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Original Paper

Curcumin Protects an SH-SY5Y Cell Model of Parkinson's Disease Against Toxic Injury by Regulating HSP90

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Key Words
Parkinson • MPP^+ • Curcumin • HSP90 • SH-SY5Y • Dopaminergic neurons

Abstract
Background/Aims: We aimed to explore the protective role of curcumin (Cur) in a cell model of Parkinson's disease (PD) and its underlying mechanism. ***Methods:*** In this study, genes concerned with PD-related keywords were screened within DiGSeE database. The association network between Cur and selected genes was downloaded from STITCH, with the interactions analyzed by STRING. We built a mitochondrial toxin 1-methyl-4-phenylpyridinium (MPP^+)-induced SH-SY5Y cell model of PD. Cell morphology was observed under an electron microscope. MTT assay was applied to detect cell proliferation rate. Western blot assay was conducted to determine the level of apoptotic markers, including cleaved caspase 3, Bcl-2-associated X protein (Bax) and B-cell lymphoma-extra-large (Bcl-xl). Tyrosine hydroxylase (TH), dopamine transporter (DAT) protein levels and dopamine (DA) concentration were identified as dopaminergic neuron markers and measured by western blotting or Enzyme-linked immunosorbent assay (ELISA). ***Results***: Cur rescued the toxicity effects of MPP^+ on SH-SY5Y cells, by controlling morphological change, promoting cell proliferation and inhibiting apoptosis. Of all PD-related genes, *HSP90* played an important role in Cur-gene network. HSP90 protein level was elevated by MPP^+, whereas Cur could reverse this effect. Silencing of *HSP90* significantly attenuated the curative effect introduced by Cur, while *HSP90* overexpression enhanced the impact of Cur on PD. ***Conclusion:*** Cur can effectively inhibit the toxic effect of MPP^+ on SH-SY5Y cells and significantly reduce the adverse effects of MPP^+ on dopaminergic neurons via up-regulation of *HSP90*.

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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by reduced dopaminergic neurons in substantia nigra pars compacta (SNc) and accumulated misfolded α-synuclein protein to generate eosinophilic protein deposits (Lewy bodies) [1, 2]. These characteristics account for clinical symptoms of PD, including bradykinesia, rigidity, tremor and postural instability; however, PD is presented as a multisystem disorder, which leads to other non-motor defects such as cognitive dysfunction and sleep disturbance during progression [3]. PD is ranked as the 2nd most common neurodegenerative disorder just after Alzheimer's disease as it affects approximately 1% of persons aged above 65 and almost 3% of those over 80 [3]. Aging being the greatest risk factor, PD patient number is still at rise and is estimated to duplicate by year 2030 [3, 4]. The etiology of PD has not been fully revealed, but a combination of genetic and environmental factors has often been involved in current literature [2]. As for the environmental approach, the development of PD models introduced by chemicals such as 1-methyl-4-phenyl-1, 2-3, 6-tetrahyrdopyridine (MPTP) or 1-methyl-4-phenyl-pyridiniumion (MPP^+) has conditioned further investigations into PD [1, 4].

Genetic studies have found genes such as SNCA, LRRK2, PINK1, DJ-1, ATP13A2 and CHCHD2 to be correlated with PD [2]. A study of van de Merwe *et al.* proposed that curcumin (Cur) protected against PD injury in a PINK1-knockdown cell model [2]. Cur, or diferuloylmethane, is a natural ingredient extracted from Curcuma longa rhizome [5, 6]. It has attracted great interest for its numerous pharmacological properties such as anti-inflammation, antioxidant, anti-tumor and anti-nerve degeneration [2, 7]. Previous studies suggested that Cur could be used in PD treatment *in vivo* [8]. For instance, Liu *et al.* demonstrated that Cur was protective in PC12 cell models of PD stimulated by A53T α-synuclein [9]. Yu *et al.* investigated via an MPP^+-introduced PD model suggested that the anti-inflammatory and anti-oxidative roles of Cur were by interacting with TLR4 and its downstream effectors [5]. Cui *et al.* emphasized its mitigation on PD dopaminergic neural damage, of which increased tyrosine hydroxylase (TH) activity was included as a parameter [8]. However, the underlying mechanism of Cur being anti-PD has not been fully understood [7].

