

Lycium barbarum polysaccharides alleviate Parkinson's disease-induced neurodegeneration by up-regulating USP10 to modulate endoplasmic reticulum stress

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Abstract

Endoplasmic reticulum stress (ERS) is a significant pathological mechanism in Parkinson's disease (PD), and prolonged ERS can ultimately lead to cellular apoptosis. This study aimed to investigate the neuroprotective effects of Lycium barbarum polysaccharides (LBP) against MPP⁺-induced damage in Neural cells. The cells were treated with MPP⁺ for 24 hours, followed by LBP treatment for another 24 hours. Western blot analysis revealed that, compared to the Control group, MPP⁺ treatment upregulated the expression of GRP78, p-IRE1 α , p-eIF2 α , ATF-6, CHOP, caspase-3 and Bax, while downregulating Bcl-2. In contrast, LBP treatment significantly counteracted these effects by inhibiting the upregulation of ERS and pro-apoptotic markers and restoring Bcl-2 expression. Knockdown of ubiquitin-specific protease 10 (USP10) attenuated the protective effects of LBP in MPP⁺-injured Neural cells. Furthermore, co-immunoprecipitation and immunofluorescence co-localization assays confirmed an interaction between USP10 and Yes-associated protein 1 (YAP1). Deubiquitination experiments indicated that USP10 reduces YAP1 ubiquitination and enhances its protein stability. Additionally, we demonstrated that USP10 suppresses the PERK/eIF2 α /ATF-4/CHOP pathway by inhibiting YAP1 ubiquitination, thereby alleviating MPP⁺-induced ERS and apoptosis. In summary, our findings indicate that LBP attenuates MPP⁺-induced neuronal injury by promoting USP10-mediated deubiquitination of YAP1 and subsequently inhibiting the PERK signaling pathway. These findings provide a novel direction and strategy for the clinical treatment of PD, as well as a new avenue for basic research in PD.

Background: Endoplasmic reticulum stress (ERS) is an important pathogenesis of Parkinson's disease (PD). Chronic ERS can cause cell apoptosis. Lycium barbarum polysaccharides (LBP) are a type of protein polysaccharide with neuroprotective, anti-inflammatory, and anti-apoptotic effects, and have potential therapeutic effects on neurodegenerative diseases.

Objective: This study aims to investigate the neuroprotective effect of LBP on MPP⁺-induced Neural cells and its potential molecular regulatory mechanism.

Method: Neural cell was induced with MPP⁺ for 24 h, followed by LBP treatment for another 24 h. Neural cells were transfected with USP10 and YAP1 overexpression plasmids and specific siRNAs. The PERK pathway inhibitor GSK2606414 was employed to counteract the effects of YAP1 knockdown. Subsequent evaluations encompassed CCK-8 assay for cell viability, Western blotting for ERS related proteins, flow cytometry for cell apoptosis and mitochondrial membrane potential, immunofluorescence for protein localization, calcium probes for intracellular calcium homeostasis. The interaction between USP10 and YAP1 was verified by co-immunoprecipitation.

Result: In neural cells, MPP⁺ induces endoplasmic reticulum (ER) swelling, vacuolization, and intracellular calcium imbalance. It concurrently upregulates the expression of endoplasmic reticulum stress markers (GRP78, p-PERK, p-eIF2 α , ATF-4, CHOP) and pro-apoptotic proteins (caspase-3, Bax), while downregulating the expression of the anti-apoptotic protein Bcl-2. These changes ultimately promote endoplasmic reticulum stress, oxidative stress, and cellular apoptosis. LBP alleviates these

changes by restoring calcium homeostasis and downregulating stress and apoptosis-related proteins, an effect that is abolished by USP10 knockdown. USP10 mediates this protection by inhibiting YAP1 ubiquitination. Notably, the PERK inhibitor GSK2606414 reversed the enhanced expression of apoptotic and stress proteins resulting from YAP1 silencing.

Conclusion: These results demonstrate that LBP exerts neuroprotective effects by alleviating MPP⁺-induced ERS and cell apoptosis. The underlying mechanism involves the upregulation of USP10-mediated deubiquitination of YAP1, which in turn suppresses the activity of the PERK signaling pathway. Our results provide a novel direction and strategy for the clinical treatment of PD, as well as a new avenue for basic research in PD.

1. Introduction

Parkinson's disease (PD) ranks as the second most prevalent neurodegenerative disorder affecting the central nervous system. Its pathological feature is the selective loss of dopaminergic (DA) neurons in the substantia nigra and striatum, with the misfolding and aggregation of α -synuclein (α -syn) forming Lewy bodies (LB)^[1–3]. The pathogenesis of PD involves a complex interplay of multiple factors, oxidative stress, endoplasmic reticulum stress, apoptosis, and mitochondrial dysfunction have been closely linked to PD development^[4–6]. Epidemiological studies show that it is estimated that there will be 25.2 million people with PD worldwide by 2050, an increase of 112% compared to 2021^[7–9]. Currently, the treatment options for Parkinson's disease include drug therapy, surgical treatment, and gene therapy, among others. However, existing treatments for PD remain limited and often yield suboptimal therapeutic outcomes^[10–13]. Studies have shown that many traditional Chinese medicines possess antioxidant, anti-inflammatory, and analgesic properties, and have significant therapeutic effects in neurological diseases such as PD^[14, 15]. Therefore, studying the role of traditional Chinese medicine in the treatment of PD and understanding its possible mechanism of action provide new targets and treatment directions for the treatment of PD.

Lycium barbarum polysaccharide (LBP) is the main active component extracted from the natural Chinese herb *Lycium barbarum* and is a protein polysaccharide^[16, 17]. Studies have shown that LBP has antioxidant, anti-apoptotic, anti-inflammatory and anti-aging functions^[17–19]. Currently, there are studies that have proved that LBP plays an important role in preventing and treating neurological diseases, heart failure, and adolescent depression^[20, 21]. The research shown that LBP can improve the cognitive function and oxidative stress levels of Alzheimer's disease model rats^[22, 23]. However, the role of LBP in PD and its underlying mechanism remain to be elucidated.

The endoplasmic reticulum (ER) is an intracellular substructure that mainly functions in protein modification, folding, secretion, and calcium homeostasis^[24, 25]. When proteins in the ER lumen are not folded properly or undergo incorrect folding and abnormal aggregation, the Unfolded Protein Response

(UPR) is activated^[26, 27]. When the UPR fails to maintain protein homeostasis, the cell enters an apoptotic program, causing neurological dysfunction^[28, 29].

Ubiquitination is a reversible post-translational modification that regulates protein degradation or function through the ubiquitin system^[30]. This process can be reversed by deubiquitinases, which cleave and remove ubiquitin chains from substrate proteins^[31]. Ubiquitin-specific peptidase 10 (USP10), a key member of the ubiquitin-specific protease family, has been shown to inhibit the ubiquitin-proteasome degradation pathway via deubiquitination, thereby maintaining protein stability and ameliorating neural injury following cerebral ischemia-reperfusion^[32]. Nevertheless, whether USP10 influences neural injury in Parkinson's disease by modulating protein ubiquitination remains unclear.

Therefore, this study aims to demonstrate that LBP exerts neuroprotective effects by upregulating USP10, which inhibits the ubiquitin-proteasome-mediated degradation of YAP1 protein, thereby activating the PERK/eIF2 α /ATF4/CHOP signaling pathway and alleviating ERS. These findings provide a novel direction and strategy for the clinical treatment of PD, as well as a new avenue for basic research in PD.

2. Materials and methods

2.1 Isolation of primary mouse nerve cells

Newborn C57BL/6 mice (5 males and 5 females, 14 days old) were euthanized, and the embryos were aseptically isolated and transferred into a culture dish placed under dissecting microscope. After immobilizing the embryonic heads, the skin and skull were carefully removed using microscissors, and the brain was bisected. The ventral base connected to the cerebellum was severed, and the hippocampus was excised with microscissors and transferred into a centrifuge tube containing pre-cooled dissection buffer. Pre-warmed trypsin was then added, and the tissue was digested in a 37°C water bath for 30 minutes. Undigested tissue fragments and debris were removed by filtration through a cell strainer. The resulting cells were collected and cultured in a 37°C, 5% CO₂ incubator. Following cell attachment, 5 μ mol/L cytarabine was added to the culture medium. On the third day, the medium was replaced with fresh complete medium for subsequent experiments.

