The role of thioredoxin-1 in suppression of endoplasmic reticulum stress in Parkinson disease

Xian-Si Zeng a,b, Jin-Jing Jia b, Yongwon Kwon c, Sheng-Dong Wang b, Jie Bai b,∗

a Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming 650500, China
b Laboratory of Molecular Neurobiology, Medical Faculty, Kunming University of Science and Technology, Kunming 650500, China
c Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

Abstract

Endoplasmic reticulum (ER) stress has been implicated in Parkinson disease. We previously reported that thioredoxin 1 (Trx-1) suppressed the ER stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; however, its molecular mechanism remains largely unknown. In the present study, we showed that 1-methyl-4-phenylpyridinium ion (MPP+) induced ER stress by activating glucose-regulated protein 78 (GRP78), inositol-requiring enzyme 1α (IRE1α), tumor necrosis factor receptor-associated factor 2 (TRAF2), c-Jun N-terminal kinase (JNK), caspase-12, and C/EBP homologous protein (CHOP) in PC12 cells. The downregulation of Trx-1 aggravated the ER stress and further increased the expression of the above molecules induced by MPP+. In contrast, overexpression of Trx-1 attenuated the ER stress and repressed the expression of the above molecules induced by MPP+. More importantly, the overexpression of Trx-1 in transgenic mice suppressed ER stress by inhibiting the activation of these molecules. We present, for the first time, the molecular mechanism of Trx-1 suppression of endoplasmic reticulum stress in Parkinson disease in vitro and in vivo. Based on our findings, we conclude that Trx-1 plays a neuroprotective role in Parkinson disease by suppressing ER stress by regulating the activation of GRP78, IRE1α, TRAF2, JNK, caspase-12, and CHOP.

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Original Contributions

Parkinson disease (PD) is a neurodegenerative disorder characterized by a progressive degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and decreased levels of dopamine in the putamen of the dorsolateral striatum. 1-Methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its active metabolite, 1-methyl-4-phenylpyridinium ion (MPP+), are commonly used to make an experimental model of PD in vitro or in vivo [1]. The molecular mechanism of PD has been intensively investigated; however, it remains largely unknown. Massive studies have indicated that oxidative stress and endoplasmic reticulum (ER) stress are involved in the pathogenesis of PD [2].

Accumulation of unfolded proteins in the ER activates a transcriptional induction of path-proteins in the ER termed the unfolded protein response (UPR). ER stress elicits two major cellular protective responses. One is the attenuation of protein synthesis, and the other is the upregulation of genes encoding chaperones that facilitate the protein folding process. A majority of the UPR signaling pathways have been unraveled [3–5]: (1) inositol-requiring enzyme 1 (IRE1) activation that leads to X-box binding protein mRNA splicing; (2) activating transcription factor 6 (ATF6) release from the ER membrane, followed by nuclear translocation and transcriptional upregulation of ER response genes; and (3) dsRNA-dependent protein kinase-like endoplasmic reticulum kinase (PERK) activation and phosphorylation of eIF2α, which leads to the inhibition of translation initiation [6–8]. With continued ER stress, terminal UPR events occur along with release of ER-sequestered calcium, IRE1 translocation, and subsequent activation of multiple proapoptotic pathways including tumor necrosis factor receptor-associated factor 2 (TRAF2), apoptosis signal-regulating kinase-1 (ASK1), and c-Jun N-terminal kinase (JNK) that ultimately lead to cell death [9–11].

Thioredoxin-1 (Trx-1) is a 12-kDa multifunctional protein having a redox-active disulfide/dithiol within its conserved active-site sequence: -Cys-Gly-Pro-Cys- [12]. Accumulating evidence has shown that Trx-1 plays a cytoprotective role against cellular damage and stressful perturbations [13]. Trx-1 has been shown to scavenge singlet oxygen and hydroxyl radicals [14]. Trx-1 can protect cells from hydrogen peroxide, UV irradiation, and focal

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Our previous study showed that Trx-1 overexpression in PC12 cells [17]. Trx-1 has been reported as a modulator of ER stress [18]. However, the mechanism remains unknown.

In this study, we explored the effect of MPP+ on the expression of IRE1α, TRAF2, JNK, and procaspase-12 in PC12 cells. Furthermore, we examined the regulation of Trx-1 on glucose-regulated protein 78 (GRP78), IRE1α, TRAF2, C/EBP homologous protein (CHOP), and JNK. Our results suggest Trx-1 plays a protective role in suppressing MPP+ /MPTP-induced ER stress.