The 90-kDa heat shock protein (Hsp90) belongs to the heat shock proteins (HSPs), which serve as molecular chaperons to assist protein folding, stabilization and activation, etc. [10, 11]. HSPs will be raised by cellular stress, but remain at a low level in healthy conditions [12]. Hsp90 is a major therapeutic target in cancer studies, but more recently it is focused for a curative role in neurodegenerative disorders [11]. In PD, Aridon *et al.* argued that Hsp70 and Hsp90 prevented protein misfolding and repressed neuron apoptosis [12]. Daturpalli *et al.* found that Hsp90 prevented α-synuclein accumulation and Lewy body formation to protect against PD [11]. Curcumin was used to treat various diseases through altering HSPs expression in many reports. For instance, Lv *et al.* had reported that curcumin inhibited heat shock protein 90 (Hsp90) increase in HCMV infection of human embryonic lung fibroblast cells through hydrogen bonds, hydrophobic interactions and conjugation to bind with Hsp90 [13]. Additionally, curcumin attenuated selenite-induced cataract through the reduction of Hsp70 and other intracellular production of reactive oxygen species [14]. Among the PD related genes, *HSP90* showed the direct interaction with curcumin in this study, besides that the combination therapy of curcumin and *HSP90* for Parkinson has not yet been investigated. In this study, we applied bioinformatic analysis to screen out Cur-and PD- related genes to investigate the underlying mechanism of Cur in preventing against PD injury. MPP^+-stimulated SH-SY5Y cell models were built for experimental study. Cell proliferation, apoptosis and morphology were taken as criteria for MPP^+ cytotoxicity. Dopaminergic neuron markers were measured by western blotting and enzyme-linked immunosorbent assay (ELISA). Our research suggested that Cur improved PD cells' survival rate, which could be improved by *HSP90* overexpression.

Materials and Methods

Bioinformatics retrieval

DiGSeE (http://210.107.182.61/geneSearch/) is a search platform for genetic basis of human diseases. With the keyword of "Parkinson", 3 data like "Parkinson disease", "Parkinson disease, secondary" and "Parkinsonian disorders" were screened out and their gene sets were selected to figure out the co-existed PD genes. The interactions between these genes and Cur were figured out and plotted via STRING (http://stitch.embl.de/), with the calculation done by Dijkstra algorithm.

SH-SY5Y cell model of PD

2.9714 mg of MPP^+ was dissolved with 1 mL of double distilled water to obtain 10 mmol/L MPP^+ solution. The solution was filtrated and stored in dark at -20 °C. Digested SH-SY5Y cells (BNCC338056, BeNa Culture Collection, Beijing, China) were grown in high-glucose Dulbecco's modified eagle medium (DMEM-H) with 10% Fetal Bovine Serum (FBS) containing glutamine and sodium pyruvate, in a 5% CO_2 humidified incubator at 37 °C. Then cells were incubated for 12 h in 96-well plates at a density of 1×10^5 / mL and 100 μL per well.

Well-cultivated SH-SY5Y PD cells were divided into several groups according to treatments of different concentrations of MPP^+ and Cur. A normal group was left with treatment of neither MPP^+ nor Cur. A group of 400 μmol/L MPP^+ treatment went in contrast with groups of 400 μmol/L MPP^+ + 10, 20 or 40 μmol/L Cur treatment. Cells morphology was observed under an electron microscope (Olympus, Tokyo, Japan) after 24 h cultivation.

Transfection

Overexpression vectors of *HSP90* (ov-HSP90) and short interference RNA siRNA of *HSP90* (si-HSP90) for transfection were purchased from GenePharma (Shanghai, China). Before transfection, the cells were starved for 6 h with 2% FBS culture condition. The transfection was performed via Lipofectamine 3000 (Life Technologies, USA) according to the manufacturer's protocols. During the transfection, cells were under free-FBS condition. After 6 h transfection, the culture was refreshed with whole medium culture (10% FBS, high-glucose DMEM). All the following experiments were conducted after 24 h transfection. The control group in Fig. 1 was transfected with nothing but transfection reagent.