2.2 Neural cell culture and processing

The nerve cells were cultured in a high-glucose DMEM (Hyclone, USA) supplemented with 10% FBS (ExCell, China) and 1% penicillin-streptomycin (Solarbor, China) in cell culture flasks. The cells were cultured in a constant temperature incubator at 37°C and 5% CO₂. Neural cells were seeded into 96-well plates and cultured for 2–3 days. Cells were treated with MPP⁺ (Beyotime, China) at concentrations of 0.2, 0.6, 0.8, 1.0, 1.2, 1.4 mmol/L and/or LBP (Beijing Pufei Co., Ltd., China) at 25, 50, 100, 200, 300, and 400 μ mol/mL in high-glucose DMEM supplemented with 10% FBS, followed by incubation for 24 hours.

In certain experiments, cells were initially exposed to 1 mmol/L MPP⁺ for 24 hours, washed three times with PBS, and then treated with 200 µmol/mL LBP for an additional 24 hours.

2.3 Cell Transfection

The pcDNA3.1 plasmid was used to generate constructs for overexpressing USP10 and YAP1 (Genechem, China), whereas specific siRNAs were employed to knock down USP10 and YAP1 (GenePharma, China). Neural cells were seeded into 96-well plates and cultured in a humidified incubator at 37°C with 5% CO₂ for 24 hours. The old medium was then aspirated and replaced with a transfection mixture prepared using Lipofectamine™ 3000 (Thermo Fisher, China) according to the manufacturer's instructions. The siRNA sequences used were as follows: USP10: forward 5'-TTTTAA ATGCCACCGAACCTATC-3', reverse 5'-CCAGCCATTCAGACCGATCT-3'; YAP1: forward 5'-GCA UCUUCGACAGUCUUCUTT-3', reverse 5'-AGAAGACUGUCGAAGAUGCTT-3'; GAPDH: forward 5'-GAGTGAACGGATTTGGCCGC-3', reverse 5'-TTGATGTTGGCGGGATCT CG-3'.

2.3 CCK-8 (Cell Counting Kit-8)

The CCK-8 kit was obtained from Beyotime Biotechnology Co., Ltd. Neural cells were seeded into 96-well plates and cultured for 24 hours. The culture medium was replaced with high-glucose DMEM containing 10% FBS. Subsequently, MPP⁺ (0.2, 0.6, 0.8, 1.0, 1.2, and 1.4 mmol/L) and LBP (25, 50, 100, 200, 300, and 400 µmol/mL) were added to the wells and incubated for 24 hours. After treatment, 10 µL of CCK-8 reagent was added to each well and the plates were incubated for another 2 hours. Finally, the absorbance at 450 nm was measured using a microplate reader (Molecular Devices, USA) to assess cell viability.

2.4 Cell apoptosis detection

Neural cells were harvested using 0.25% trypsin (Hyclone, USA), washed with PBS, and seeded into 96-well plates. Subsequently, 5 µL of Annexin V-FITC and 5 µL of PI (Multisciences, Shanghai, China) was added to each well, and the cells were incubated at room temperature in the dark for 20 minutes. Apoptosis was then analyzed using a flow cytometer (Beckman Coulter Company, USA).

2.5 Mitochondrial membrane potential ($\Delta\Psi_m$) detection

Neural cells were seeded into 6-well plates. After treatment, the cells were digested using 0.25% trypsin and washed with PBS. According to the manufacturer's instructions, a working solution of TMRE (Beyotime, China) was prepared by diluting 1 µL of the reagent in 1 mL of buffer. Then, 1 mL of the TMRE working solution was added to each well, and the cells were incubated in the dark at 37°C with 5% CO₂ for 30 minutes. Following incubation, the cells were washed, replenished with fresh culture medium, collected, and resuspended in PBS. The mitochondrial membrane potential ($\Delta\Psi_m$) was subsequently measured using flow cytometry.

2.6 ROS detection

The ROS detection kit was obtained from Beyotime Biotechnology Co., Ltd. Neural cells were seeded into 6-well plates. Following digestion, the DCFH-DA stock solution was diluted with 1×PBS to a final concentration of 10 μmol/L according to the manufacturer's instructions to prepare the working solution. Then, 1 mL of the DCFH-DA working solution was added to each well. The plates were incubated at 37°C under 5% CO₂ for 25 minutes. After incubation, the cells were washed three times with PBS. Finally, fluorescence intensity was measured using a flow cytometer.

2.7 Western blotting

Proteins were extracted using RIPA lysis buffer (Beyotime, China) and quantified using a BCA protein assay kit (Kangwei Century, China). Equal amounts of protein were separated by sodium dodecyl SDS-PAGE (Beyotime, China) and subsequently transferred onto PVDF membranes. The membranes were then blocked by incubation with 5% skim milk at room temperature for 2 hours. Following blocking, membranes were incubated overnight at 4°C with specific primary antibodies against: GRP78 (cat. no. abs130538, Absin, 1:1000), p-IRE1α (cat. no. ab48187, Abcam, 1:1000), p-eIF2α (cat. no. ab32157, Abcam, 1:1000), ATF-6 (cat. no. abs135529, Absin, 1:1000), CHOP (cat. no. abs131376, Absin, 1:1000), caspase-3 (cat. no. abs111175, Absin, 1:1000), bcl-2 (cat. no. abs131701, Absin, 1:1000), Bax (cat. no. abs130057, Absin, 1:1000), USP10 (cat. no. abs158903, Absin, 1:1000), YAP1 (cat. no. abs155298, Absin, 1:1000), β-actin (cat. no. abs132001, Absin, 1:10000), p-PERK (cat. no. AP328, Beyotime, 1:1000), and ATF-4 (cat. no. abs131517, Absin, 1:1000). The membrane was washed with TBST and then incubated with the secondary antibody (1:10000) at room temperature for 2 hours. Finally, protein bands were visualized using an enhanced chemiluminescence (ECL) detection system, and gray values were quantified using Image J software.

2.9 Immunofluorescence

Neural cells were seeded onto sterile coverslips. Following drug treatment, the cells were washed with PBS and fixed with 4% PFA at room temperature for 30 minutes. After fixation, the cells were washed again and blocked with 10% donkey serum (Jackson, USA) in PBST for 1 hour at room temperature. Subsequently, the cells were incubated overnight at 4°C with anti-USP10(abs158903, Absin, 1:500) and anti-YAP1(abs155298, Absin, 1:500) antibodies diluted in PBST. The next day, after discarding the primary antibodies, the cells were thoroughly washed three times with PBS. Cell were incubated in the dark with fluorescently-labeled secondary antibodies and DAPI solution diluted in PBST, for 2 hours and 30 minutes respectively at room temperature. Following a final wash step, the coverslips were mounted using antifade mounting medium. Fluorescence signals were finally visualized under optical microscope (Olympus, Japan).

2.10 Co-immunoprecipitation (Co-IP) assay

Cells were lysed with a lysis buffer supplemented with protease inhibitors to extract total protein. Protein A/G magnetic beads (Santacruz Biotechnology, USA) were incubated with anti-USP10, anti-YAP1, or anti-IgG antibody at 4°C for 2 hours to facilitate antibody binding. The antibody-bound beads were then collected using a magnetic separation rack and incubated with the total protein lysate at 4°C to enable

specific immunoprecipitation of USP10, YAP1, or IgG. Subsequently, the bead-protein complexes were magnetically captured and thoroughly washed with washing buffer. Finally, the immunoprecipitated samples were resuspended in SDS-containing loading buffer, heated at 95°C for 5–10 minutes to dissociate the proteins from the beads and prepared for Western blot analysis.

