducing mitochondrial outer membrane permeabilization [9]. Thus, inhibiting the

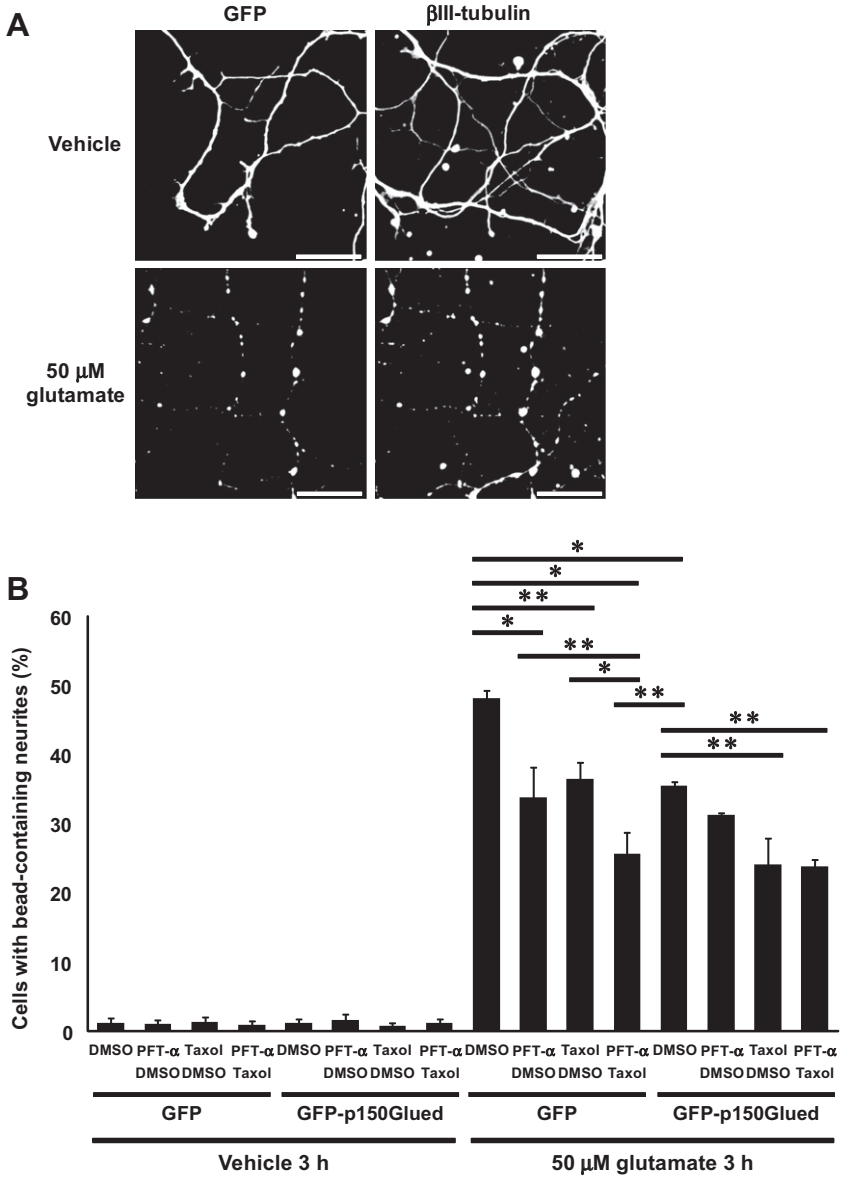

Fig. 4. PFT-α and taxol cooperatively suppress axon degeneration. (A) Representative images of GFP vector-transfected 8 DIV neurites treated with 50 μM glutamate or vehicle for 3 h and labeled for GFP and βIII-tubulin. Bars: 20 μm. (B) Quantification of neurons containing beading neurites (>197 cells counted for each condition). Vector and GFP-p150Glued WT-transfected neurons treated with 50 μM glutamate, 10 μM PFT-α, 100 nM taxol, DMSO, and vehicle as indicated for 3 h. In glutamate-treated neurons transfected with vector and added with DMSO, 10 μM PFT-α, 100 nM taxol, and 10 μM PFT-α + 100 nM taxol, showed 48.1%, 33.8%, 36.5%, and 25.7%, respectively ($^{*}p < 0.01$, $^{**}p < 0.05$). In glutamate-treated neurons transfected with GFP-p150Glued WT and added with DMSO, 10 μM PFT-α, 100 nM taxol, and 10 μM PFT-α + 100 nM taxol, showed 35.4%, 31.2%, 24.1%, and 23.8%, respectively ($^{**}p < 0.05$). Statistics represent mean ± SEM of 3 independent experiments.