MTT assay

MTT assay was used to detect the effects of drugs on cell viability. MTT Cell Proliferation Kit and Cytotoxicity Assay Kit were purchased from Sangon Biotech (Shanghai, China) and were used to perform MTT assay. After 12 h treatment of 0 μmol/L, 50 μmol/L, 100 μmol/L, 200 μmol/L, 400 μmol/L or 800 μmol/L (μM) MPP^+ with or without 40 μmol/L curcumin (Cur), the viability of SH-SY5Y cell was identified via MTT assay. 1 mg MTT was added to each milliliter of medium and incubated at 37 °C for 4 h. After 4 h, the medium in the plate was discarded, and 200 μL dimethylsulphoxide (DMSO) was added to each well before one-minute shaking for dissolution in a microplate reader (BioRad Model 680; Bio-Rad, Hercules, CA, USA). The absorbance of each well was measured at 570 nm, and the cell growth curve was plotted based on an average of five wells. The absorbance of control group (0 μmol/L MPP^+ or only transfection reagent) was normalized as 100% and the other groups were compared with it. The experiment was repeated in triplicate.

Western blot

SH-SY5Y PD cells treated with or without Cur were lysed in radio-immunoprecipitation assay (RIPA) buffer (BioVision, Milpitas, CA, USA). Then cells were sonicated and centrifuged for complete lysis. Total protein in supernatants was quantified using BCA-200 protein assay kit (Pierce Biotechnology, Rockford, IL, USA). 20 μL of samples were mixed with loading buffer and dithiothreitol (DTT) at a ratio of 8:10:2, and then boiled for 5 min. The protein was separated with 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a polyvinylidene fluoride (PVDF) membrane (500 mA). The membrane was sealed up in Tris Buffered Saline Tween (TBST) with 5% skim milk at room temperate for 1 h, and subsequently cultured with primary antibodies including anti-Tyrosine Hydroxylase (ab112, 0.75μg/mL ; Abcam Inc., Cambridge, MA, USA), anti-Dopamine Transporter (ab111468, 1 μg/mL),

anti-Hsp90 (ab13495, 0.5 μg/mL), anti-cleaved-Caspase 3 (ab2302, 1 μg/mL), anti-Bcl-XL (ab2568, 1 μg/mL), anti-Bax (ab32503, 0.1 μg/mL), anti-β-actin (ab179467, 0.1 μg/mL) and anti-Tubulin (ab6160, 1 μg/mL) at 4 °C for one night. After TBST-washing in triplicate, the membrane was incubated for 1 h at room temperature with secondary antibody Goat anti-Rabbit IgG H&L (HRP) (ab6721, 0.1 μg/mL). Protein bands were visualized using enhanced chemiluminescence (Santa Cruz Biotechnology; Santa Cruz, CA, USA). Quantity One software (Bio-Rad) was used for image analysis. The results were analyzed by Image Pro Plus 5.0 (Media, Cybernetics, USA). Tubulin and β-actin were included as internal controls. The experiment was repeated in triplicate.

ELISA

Human Dopamine (DA) ELISA Kit (MBS045009; MyBioSource, San Diego, CA; sensitivity: 1.0 pg/mL) was used for determination of DA concentrations in SH-SY5Y cells treated with variations of MPP^+ and Cur for 24 h according to the manufacturer's instructions. We measured sample absorbance at 450 nm. The experiment was repeated in triplicate.

Data processing

Data were shown as mean ± SEM (standard error of mean). All analysis was done in SPSS 10.0 software. Comparisons were conducted by one-way or two-way ANOVAs, along with Newman-Keuls or Bonferroni correction for multiple comparison tests. $P<0.05$ was regarded as statistically significant.

Results

Curcumin relieved the alternation of SH-SY5Y cells introduced by MPP^+

Normal SH-SY5Y cells were spindle or polygonal, being transparent and of high refractive index. Each cell had reticular connection with surrounding cells and disclosed numerous elongations. After 24 h treatment with 400 μmol/L MPP^+, cell connections disappeared or were broken, and the cell bodies shrunk. When higher concentration (40 μmol/L) of Cur was cultivated with PD cell models, fewer cells shrank, the elongation of cells was retrieved, and the intercellular connections increased (Fig. 2A). The above observations indicated that Cur could prevent the toxic effect of MPP^+ on cellular morphology.