2.11 Statistical analysis

The data in this study were all statistically analyzed using GraphPad Prism software. All data were presented as the mean $\pm$ standard deviation (SD) of three independent experiments. One-way analysis of variance was used for multiple comparisons, followed by Bonferroni correction for post-hoc tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1 LBP mitigates injury of Neural cells induced by MPP⁺

To investigate the neuroprotective effect of LBP, we treated Neural cells with varying concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4 mmol/L) of MPP⁺ to simulate neuronal damage in PD. According to the CCK-8 assay, 0.6 and 0.8 mmol/L MPP⁺ has a relatively minor inhibitory effect on cell viability, while 1 mmol/L MPP⁺ induced approximately 50% cell death, 1.2 and 1.4 mmol/L MPP⁺ caused more than half of the cells death (Fig. 1A). Therefore, 1 mmol/L MPP⁺ was selected for subsequent experiments. Next, we evaluated the effects of LBP at concentrations of 25, 50, 100, 200, 300, and 400 μ mol/mL on cell viability. The results revealed that LBP decreased cell viability at concentrations exceeding 300 μ mol/mL in a concentration-dependent manner (Fig. 1B). LBP alleviated the MPP⁺-induced decline in cell viability in a concentration-dependent manner, with most significant effects observed at 200 μ mol/mL (Fig. 1C). Furthermore, flow cytometry analysis revealed that LBP alleviated MPP⁺-induced apoptosis in a concentration-dependent manner, with a significant protective effect observed at 100 μ mol/mL and a most significant protective effect at 200 μ mol/mL (Fig. 1D). Therefore, 200 μ mol/mL LBP was selected for subsequent experiments.

3.2 LBP mitigated ERS and apoptosis induced by MPP⁺ in Neural cells.

LBP is a protein glycoprotein composed of six monosaccharides, various trace elements, and amino acids. Studies have shown that Codonopsis polysaccharides, which have a similar structure to LBP, exert neuroprotective effects in Alzheimer's disease by regulating the ERS response^[33]. Therefore, next we explored whether LBP could exert neuroprotective effects by regulating ERS.

We first assessed the effect of LBP on MPP⁺-induced ERS using Western blot analysis. The results revealed that, compared with the Control group, MPP⁺ treatment alone markedly upregulated the expression of GRP78, p-IRE1 α , p-eIF2 α , ATF-6, and CHOP proteins. Compared with the MPP⁺ group, MPP⁺-induced upregulation of GRP78, p-eIF2 α , and CHOP proteins, while the expression levels of p-

IRE1 α and ATF-6 showed no significant changes (Fig. 2A). Electron microscopy observations further demonstrated substantial swelling and vacuolation of ER in the MPP⁺-treated group, whereas co-treatment with LBP markedly ameliorated these MPP⁺-induced ultrastructural alterations (Fig. 2B).

Intracellular calcium signals were detected by calcium fluorescent probe. The results revealed that, compared with the Control group, Ca²⁺ fluorescence intensity was significantly increased in the MPP⁺-treated group. compared with the MPP⁺ group, the MPP⁺-induced elevation in Ca²⁺ intensity was markedly attenuated by LBP treatment (Fig. 2C).

Excessive ERS often activates apoptotic signaling, ultimately leading to cell death. Therefore, we explored the effect of LBP on cell apoptosis caused by MPP⁺. Western blot analysis revealed that, compared with the Control group, MPP⁺ treatment alone upregulated the expression of the pro-apoptotic proteins caspase-3 and Bax, while downregulating the anti-apoptotic protein Bcl-2. In contrast, LBP treatment counteracted these MPP⁺-induced changes (Fig. 2D). Immunofluorescence staining indicated that MPP⁺ treatment alone increased ROS levels, the effect that was significantly attenuated by LBP co-treatment (Fig. 2E). Since severe ERS can impair mitochondrial function, we further assessed the mitochondrial membrane potential ($\Delta\Psi_m$) using flow cytometry. The results showed a pronounced loss of $\Delta\Psi_m$ following MPP⁺ exposure, which was effectively restored by LBP (Fig. 2F).

Collectively, these results demonstrate that LBP alleviates MPP⁺-induced ERS and apoptosis. This protective effect is achieved through downregulation of associated proteins expression, amelioration of ER pathological changes, and maintenance of intracellular calcium homeostasis.

3.3 Knockdown of USP10 weakens the protective effect of LBP

Previous study have shown that the polysaccharide of *Amanita bisporigera*, which has a similar structure to LBP, can mediate the inflammatory response by regulating the USP10/NF- κ B/Nrf2 signaling pathway^[34]. USP10 is an anti-apoptotic factor that can exert neuroprotective effects by regulating ERS-mediated cell apoptosis^[35]. Therefore, we sought to determine whether LBP alleviates MPP⁺-induced cell damage by regulating USP10.

Firstly, we examined the effect of MPP⁺ and LBP treatment on USP10 protein expression using Western blot analysis. The results revealed that, compared with the Control group, USP10 expression was significantly decreased in the MPP⁺-treated group; compared with the MPP⁺ group, LBP treatment rescued the MPP⁺-induced reduction in USP10 expression (Fig. 3A). To further investigate whether USP10 mediates the protective effect of LBP, we knocked down USP10 using siRNA and assessed the resulting phenotypic changes. Western blot analysis indicated that si-USP10#3 exhibited the highest knockdown efficiency compared to the si-USP10#1 and si-USP10#2 (Fig. 3B). Compared with the si-NC group exposed to MPP⁺ and LBP, USP10 expression was significantly reduced in the si-USP10#3-transfected group (Fig. 3C). Furthermore, CCK-8 assays demonstrated that cell viability was significantly decreased in the MPP⁺ group compared to the Control group. LBP treatment attenuated the MPP⁺-

induced reduce of cell viability. Importantly, USP10 knockdown abolished the protective effect of LBP on cell viability when compared with the si-NC groups under MPP⁺ and LBP co-treatment conditions (Fig. 3D). Compared to the Control group, the expression of GRP78, p-PERK, p-eIF2 α , ATF-4, and CHOP proteins was significantly up-regulated in the MPP⁺ group. LBP attenuated this MPP⁺-induced upregulation of ERS-related proteins. Moreover, knockdown of USP10 abolished the protective effect of LBP, preventing the reduction in the expression of these proteins (Fig. 3E).

Calcium probe assays indicated a significant increase in calcium ion fluorescence intensity in the MPP⁺ group compared to the Control. This effect was mitigated by co-treatment with LBP. However, USP10 knockdown disrupted the ability of LBP to restore calcium homeostasis (Fig. 3F). Western blot analysis showed that LBP antagonized the MPP⁺-induced upregulation of caspase-3 and Bax and downregulation of Bcl-2. Conversely, USP10 knockdown attenuated this protective effect of LBP, leading to increased levels of caspase-3 and Bax and decreased Bcl-2 (Fig. 3G). Immunofluorescence results demonstrated that MPP⁺ induced a marked increase in intracellular ROS levels. LBP reduced this MPP⁺-induced ROS elevation, whereas USP10 knockdown inhibited the antioxidant effect of LBP (Fig. 3H).

In summary, these findings indicate that USP10 knockdown attenuates the protective effects of LBP against MPP⁺-induced endoplasmic reticulum stress and apoptotic signaling.

3.4 Overexpression of USP10 alleviates MPP⁺-induced neurotoxicity

To further investigate the role of USP10 in MPP⁺-induced neuronal injury, we overexpressed USP10 via cell transfection of pcDNA-USP10, using the pcDNA3.1 empty vector as a negative control. The results revealed that, compared with the Control group, MPP⁺ treatment significantly decreased cell viability. In contrast, USP10 overexpression markedly mitigated MPP⁺-induced reduction in cell viability (Fig. 4A). Western blot analysis showed that MPP⁺ treatment significantly up-regulated the expression of GRP78, p-IRE1 α , p-eIF2 α , ATF-6, and CHOP compared to the Control group. Compared with the vector group, overexpression of USP10 significantly attenuated MPP⁺-induced upregulation of ERS-related proteins (Fig. 4B). Furthermore, using calcium fluorescence probe, we demonstrated that MPP⁺ exposure notably increased intracellular calcium ion fluorescence intensity compared to the Control. Overexpression of USP10 effectively reduced MPP⁺-induced elevation in calcium levels (Fig. 4C).