Materials and methods

Materials

MPP+, MPTP-HCl, and SP600125 were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). Trx-1 siRNA was chemically synthesized by Shanghai GeneChem Corp., Ltd. (Shanghai, China). Antibodies (IRE1α, TRAF2, p-JNK, GRP78, CHOP, procaspase-12, tyrosine hydroxylase (TH), and β-actin) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-mouse α-tubulin antibody was owned by our laboratory and green fluorescent protein (GFP)-tagged human Trx-1 plasmid was made by Yongwon Kwon.

Cell culture

PC12 cells of the rat pheochromocytoma tumor cell line were purchased from the Kunming Institute of Zoology (Kunming, China). PC12 cells were maintained in RPMI 1640 medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin) at 37 °C in a humid atmosphere containing 5% CO2.

SiRNA preparation and cell transfection

The sequences of Trx-1 siRNA and negative control siRNA were as follows: Trx-1 sense, 5′-CAGAAGUUGCUGCCAGACUGUt-3′, and antisense, 5′-ACGUCUGCCAGCACAUCCUt-3′; negative control sense, 5′-UUUCGGAGACUGUCCAGUtt-3′, and antisense, 5′-ACGU-GACACGUUCGGGAAtt-3′; Trx-1 siRNA and negative control siRNA were diluted to 20 μM with a universal buffer.

PC12 cells were plated in six-well plates at a density of 2 × 10^5 and allowed to adhere for 12 h. The contents of 5 μl siRNA and 5 μl Lipofectamine 2000 per well were diluted separately in serum-free Opti-MEM for a final volume of 250 μl, gently mixed, and incubated for 5 min at room temperature. Then, the diluted siRNA solution and the diluted Lipofectamine 2000 were mixed gently and incubated for 20 min at room temperature. The diluted plasmid solution and the diluted Lipofectamine 2000 were mixed gently and incubated for 20 min at room temperature. The diluted plasmid/Lipofectamine 2000 complex was added to the plates. After transfection with plasmid for 24 h, cells were stimulated with MPP+ for 24 h or 15 min and then harvested for assay.

Trx-1 overexpression in PC12 cells

GFP-tagged human Trx-1 plasmid (4 μg) and 10 μl Lipofectamine 2000 per well were diluted separately in serum-free Opti-MEM for a final volume of 250 μl, gently mixed, and incubated for 5 min at room temperature. Then, the diluted plasmid solution and the diluted Lipofectamine 2000 were mixed gently and incubated for 20 min at room temperature. The diluted plasmid/Lipofectamine 2000 complex was added to the plates. After transfection with plasmid for 24 h, the cells were stimulated with MPP+ for 24 h or 15 min and then harvested for assay.

Cell viability

PC12 cells were seeded into a 96-well plate overnight and then were incubated with Trx-1 siRNA or GFP-tagged human Trx-1 for 24 h followed by culture in the absence or presence of MPP+ in a final volume of 0.2 ml for 24 h. Thereafter, cell viability was measured using the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to the manufacturer’s instructions. The viability was calculated as the mean OD of one group/mean OD of the control.

Animal experiments

Male C57BL/6 wild-type (WT) and C57BL/6 human-Trx-1 transgenic (hTrx-1 Tg) mice, 8 weeks of age, were used in the experiments. Mice were housed in plastic cages and maintained on a 12-h light/dark cycle and had free access to food and water. The dose and time of MPTP-HCl treatment were selected as reported previously [18]. To demonstrate the protective effect of Trx-1 in Parkinson disease in vivo, mice were divided into four groups (n = 5 per group). WT and hTrx-1 Tg mice of control groups were administered saline. WT and hTrx-1 Tg mice of MPTP groups were treated with MPTP-HCl (20 mg/kg, intraperitoneal injection, twice daily for 7 days). Mice were sacrificed 2 h after the last treatment by cervical vertebra dislocation, and then heart perfusion was performed using saline. The SNpc was rapidly dissected out, frozen, and stored in a deep freezer at −80 °C until the assays. All procedures were performed in accordance with guidelines set for the use of experimental animals by the local committee on animal care and use (No. LA2008305).