SH-SY5Y cell viability was measured by MTT assay after 12 h incubation in 0, 50, 100, 200, 400 or 800 μM MPP^+. As shown in Fig. 2B, 400 μM MPP^+ reduced survival rate by half and 800 μM MPP^+ reduced survival rate to approximately 25%. These results indicated that MPP^+ had pronounced roles in inhibiting cell survive ($$$$P<0.0001$). Treatment with Cur (40 μM) significantly attenuated the toxicity of MPP^+, indicated by cell activity maintained at a comparatively high scale with accelerated MPP^+ concentration (Fig. 2B, $*P<0.05$, $**<0.01$). The concentration of DA in SH-SY5Y cells detected by ELISA presented the same trend (Fig. 2C, $P<0.05$). Western blot was conducted to detect the protein level of pro-apoptotic factors cleaved-caspase 3, Bax and anti-apoptotic Bcl-xl after 24 h cultivation in MPP^+ (400 μM) with or without Cur (40 μM). Comparing normal group, MPP^+ (400 μM) significantly increased cleaved-caspase 3 and Bax level and cut down Bcl-xl level, which indicated promotion in apoptosis rate. Cur (40 μM) in turn reversed this trend (Fig. 2D, $P<0.05$). The results of Western blot on TH and DAT were shown in Fig. 2E. MPP^+ (400 μM) could induce cytotoxicity, which significantly decreased the protein level of TH and DAT. The addition of Cur (40 μM) could reduce MPP^+-introduced cytotoxicity as reflected by recovered protein level of TH and DAT. Therefore, Curcumin protected dopaminergic neurons against MPP^+ toxicity.

HSP90 was identified as the core gene correlated with Cur

"Parkinson Disease", "Parkinson Disease, Secondary" and "Parkinsonian Disorders" in Nervous System Diseases were put into the database of DiGSeE to probe for PD-related genes. After deletion of duplicate values, 491 related genes were selected by "Parkinson Disease", 64 by "Parkinson Disease, Secondary", and 1101 by "Parkinsonian Disorders" (Table S1 - for all supplemental material see www.karger.com/10.1159/000495326).