3.5 Inhibition of YAP1 ubiquitination by USP10 mitigates MPP⁺-induced ERS and neural cell apoptosis.

The above results indicated that LBP attenuated MPP⁺-induced ERS and apoptosis through upregulation of USP10. Next, we sought to elucidate the underlying mechanism of USP10. Using the BioGRID database, we identified potential interacting proteins of USP10, among which Yes-associated protein 1 (YAP1) emerged as a candidate of interest. YAP1 is a transcriptional co-activator and a key effector factor of the Hippo signaling pathway. Studies have shown that YAP can protect hippocampal neurons

from ischemia-reperfusion damage and exert neuroprotective effects^[36]. Based on this, we sought to investigate whether USP10 exerts its protective effects against MPP⁺-induced injury by activating YAP1.

To investigate the interaction between USP10 and YAP1, we first performed Co-IP assays. As shown in Fig. 5A, USP10 specifically co-precipitated with YAP1, and reciprocally, YAP1 also co-precipitated with USP10. Furthermore, immunofluorescence analysis revealed notable co-localization of USP10 and YAP1 in the cytoplasm (Fig. 5B). Given that USP10 is a known deubiquitinase, we further explored whether it regulates YAP1 in an ubiquitination-dependent manner. In vitro ubiquitination assays demonstrated that overexpression of USP10 markedly reduced the ubiquitination level of YAP1, whereas knockdown of USP10 significantly enhanced YAP1 ubiquitination (Fig. 5C).

To further investigate whether USP10 exerts its neuroprotective effects via YAP1, we overexpressed YAP1 by cell transfection of pcDNA-YAP1, using the pcDNA3.1 vector as a negative control. As confirmed by the results, MPP⁺ treatment reduced YAP1 protein expression compared with the Control group. In contrast, USP10 overexpression significantly up-regulated YAP1 levels in the presence of MPP⁺ compared to the MPP⁺ and empty vector co-treatment group. Furthermore, concomitant overexpression of USP10 and YAP1 under MPP⁺ treatment resulted in a further significant increase in YAP1 protein expression compared to the MPP⁺ and USP10 overexpression co-treatment group (Fig. 5D). CCK-8 results revealed a significant reduction in cell viability in the MPP⁺ treatment group compared with the Control group. In contrast, USP10 overexpression significantly increased cell viability in the presence of MPP⁺ compared to the MPP⁺ and empty vector co-treatment group. Furthermore, concomitant overexpression of USP10 and YAP1 under MPP⁺ treatment resulted in a further significant increase in cell viability compared to the MPP⁺ + and USP10 overexpression co-treatment group (Fig. 5E).

Western blot analysis revealed that, compared with the control group, MPP⁺ treatment increased the expression of GRP78, p-PERK, p-eIF2 α , ATF-4, and CHOP proteins. Compared to the MPP⁺ and empty vector co-treatment group, USP10 overexpression reduced the expression of ERS-related proteins in the presence of MPP⁺. Furthermore, compared to the MPP⁺ treatment and USP10 overexpression group, concomitant overexpression of USP10 and YAP1 resulted in a further increase in ERS-related protein expression (Fig. 5F). Flow cytometry results indicated that, compared with the control group, MPP⁺ treatment promoted neuronal apoptosis. Compared to the MPP⁺ and empty vector co-treatment group, USP10 overexpression suppressed neuronal apoptosis in the presence of MPP⁺. In contrast, compared to MPP⁺ and USP10 overexpression co-treatment group, concomitant overexpression of USP10 and YAP1 further reduced neuronal apoptosis in the presence of MPP⁺ (Fig. 5G). Collectively, these results demonstrate that USP10 inhibits proteasomal degradation of YAP1 via deubiquitination, thereby mitigating MPP⁺-induced ERS and apoptosis.

3.6 YAP1 Attenuates MPP⁺-Induced ERS and Apoptosis by Suppressing the PERK/eIF2 α /ATF-4/CHOP Pathway

Next, we further explored whether YAP1 regulates the ERS and apoptosis of nerve cells by modulating the PERK/eIF2 α /ATF-4/CHOP pathway.

Western blotting results showed that, compared with the si-NC group, si-YAP1#1, si-YAP1#2, and si-YAP1#3 treatments significantly suppressed YAP1 protein levels, among which si-YAP1#1 exhibited the most potent inhibition (Fig. 6A). To investigate whether YAP1 mediates MPP⁺-induced cytotoxicity by regulating the PERK pathway, we performed a rescue experiment using the PERK pathway inhibitor GSK2606414. CCK-8 results demonstrated that 2 and 2.5 μ mol/L GSK2606414 reduced cell viability, whereas concentrations of 0.1–1.5 μ mol/L showed no cytotoxicity. Therefore, 1.5 μ mol/L GSK2606414 was used for subsequent analysis (Fig. 6B). Western blot analysis showed that, compared with the Control group, the MPP⁺-treated group exhibited significantly elevated levels of p-PERK, p-eIF2 α , ATF-4, and CHOP proteins. Compared to the MPP⁺ and si-NC co-treatment group, PERK pathway relative protein expression was significantly increased in the MPP⁺ and si-YAP1 co-treatment group. Compared to the MPP⁺ and si-YAP1 co-treatment group, PERK pathway relative protein expression was significantly reduced in the MPP⁺, si-YAP1 and GSK2606414 co-treatment group (Fig. 6C).

CCK-8 assay result showed that MPP⁺ treatment decreased the cell viability, and YAP1 knockdown further enhanced this reduction. In contrast, treatment with GSK2606414 significantly rescued the decline in the cell viability induced by YAP1 knockdown (Fig. 6D). Western blot analyses demonstrated that the expression of caspase-3 and Bax proteins was significantly upregulated, while Bcl-2 was downregulated in MPP⁺-treated cells. YAP1 knockdown further intensified these changes. Conversely, GSK2606414 treatment significantly attenuated the alterations in apoptosis-related protein expression caused by YAP1 knockdown (Fig. 6E). Further flow cytometry analysis revealed that the apoptosis rate was significantly increased following MPP⁺ treatment, and this effect was further exacerbated by YAP1 knockdown. However, GSK2606414 treatment markedly suppressed the increase in apoptosis mediated by YAP1 knockdown (Fig. 6F). Taken together, these results demonstrate that YAP1 alleviates MPP⁺-induced ERS and apoptosis by inhibiting the PERK/eIF2 α /ATF-4/CHOP signaling pathway.

4. Discussion

In this study, we demonstrated the protective effect of LBP on MPP⁺-induced damage in neural cell cells. Furthermore, we explored the underlying mechanism through which LBP exerts its protective role in MPP⁺-induced Neural cell. Ultimately, our findings revealed that LBP provides neuroprotection by upregulating USP10, thereby inhibiting YAP1 ubiquitination, and by suppressing the PERK/eIF2 α /ATF-4/CHOP signaling pathway, which alleviates MPP⁺-induced ERS and apoptosis.

The pathological hallmark of LB in PD primarily consists of misfolded and phosphorylated α -syn, along with other aggregated proteins and abnormal organelles. Currently, misfolded α -syn is widely recognized as a major driver of PD pathology and neurodegeneration, associated with multiple mechanisms including impaired ER function and proteasome dysfunction^[29, 37, 38]. In postmortem midbrain samples from individuals with early-stage PD, elevated levels of the ER sensor PERK and its activated

downstream target p-eIF2 α were explored, suggesting that ERS plays a significant role in PD pathogenesis^[39]. Recent studies have demonstrated that modulation of the ERS can effectively mitigate neuronal damage in PD. For instance, Yasser et al. reported that administration of Sinapic acid reduced loss of DA neurons and α -syn aggregation in a rotenone-induced rat model of PD, likely through regulation of the ERS-mediated UPR pathway^[40]. Liu et al. demonstrated in a study that transient receptor potential vanilloid 4 (TRPV4) facilitated apoptosis in MPP⁺-induced Neural cell by ERS^[41]. Fan et al. reported that the interaction between the antioxidant protein DJ-1 (Parkin7) and endoplasmic reticulum-mitochondria contact sites contributes to ERS in the pathogenesis of PD^[42].

In this study, we demonstrated that MPP⁺ treatment induced significant ERS in neurons, as evidenced by the upregulation of ERS-related proteins (GRP78, p-IRE1 α , p-eIF2 α , ATF-6, and CHOP), accompanied by marked ultrastructural changes including ER swelling and vacuolation, along with a significant increase in intracellular calcium levels. Concurrently, MPP⁺ exposure promoted neuronal apoptosis, elevated intracellular ROS, and impaired mitochondrial function.