production of these pro-apoptotic proteins would benefit neurons, including mitochondrial function. As the transactivated gene products by p53 have opposing functions, excitotoxicity likely weighs more on inducing self-destructive pathways than on protective pathways involving p53 transactivation. Clarifying the molecular mechanisms downstream of the inhibition of p53 transactivation would provide novel insights to how defects in neurons triggered by excitotoxicity could be improved.

Axon degeneration and mitochondrial dysfunction are highly correlated [19], and our results are consistent with this notion since we observed significantly suppressed axon degeneration by simultaneously inhibiting of p53 transactivation and stabilizing microtubules. Consistent with our previous findings, microtubule stabilization alone suppressed excitotoxicity-induced axon degeneration [7]. This protective effect was also observed at a similar level with the inhibition of p53 transactivation alone. Thus, the protective effect on axonal integrity and morphology by simultaneous inhibition of p53 transactivation and microtubule stabilization is synergistic. On the other hand, microtubule stabilization showed no notable effect on excitotoxicity-induced mitochondrial dysfunction yet showed an additive protective effect in concert with the inhibition of p53 transactivation. These contrasting effects by microtubule stabilization could be explained by distinct downstream events. Excitotoxicity triggers excess influx of calcium and individual mitochondrion varies for their calcium uptake depending on the spatial proximity to the calcium source [20]. As calcium overload triggers mitochondrial dysfunction which is accompanied by events, including mitochondrial outer membrane permeabilization and release of pro-apoptotic proteins, microtubule stabilization could permit the relocation of mitochondria to areas with lower calcium concentration to become competent to suppress excitotoxicity-induced mitochondrial dysfunction in a condition

when the production of pro-apoptotic proteins by p53 is inhibited. On the contrary, protective effect on axonal integrity and morphology is achieved by microtubule stabilization perhaps through the facilitation of retrograde transport of pro-survival enzymes, for example activated forms of Erk1/2 MAP kinases, to activate protective signaling cascades against axon degeneration [21].

p53 associates with mitochondria and induces mitochondrial outer membrane permeabilization which leads to the release of pro-apoptotic factors, Bax and Bak, from the mitochondrial and triggers apoptosis [9]. PFT-μ inhibits the association of p53 with mitochondria by reducing the affinity of p53 with anti-apoptotic proteins, Bcl-XL and Bcl-2, without affecting p53 transactivation function [15]. Thus, PFT-μ selectively inhibits the mitochondrial branch of the p53 pathway and is considered as a protective compound against hematopoietic radiation syndrome, a side effect in cancer therapy [15]. Acknowledging this beneficial effect of PFT-μ, we observed robust deleterious effect of PFT-μ on axons of cultured hippocampal neurons. The molecular and mechanistic features of this degenerative effect on axons are unknown, however, it should be taken to account that PFT-μ could elicit neurological defects in addition to protecting the blood system.

Association of WT and the truncated form of p150Glued to mitochondria was elevated with simultaneous inhibition of p53 transactivation and microtubule stabilization. We hypothesized that overexpression of WT p150Glued could further improve axonal integrity and morphology in concert with the inhibition of p53 transactivation and microtubule stabilization. However, inhibition of p53 transactivation did not show cooperative effect with overexpression of WT p150Glued to suppress axon degeneration. The degree of suppression of axon degeneration by overexpression of WT p150Glued with microtubule stabilization or with simultaneous inhibition of p53 transactivation and microtubule stabilization was similar to that by overexpression of control GFP with simultaneous inhibition of p53 transactivation and microtubule stabilization. These results suggest that overexpression of WT p150Glued and inhibition of p53 transactivation function in a linear pathway and that p150Glued functions upstream of inhibition of p53 transactivation as p150Glued truncation is not suppressed by PFT-α treatment. Clarifying the significance of p150Glued association with mitochondria and the molecular relationship with the p53 transactivation-dependent pathway would provide novel insight of how axon degeneration process is regulated in the context of excitotoxicity.

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