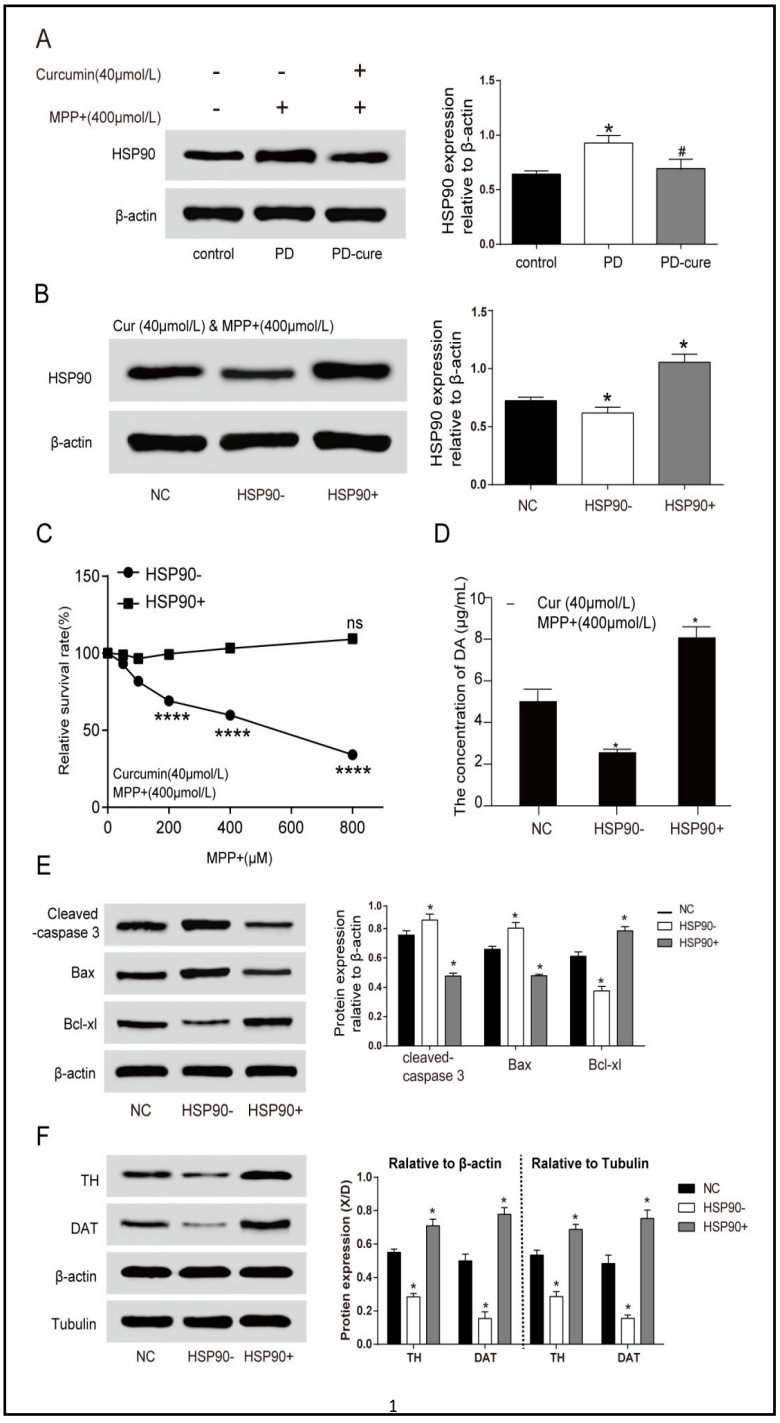

Fig. 1. HSP90 played an important role for Cur to alleviate MPP⁺-induced defects. (A) Western blot results showed that Hsp90 protein level was upregulated at an MPP⁺ condition, and this increase was relieved by Cur treatment. 1way-ANOVA: F (2, 6) = 48.69, P<0.0001. * P<0.05 compared to normal group and # P<0.05 compared to PD group. (B) Western blot was performed to determine the protein level of Hsp90 after overexpression or downregulation of HSP90. The result indicated successful transfection. 1way-ANOVA: F (2, 6) = 56.50, P<0.0001. (C) MTT assay was put forward to test the influence of HSP90 expression on cell survival rate, relative to corresponding MPP⁺ group. Overexpression of HSP90 retained relative survival rate of SH-SY5Y cells. 1way-ANOVA: F (4, 20) = 61.10, P<0.0001 (HSP-), F (4, 20), P=0.8404 (HSP+). (D) DA concentration was detected by ELISA. Silencing of HSP90 reduced DA concentration while upregulation of HSP90 uplifted DA concentration. 1way-ANOVA: F (2, 6) = 26.32, P<0.001. (E) The protein level of apoptosis-related protein cleaved caspase 3, Bax and Bcl-xl were detected via western blot. Cleaved caspase 3 and Bax levels increased but Bcl-xl decreased after knockdown of HSP90, suggesting facilitated apoptosis. Overexpression of HSP90 cut down caspase 3 and Bax while raises up Bcl-xl, suggesting blocked apoptosis. 1way-ANOVA: F (4, 18) = 171.9, P<0.0001 (cleaved-caspase 3), F (4, 18) = 41.49, P<0.0001 (Bax), F (4, 18) =51.72, P<0.0001 (Bcl-xl). (F) Protein levels of dopaminergic neuron markers TH and DAT were detected via western blot. Both were downregulated by HSP90- yet upregulated by HSP90+. 1way-ANOVA: F (2, 12) = 12.39, P<0.001 (TH) and F (2, 12) = 10.81, P<0.001 (DAT). NC group only transfected with transfected reagent. HSP90- means HSP90 knockdown with HSP90 siRNA transfection and HSP90+ means HSP90 overexpression with HSP90 overexpressed vector transfection. * P<0.05, ****P<0.0001, ns = no significance compared to NC group.