LBP is the primary active component of *Lycium barbarum*, possesses diverse biological activities, including antioxidant, anti-apoptotic, anti-inflammatory, and anti-aging effects. Accumulating evidence indicates that LBP exerts a protective role in various neurodegenerative diseases. For instance, Lu et al. reported that LBP ameliorated cognitive dysfunction in an A β -induced rat model of Alzheimer's disease by modulating oxidative stress^[22]. Similarly, Yang et al. demonstrated that LBP alleviated light-induced oxidative stress, mitochondrial dysfunction, and apoptosis in hippocampal neurons^[20]. Further studies have shown that LBP can mitigate MPTP and 6-OHDA-induced α -syn aggregation, nigrostriatal degeneration, and neuronal apoptosis^[43–45]. Building upon this evidence, we investigated the effects of LBP on MPP⁺-induced ERS and apoptosis in primary neurons. Our results showed that LBP treatment significantly suppressed the MPP⁺-induced upregulation of ERS-related proteins GRP78, p-eIF2 α , and CHOP. In contrast, the expression levels of p-IRE1 α and ATF-6 remained unaltered, suggesting that the neuroprotective effect of LBP is mediated specifically via the PERK pathway. Furthermore, LBP treatment effectively attenuated MPP⁺-induced cell apoptosis, reduced the accumulation of ROS and calcium ions, and restored mitochondrial function.

USP10, identified as the target of LBP, is a deubiquitinating enzyme. While earlier studies have primarily focused on the role of USP10 in tumors, emerging evidence has linked it to the pathogenesis of degenerative disorders, including PD. Notably, Anisimov et al. recently demonstrated that USP10 is involved in the degradation of α -syn in PD^[46, 47]. Emerging evidence indicates that USP10 can suppress dopamine-induced, ROS-dependent neuronal apoptosis by enhancing Nrf2 activity^[48]. In this study, we found that overexpression of USP10 significantly alleviated MPP⁺-induced cellular damage. This was demonstrated by the downregulation of ERS and pro-apoptotic proteins, improved cell viability, reduced apoptosis, and diminished intracellular accumulation of calcium and ROS. Conversely, USP10 knockdown attenuated the protective effects of LBP against MPP⁺-induced injury. This was evidenced by the upregulation of ERS and apoptosis-related proteins, accompanied by a further decline in cell viability,

increased apoptosis, and elevated levels of calcium and ROS. In summary, our findings indicate that USP10 upregulation enhances the protective role of LBP in alleviating MPP⁺-induced ERS, oxidative stress, and apoptotic cell death.

Previous studies have established YAP1 as a key regulator of cell proliferation, differentiation, and apoptosis. Its upregulation has been shown to inhibit ERS-induced apoptosis in vascular smooth muscle cells^[49]. Furthermore, Yang et al. reported that YAP1 overexpression suppresses ERS, alleviates mitochondrial dysfunction, and reduces neuroinflammation by upregulating Sirt3 and inhibiting the JNK pro-apoptotic pathway^[50]. Our study demonstrates that USP10 alleviates MPP⁺-induced ERS and apoptosis by inhibiting YAP1 ubiquitination. Overexpression of YAP1 enhances the inhibitory effects of USP10 on MPP⁺-induced cell viability decline and upregulation of ERS and apoptosis-related proteins. Conversely, YAP1 knockdown inhibits the neuroprotective effect of USP10 against MPP⁺-induced injury through activation of the PERK pathway. Critically, the PERK pathway inhibitor GSK2606414 reverses the pro-apoptotic and anti-proliferative effects resulting from YAP1 knockdown. These findings indicate that YAP1 primarily facilitates the protective role of USP10 against MPP⁺-induced ERS and apoptosis in neuronal cells by suppressing the activity of the PERK pathway.

Conclusion

Our findings indicate that LBP alleviates MPP⁺-induced ERS and cell apoptosis by upregulating the deubiquitinase USP10. Mechanistically, we demonstrate that USP10 inhibits the ubiquitination of YAP1, which in turn suppresses the activation of PERK/eIF2 α /ATF-4/CHOP signaling pathway. This cascade ultimately attenuates ERS and promotes cell survival. These results not only decipher a novel neuroprotective mechanism but also position LBP as a viable candidate for PD drug development.

Declarations

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CRedit authorship contribution statement:

Yan Zhang (Co-First Author): Conceptualization, Methodology, Formal analysis, Investigation, Writing—Original Draft.

Bonian Ma (Co-First Author): Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing—Original Draft.

Guichao Zuo: Resources, Data Curation, Visualization.

Zhengying Jing: Investigation, Data Curation, Visualization.

Hui Dong (Corresponding author): Writing—Review & Editing, Supervision, Project administration.

Guisheng Chen (Corresponding author): Conceptualization, Writing–Review & Editing, Supervision, Project administration.

Availability of data and materials: Due to privacy protection, the data provided in this study are available upon request from the corresponding author.

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval: This study was approved by the Ethics Committee of Ningxia Medical University (Approval No. KYLL-2021-659). All experiments were performed in accordance with relevant guidelines and regulations.