Behavioral tests

Locomotor activity was measured in a plastic box (38.5 × 27.5 × 15 cm). Mice were divided into four groups (n = 5 per group). WT and hTrx-1 Tg mice of control groups were administered saline. WT and hTrx-1 Tg mice of MPTP groups were treated with MPTP-HCl (20 mg/kg, intraperitoneal injection, twice daily for 7 days). The locomotor activity was measured for 15 min after the last treatment. Limb impairment was assessed by a traction test, as described previously [19]. Mice were hung from a horizontal wire by their forepaws. A mouse was scored as 3 for gripping the wire with both hind paws, 2 for gripping the wire with one hind paw, and 1 for not gripping the wire with either hind paw.

Immunohistochemistry

Visualization of DA neurons was performed in mouse SNpc sections. Mice were sacrificed by cervical vertebra dislocation, and then heart perfusion was performed using saline solution. Brains were rapidly extracted and fixed in 4% paraformaldehyde for 24 h and then dehydrated in an ethanol series (from 70% to 100%), became transparent in xylene, were embedded in paraffin, and were serially sectioned at 15 μm. Brain sections were deparaffinized with xylene and rehydrated through an ethanol series (from 100% to 80%). Antigen retrieval was performed by soaking in boiling citrate buffer (pH 6.0) for 2 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide (H2O2) in methanol for 10 min, followed by incubation with 10% normal goat serum for 1 h at room temperature. DA neurons were visualized using an

ischemic brain damage [15,16].
anti-TH antibody (1:100). Specifically, brain sections were incubated with the primary antibodies at 4°C overnight and with biotin-conjugated goat anti-mouse Ig (1:500) at room temperature for 1 h in the dark. After being incubated with 3,3′-diaminobenzidine, brain sections were restained in hematoxylin. Images were taken using a Leica DM1000 (Leica, Beijing, China). The loss of DA neurons after MPTP-HCl treatment was defined as the variation in the brown staining.

Western blot

Protein lysates were prepared using a solubilizing solution (20 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% NP-40, 1 mM EDTA, 1 mM phenylmethanesulfonyl fluoride, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na3VO4, 1 mM β-glycerol phosphate, and 1 mg/ml leupeptin). Protein concentration was determined using the Bio-Rad protein assay reagent (Hercules, CA, USA). Equal quantities of protein were separated by 12% (for IRE1α, TRAF2, p-JNK, GRP78, CHOP, procaspase-12, and TH) or 15% (for Trx-1) SDS–PAGE and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Billerica, MA, USA). The membrane was soaked in 10% skim milk (in phosphate-buffered saline, pH 7.2, containing 0.1% Tween 20) or 3% bovine albumin V (in Tris-buffered saline, pH 7.2, containing 0.1% Tween 20) overnight at 4°C and then incubated with primary antibodies (1:1000) followed by peroxidase-conjugated anti-mouse or anti-rabbit IgG (1:10,000) (KPL, Gaithersburg, MD, USA). The epitope was visualized by an ECL Western blot detection kit (Millipore). Densitometry analysis was performed using ImageJ software.

Data analysis

Data are expressed as means ± SD. Statistical analysis was performed using SPSS software. One-way ANOVA followed by a post hoc multiple comparison test was used to compare control and treated groups. P values less than 0.05 were considered statistically significant. All blots are representative of experiments that were performed at least three times.

Results

MPP⁺ activated IRE1α, TRAF2, JNK, and caspase-12 in PC12 cells

To examine the involvement of ER stress in MPP⁺-induced toxicity in this study, we examined IRE1α expression after MPP⁺ treatment. As shown in Fig. 1A, IRE1α level was increased after MPP⁺ treatment for 24 h in a time-dependent manner. The expression of TRAF2 was also examined by Western blot in PC12 cells. Our results showed that MPP⁺ induced the expression of TRAF2 in a time-dependent manner (Fig. 1B). Further, JNK was activated in very short time (Fig. 1C). We verified that MPP⁺ induced procaspase-12 cleavage (Fig. 1D). To confirm whether procaspase-12, the executor of ER pathway-induced apoptosis, is activated by JNK, PC12 cells were pretreated with SP600125, a JNK

![Fig. 1.](image-url)
inhibitor, for 30 min followed by MPP+ treatment for 24 h. The results showed that the activation of procaspase-12 was suppressed by SP600125 (Fig. 1E). Taken together, these results suggest that the expression of IRE1α and TRAF2 and JNK is induced by MPP+ and that procaspase-12 is activated by JNK in this study.