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Figures

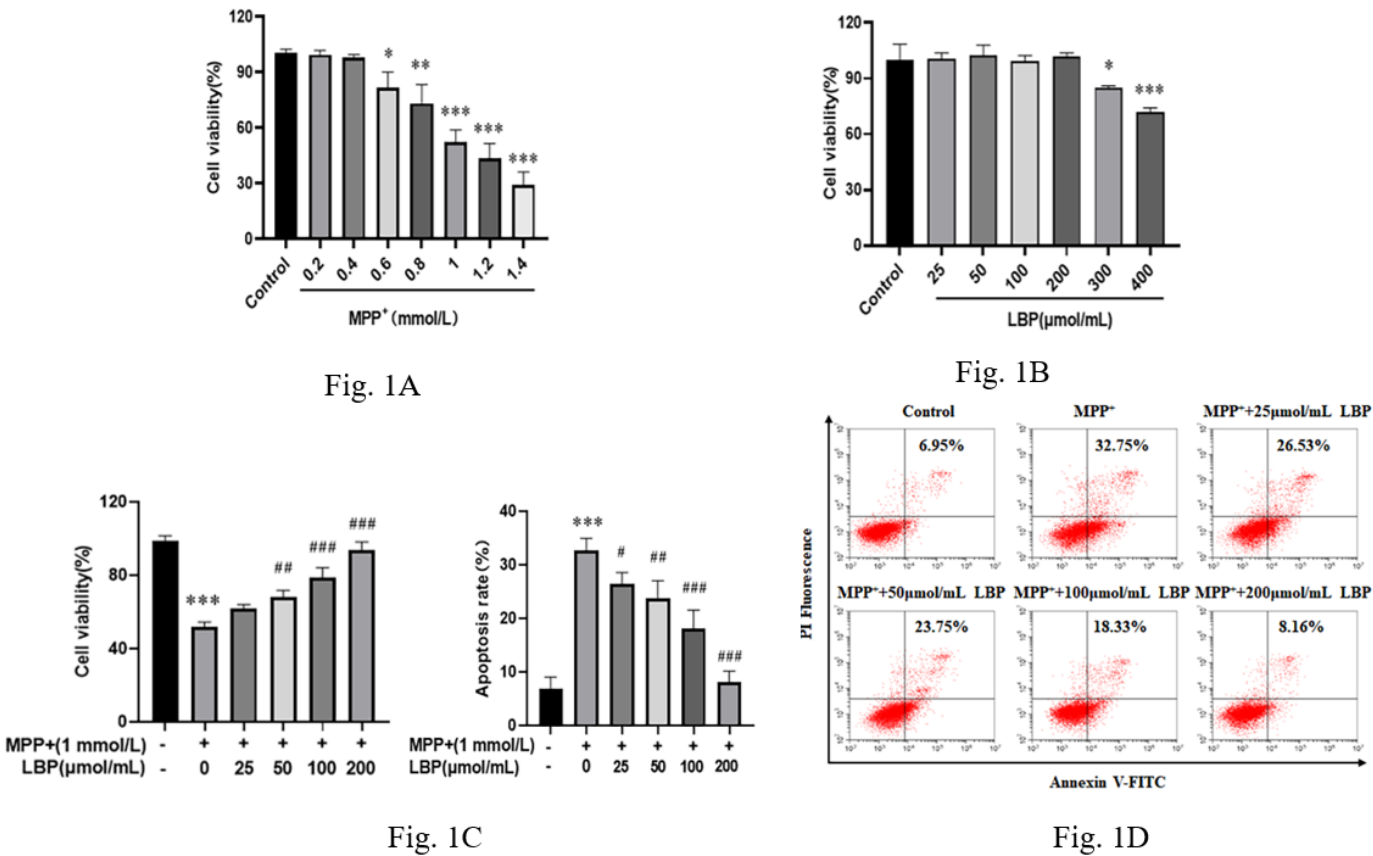


Figure 1

LBP attenuates MPP⁺-induced neurotoxicity in primary neurons. (A) CCK-8 assay of cell viability under different concentrations of MPP⁺ (0.2, 0.4, 0.6, 0.8, 1, 1.2 and 1.4 mmol/L) treatment. (B) CCK-8 assay of cell viability under different concentrations of LBP (25, 50, 100, 200, 300 and 400 µmol/mL) treatment. (C) CCK-8 assay of cell viability, after 1 mmol/L MPP⁺ treatment 24 hours, followed by co-treatment with different concentrations of LBP (25, 50, 100 and 200 µmol/mL) for another 24 hours. (D) Flow cytometry assay of cell apoptosis rate, after 1 mmol/L MPP⁺ treatment 24 hours, followed by co-treatment with different concentrations of LBP (25, 50, 100 and 200 µmol/mL) for another 24 hours. Data are expressed as mean ± SD; **P* < 0.05; ***P* < 0.01; ****P* < 0.001, compared with Control group; #*P* < 0.05; ##*P* < 0.01; ###*P* < 0.05, compared with MPP⁺ group. n = 3-5.

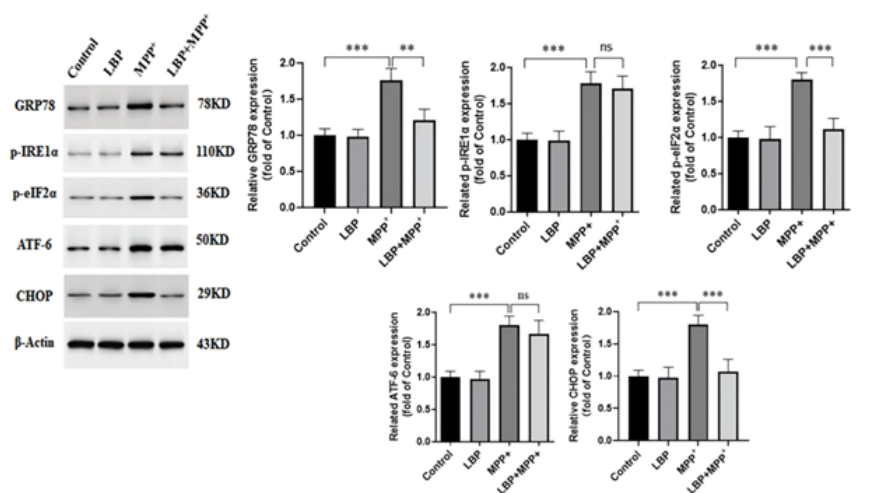


Fig. 2A

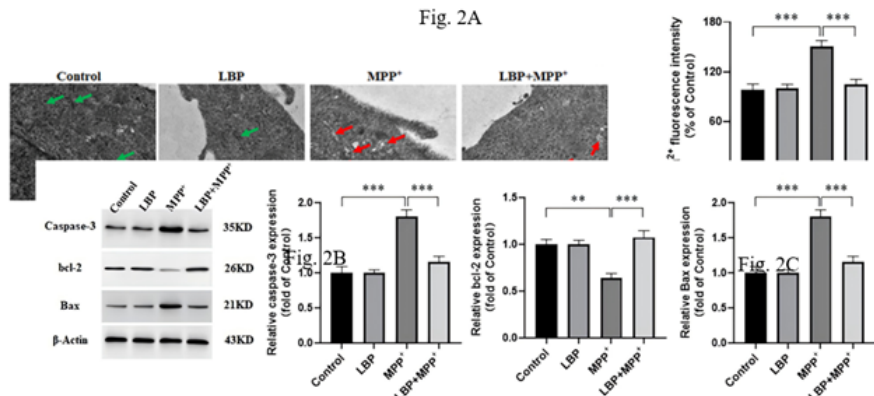


Fig. 2D

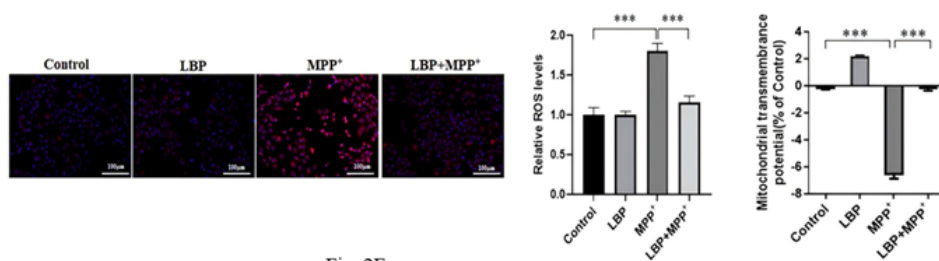


Fig. 2E

Fig. 2F

Figure 2

LBP ameliorated MPP⁺-induced ERS and apoptosis. (A) Western blot analysis of ERS-related proteins expression (GRP78, p-IRE1α, p-eIF2α, ATF-6, CHOP). (B) Electron microscopic analysis of endoplasmic reticulum morphology. Green arrows indicate normal endoplasmic reticulum, while red arrows indicate stress-injured endoplasmic reticulum. Scale bar = 1 μm. (C) Fluo-4 AM fluorescence probe analysis of intracellular calcium levels. (D) Western blot analysis of apoptosis-related proteins expression (caspase-

3, Bcl-2, Bax). (E) DCFH-DA fluorescent probe detection of intracellular ROS levels. (F) Flow cytometry detection of mitochondrial membrane potential ($\Delta\Psi_m$). Scale bar = 100 μm . Data are presented as mean $\pm$ standard deviation. $**P < 0.01$, $***P < 0.001$.

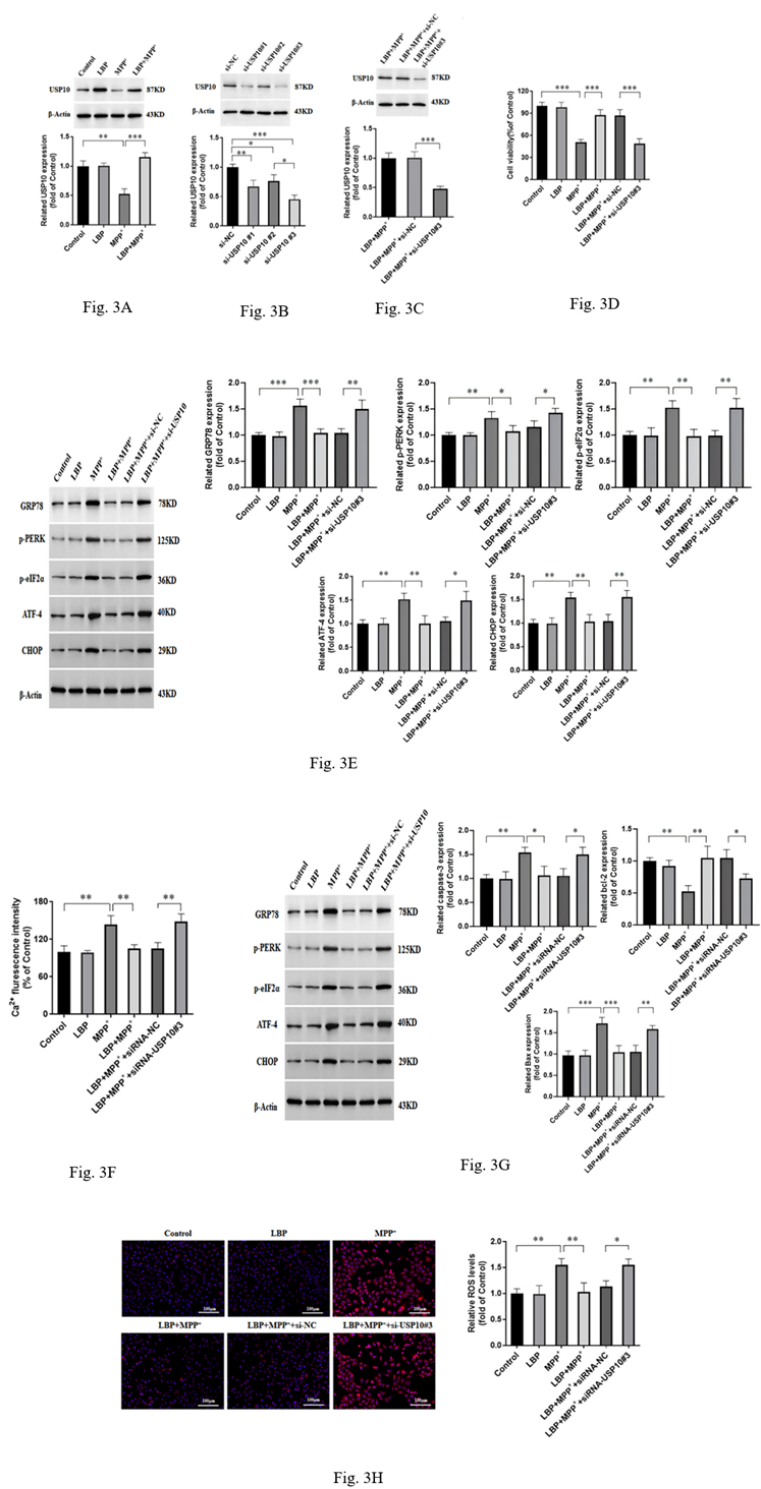


Figure 3

USP10 down-regulation attenuated the protective effects of LBP against MPP⁺-induced ERS and apoptosis. (A-C) Western blot analysis of USP10 protein expression. (D) CCK-8 assay detection of cell

viability. (E) Western blot analysis of ERS related proteins expression (GRP78, p-PERK, p-eIF2 α , ATF-4, and CHOP). (F) Fluorescent calcium probe detection of intracellular calcium levels. (G) Western blot analysis of apoptosis-related proteins (caspase-3, bcl-2 and Bax). (H) DCFH-DA fluorescent probe detection of intracellular ROS levels. Scale bar = 100 μ m. Data are presented as mean $\pm$ standard deviation. * P < 0.05, ** P < 0.01, *** P < 0.001.

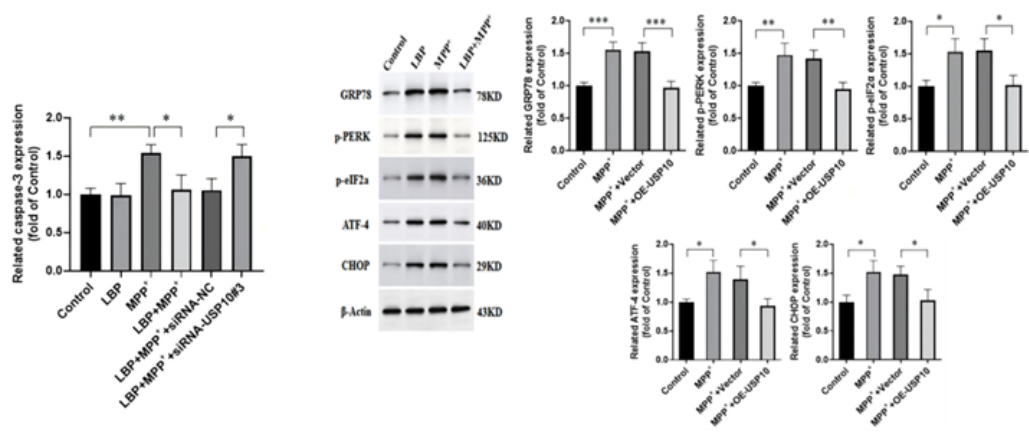


Fig. 4A

Fig. 4B

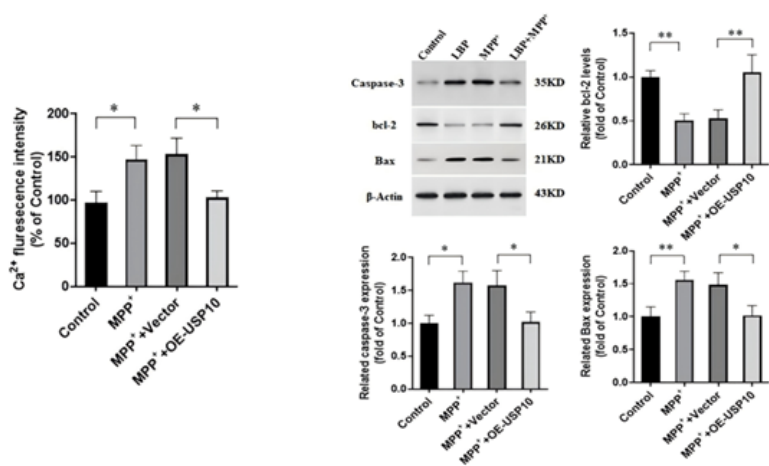


Fig. 4C

Fig. 4D

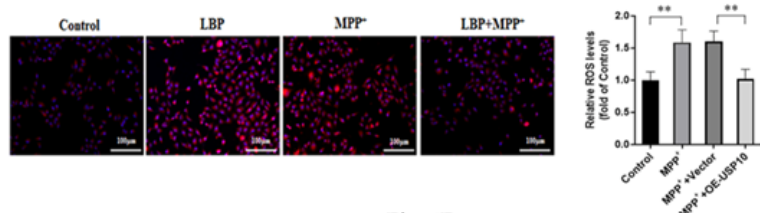


Fig. 4E

Figure 4

USP10 upregulation alleviated MPP⁺-induced ERS and cell apoptosis. (A) CCK-8 assay detection of cell viability. (B) Western blot analysis of ERS related proteins expression. (C) Fluorescent calcium probe detection of intracellular calcium levels. (D) Western blot analysis of apoptosis-related proteins (caspase-3, bcl-2 and Bax). (E) DCFH-DA fluorescent probe detection of intracellular ROS levels. Scale bar = 100 μ m. Data are presented as mean $\pm$ standard deviation. * P < 0.05, ** P < 0.01, *** P < 0.001.