**Trx-1 inhibited the toxicity of MPP+ in PC12 cells**

MPP+ decreases viability of PC12 cells. We duplicated that MPP+ decreased Trx-1 expression in a time-dependent manner (Fig. 2A) and MPTP decreased Trx-1 expression in SNpc of WT C57BL/6 mice (Fig. 2B). To clarify if Trx-1 plays an important role in regulating the toxic effects of MPP+, Trx-1 expression was downregulated by siRNA and upregulated by transfecting GFP-tagged human Trx-1. Trx-1 siRNA reduced the expression of Trx-1 (Fig. 2C). The Trx-1 overexpression in the PC12 cells was observed by fluorescence microscope (Fig. 2E) and verified by Western blot (Fig. 2F) after transfection for 24 h. SH-SY5Y cells were used as a positive control. Then the effect of Trx-1 on MPP+ toxicity in PC12 cells was examined by MTT assay. As shown in Fig. 2D and G, no apparent cytotoxicity was observed in PC12 cells transfected with Trx-1 siRNA or GFP-tagged human Trx-1. Downregulation of Trx-1 expression by siRNA increased the susceptibility of PC12 cells to MPP+-induced toxicity (Fig. 2D). In contrast, Trx-1 overexpression partly inhibited MPP+-induced cell toxicity (Fig. 2G). These results suggest that Trx-1 plays an important role in regulating MPP+-induced toxicity.

**Trx-1 siRNA aggravated MPP+-induced ER stress**

We investigated the effect of downregulation of Trx-1 expression by siRNA on MPP+-induced ER stress. The ER stress-associated GRP78, the marker of ER stress, was increased after Trx-1 siRNA treatment followed by MPP+ treatment (Fig. 3A), suggesting that Trx-1 siRNA aggravated ER stress induced by MPP+. We also tested the effect of Trx-1 siRNA on the expression of IRE1α, TRAF2, JNK, caspase-12, and CHOP. Accordingly, Trx-1 siRNA further increased the expression of IRE1α and TRAF2 (Fig. 3B and C) and induced activation of JNK, procaspase-12 cleavage, and CHOP expression (Fig. 3D–F). These data suggest that Trx-1 siRNA aggravates MPP+-induced ER stress via induction of GRP78, IRE1α, TRAF2, JNK, caspase-12, and CHOP.

**Trx-1 overexpression attenuated MPP+-induced ER stress**

We further examined the effect of Trx-1 overexpression on MPP+-induced ER stress. The PC12 cells were transfected with GFP-tagged human Trx-1. As shown in Fig. 4A, Trx-1 overexpression inhibited the increasing levels of GRP78 by MPP+. Moreover, MPP+-induced activation of IRE1α, TRAF2, JNK, caspase-12 cleavage, and CHOP expression was inhibited by overexpression of Trx-1 (Fig. 4B–F). These data suggest that Trx-1 overexpression attenuated MPP+-induced ER stress by inhibiting expression of IRE1α, TRAF2, and JNK; caspase-12 cleavage; and CHOP expression.

![Fig. 2. Regulation of Trx-1 in toxicity of MPP+ in PC12 cells.](image-url)

(A) MPP+ decreased the expression of Trx-1. (B) MPTP decreased the expression of Trx-1 in SNpc of mice (n = 5). (C) Western blot analysis of Trx-1 expression after transfection with negative control siRNA or 50 nM Trx-1 siRNA. (D) Effects of Trx-1 siRNA combined with MPP+ on the viability of PC12 cells. PC12 cells were either untreated or treated with 0.3 mM MPP+ after the siRNA transfection. Cell viability was measured by the MTT assay. (E) PC12 cells were transfected with GFP-tagged human Trx-1 for 24 h and the efficiency of transfection was observed by fluorescence microscope. (F) PC12 cells were transfected with GFP-tagged human Trx-1 for 24 h and Trx-1 overexpression was verified by Western blot. SH-SY5Y cells were used as a positive control. (G) Effects of Trx-1 overexpression followed by MPP+ exposure on the viability of PC12 cells. Cell viability was measured by the MTT assay. Similar experiments were repeated three times.

Statistical significance: n.s. > 0.05, *P < 0.05, **P < 0.001.
Trx-1 inhibited the MPTP-induced ER stress in vivo

We further examined whether Trx-1 could inhibit the toxicity of MPTP in mice. The human Trx-1 was stably expressed in the SNpc of transgenic mice (Fig. 5A). The expression of mTrx-1 in SNpc was decreased by MPTP in wild-type mice but not in the transgenic mice (Fig. 5B), whereas the expression of hTrx-1 in SNpc was decreased by MPTP (Fig. 5C). Locomotor deficit is an important hallmark of PD. The motor activity in the WT mice treated with MPTP was reduced significantly compared to WT mice treated with saline (Fig. 5D); in contrast, motor activity in the hTrx-1-overexpressing Tg mice treated with MPTP was not reduced (Fig. 5D). In this study, we also examined the effects of Trx-1 on the impairment of limb movement caused by MPTP. As shown in Fig. 5E, overexpression of Trx-1 ameliorated MPTP-induced impairment of limb movement assessed by traction tests. We further examined the expression of TH in SNpc to assess the loss of DA neurons by immunohistochemistry and Western blot analysis. As shown in Fig. 5F and G, TH expression in the SNpc was significantly decreased in WT mice after treatment with MPTP. However, overexpression of Trx-1 restored TH expression in hTrx-1 Tg mice treated with MPTP. These results indicate that overexpression of Trx-1 protects DA neurons from the neurotoxicity of MPTP. Concordant with the results in PC12 cells, overexpression of Trx-1 significantly inhibited the increase in GRP78 in SNpc in hTrx-1 Tg mice (Fig. 5H), suggesting that overexpression of Trx-1 attenuated MPTP-induced ER stress in hTrx-1 Tg mice. Moreover, MPTP-induced activation of IRE1α, TRAF2, JNK, caspase-12 cleavage, and CHOP was suppressed in SNpc in hTrx-1 Tg mice (Fig. 5I–M). These data indicate that Trx-1 has neuroprotective roles in suppressing MPTP-induced neurotoxicity by regulating the activation of GRP78, IRE1α, TRAF2, JNK, caspase-12, and CHOP.

Discussion

PD is the second most common neurodegenerative disorder, affecting approximately 6 million people worldwide [20]. However, there are no proven interventions to slow down the underlying degenerative process [21] because of the unclear mechanism.

Numerous studies have indicated that ER stress is involved in neuronal death in PD [21]. The ER-resident chaperone GRP78 is a marker for ER stress that plays a crucial role in the regulation of the ER dynamic homeostasis [22]. Under nonstressed conditions, GRP78 binds and represses ER transmembrane proteins, including IRE1, PERK, and ATF6. In response to ER stress, GRP78 dissociates from these transmembrane proteins, which leads to their activation and triggers the UPR [23–26]. IRE1 is hypothesized to be able to initiate apoptosis [27] and is clearly connected to cell death [28] because activated IRE1α has been shown to recruit the adaptor molecule TRAF2 [11,29]. IRE1α binds to TRAF2 and activates JNK through its kinase activity [10,11,29]. IRE1α, TRAF2, and JNK were activated (Fig. 1A–C), which suggests that ER stress is induced by MPP⁺ in this study. It has been reported that ER stress stimulus-induced cell death is mediated by the activation of caspase-12 [30]. The expression of procaspase-12 was also decreased significantly after MPP⁺ treatment in PC12 cells (Fig. 1D). Moreover, MPP⁺-induced procaspase-12 cleavage was inhibited by the JNK
inhibitor SP600125 (Fig. 1E), suggesting that caspase-12 is downstream of JNK. Thus, these data suggest that MPP⁺ induces ER stress by activating GRP78, IRE1α, TRAF2, JNK, and caspase-12.

Trx-1 expression is significantly decreased by MPP⁺ treatment in vitro [17,31]. In this study, MPP⁺ also reduced Trx-1 expression in a time-dependent manner in PC12 cells (Fig. 2A) and MPTP decreased Trx-1 expression in SNpc of WT C57BL/6 mice (Fig. 2B). Our previous study showed that inducers of Trx-1, such as panaxatriol saponins extracted from *Panax notoginseng* [32], provided neuroprotection against MPTP-induced neurotoxicity in vitro and in vivo [2]. Overexpression or administration of Trx-1 attenuates MPP⁺-induced neurotoxicity [17]. Therefore, we examined the effects of downregulation and overexpression of Trx-1 on MPP⁺-induced neurotoxicity in vitro and in vivo [2]. Overexpression or administration of Trx-1 attenuates MPP⁺-induced neurotoxicity [17]. Therefore, we examined the effects of downregulation and overexpression of Trx-1 on MPP⁺-induced toxicity in PC12 cells. Downregulation of Trx-1 by siRNA increased the susceptibility of PC12 cells to MPP⁺-induced toxicity (Fig. 2D). In contrast, Trx-1 overexpression inhibited the toxicity (Fig. 2C). These results suggest that Trx-1 plays an important role in regulating MPP⁺-induced toxicity.