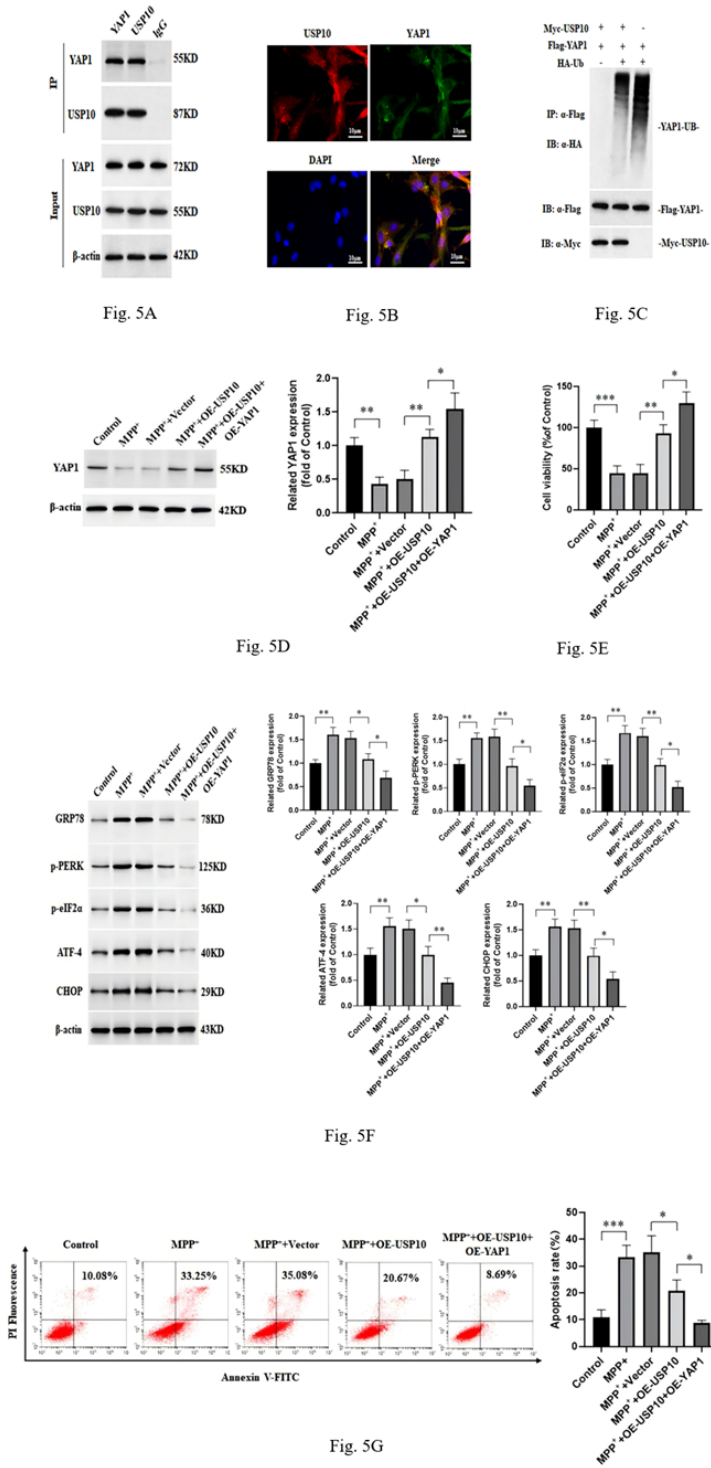


Figure 5

USP10 deubiquitinates YAP1 and ameliorates MPP⁺-induced ERS and apoptosis. (A) Co-immunoprecipitation analysis of interaction between USP10 and YAP1. (B) Immunofluorescence detection of subcellular co-localization of USP10 and YAP1. (C) Ubiquitination experiment detection of ubiquitination of YAP1. (D) Western blot analysis of YAP1 expression following MPP⁺ treatment. (E) CCK-8 assay detection of cell viability. (F) Western blot analysis of ERS related proteins expression. (G) Flow cytometry detection of cell apoptosis rate. Data are presented as mean ± SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

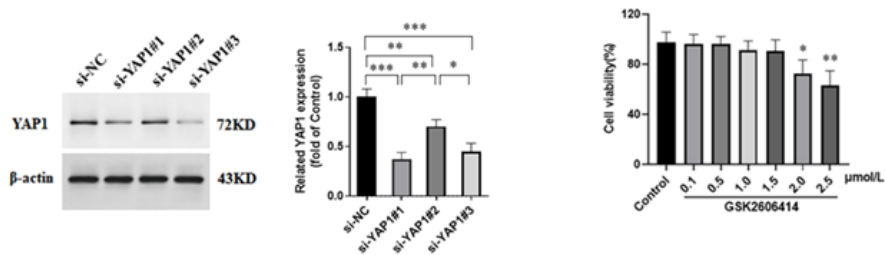


Fig. 6A

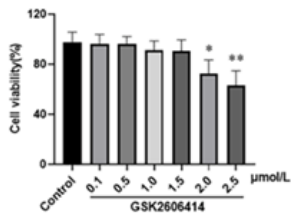


Fig. 6B

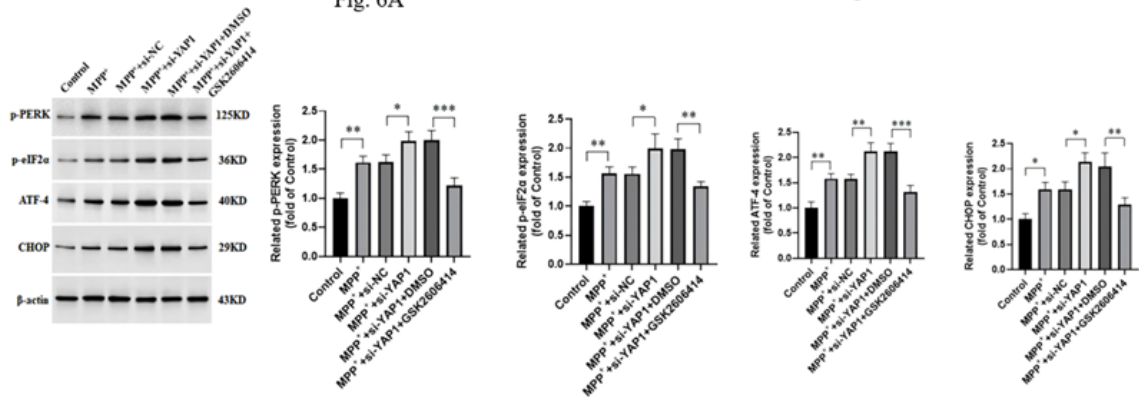


Fig. 6C

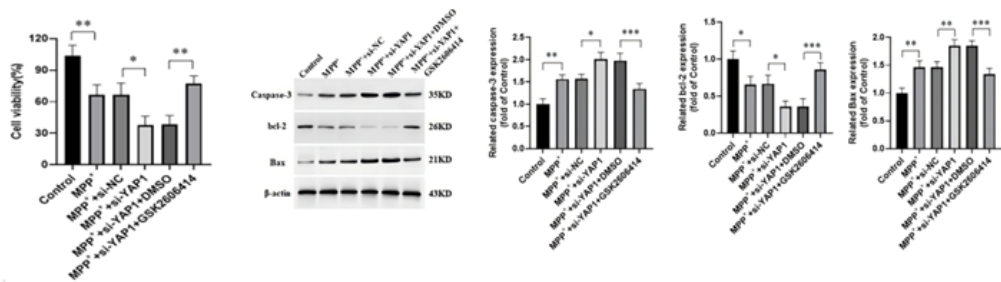


Fig. 6D

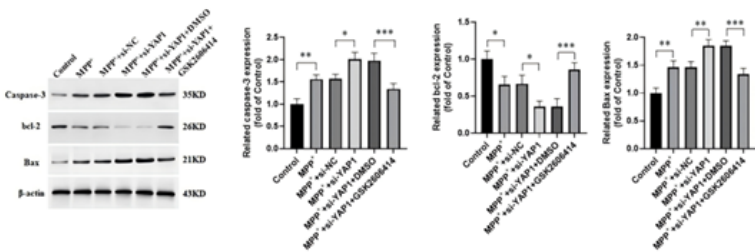


Fig. 6E

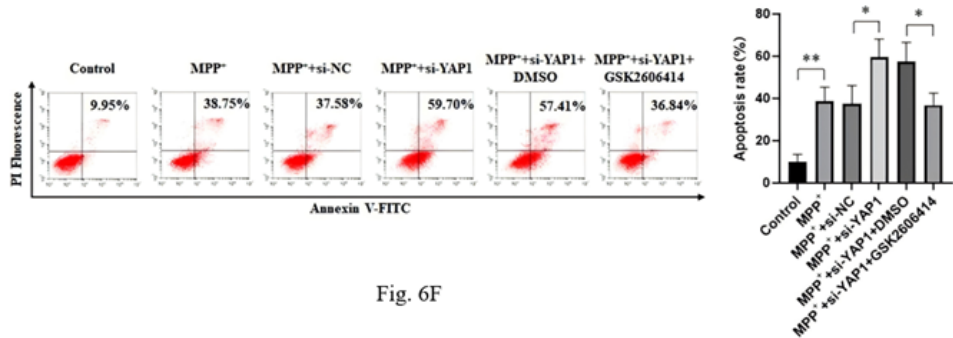


Fig. 6F

Figure 6

YAP1 alleviates MPP⁺-induced ERS and apoptosis by inhibiting the PERK pathway. (A) Western blot analysis of YAP1 knockdown efficiency. (B) CCK-8 assay detection of cell viability. (C) Western blot analysis of p-PERK, p-eIF2 α , ATF-4, and CHOP proteins expression. (D) CCK-8 assay detection of cell viability. (E) Western blot analysis of apoptosis-related proteins. (F) Flow cytometry detection of cell apoptosis rate. Data are presented as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.