The signal pathway in ER stress was then examined. The downregulation of Trx-1 by siRNA further enhanced the expression of GRP78 (Fig. 3A). In contrast, the overexpression of Trx-1 inhibited the increase in GRP78 induced by MPP⁺ treatment (Fig. 4A). Trx-1 siRNA increased the expression of IRE1α and TRAF2, as well as the activation of JNK and procaspase-12 (Fig. 3B–E). Moreover, the overexpression of Trx-1 suppressed the activation of IRE1α, TRAF2, JNK, and caspase-12 by induced by MPP⁺ (Fig. 4B–E). Meanwhile, the hTrx-1 Tg mice also exhibited a role for hTrx-1 in suppressing the expression of IRE1α, TRAF2, JNK, and caspase-12 induced by MPTP (Fig. 5I–L).

In addition to activation of the JNK pathway and ER-associated caspase-12 [33–36], CHOP was usually activated during ER stress. Severe ER stress has been linked with cell death through increased expression of CHOP, a transcription factor that sensitizes the cells to apoptosis. The IRE1α/TRA2 pathway is known to enhance CHOP activity at the posttranscriptional level [37]. Moreover, knockdown of CHOP reduced caspase-12 cleavage [38]. Consistently, our results showed that CHOP expression was induced by MPP⁺/MPTP, which was regulated by Trx-1 expression (Figs. 3F, 4F, and 5M). Our data suggest that Trx-1 regulates MPP⁺/MPTP-induced ER stress by repressing the expression of IRE1α, TRAF2, JNK, caspase-12, and CHOP (Fig. 6).

It should be noted that activation of JNK by IRE1α and TRAF2 required the presence of ASK1 [10]. Moreover, IRE1α also stimulates activation of ASK1, which causes activation downstream of stress kinases JNK and p38MAPK, which promote apoptosis [39]. Continuously, p38MAPK activates CHOP via phosphorylation of its transactivation domain [40]. Trx-1 has been reported to act as an endogenous inhibitor of ASK1 [41]; when Trx-1 is reduced it binds ASK1. Thus, overexpression of Trx-1 may repress JNK activation by suppressing ASK1. The regulation of CHOP by Trx-1 may be due to Trx-1 suppressing the activity of p38MAPK [42]. Interestingly, induction of CHOP was reported to perturb the cellular redox state by depleting cellular glutathione [43]. CHOP also activates ER oxidoreductin 1, which encodes an ER oxidase. Moreover, ER-stressed CHOP⁻/⁻ cells are relatively hypo-oxidizing [34]. Excessive free radical formation or antioxidant deficiency may be a possible mechanism for the toxicity of MPP⁺/MPTP [44]. Thus, Trx-1 may also play roles in regulating hypo-oxidizing and
Fig. 5. Inhibition by Trx-1 overexpression of behavioral deficits and ER stress-related proteins induced by MPTP. (A) Western blot analysis of hTrx-1 expression in mouse SNpc. The mice were divided into four groups (n = 5 animals per group). WT and hTrx-1 Tg mice of control groups were administered saline. WT and hTrx-1 Tg mice of MPTP groups were treated with MPTP-HCl (20 mg/kg, intraperitoneal injection, twice daily for 7 days). (B) The hTrx-1 overexpression significantly inhibited the decrease in mTrx-1 by MPTP in SNpc. (C) The expression of hTrx-1 was decreased by MPTP. (D) Ambulatory activity was measured for 15 min after the treatments. (E) Limb impairment was assessed by traction test. (F) Immunohistochemistry of TH expression in SNpc. (G) Western blot of TH expression in SNpc. (H) Trx-1 repressed the increase in GRP78 in SNpc in hTrx-1 Tg mice. (I) Trx-1 repressed the increase in IRE1α in SNpc in hTrx-1 Tg mice. (J) Trx-1 repressed the increase in TRAF2 in SNpc in hTrx-1 Tg mice. (K) Trx-1 inhibited the activation of JNK in SNpc in hTrx-1 Tg mice. (L) Trx-1 inhibited the activation of procaspase-12 in SNpc in hTrx-1 Tg mice. (M) Trx-1 repressed the increase in CHOP in SNpc in hTrx-1 Tg mice. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001.
resisting oxidative stress. This molecular mechanism needs to be further studied.

In summary, Trx-1 plays a neuroprotective role in Parkinson disease by suppressing ER stress by regulating the activation of GRP78, IRE1α, TRAF2, JNK, caspase-12, and CHOP. So Trx-1 may be a potential target for regulating ER stress in PD.

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