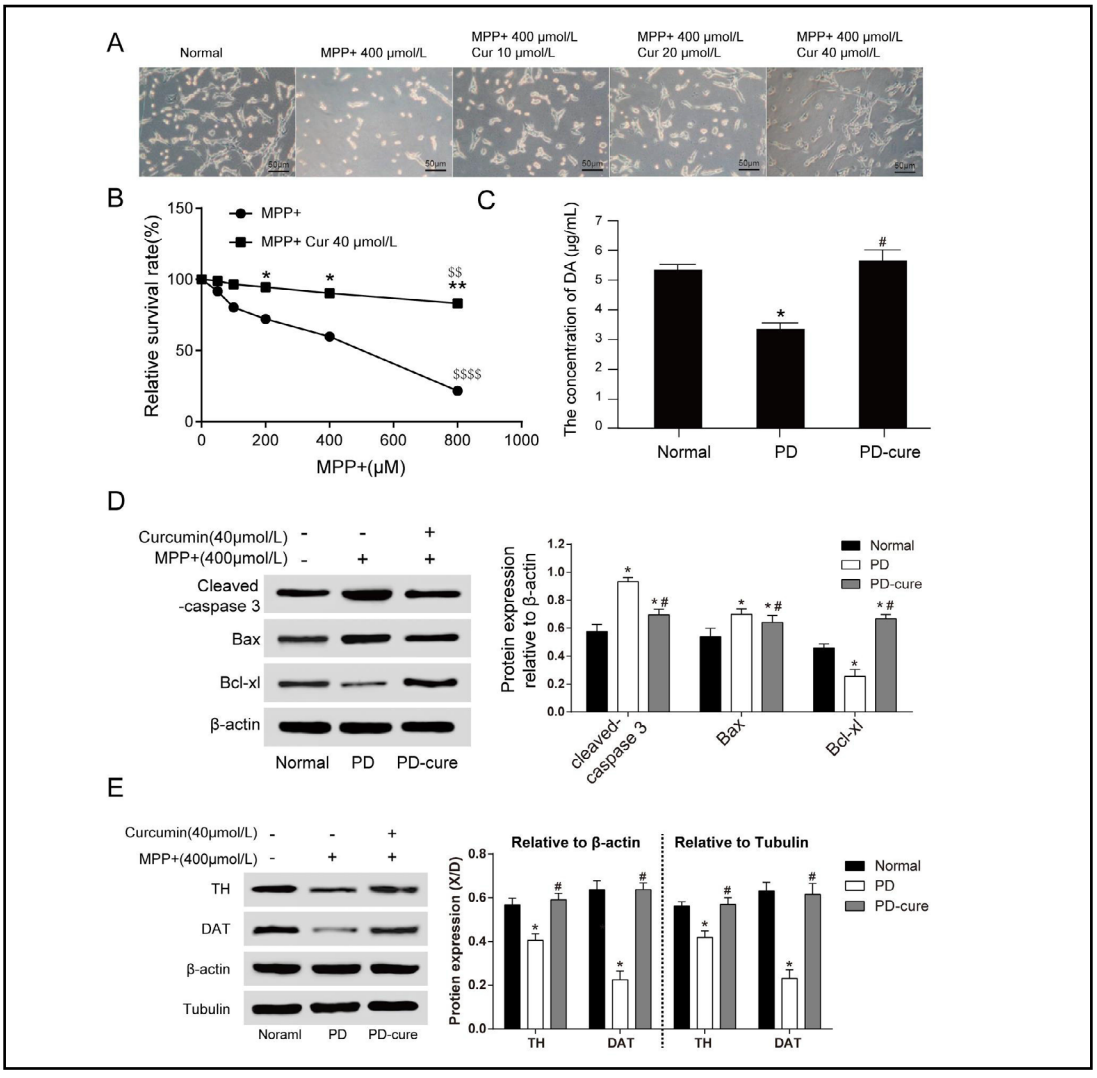

Fig. 2. Cur relieved the toxicity of MPP⁺ on SH-SY5Y cells. (A) SH-SY5Y cells were treated with 1-methyl-4-phenylpyridinium (MPP⁺, 400 μM) and curcumin (Cur) of different concentrations (0-40 μM) for microscope morphology observation. (B) Cells were treated with or without 40 μM Cur, and cell survival rate was determined in MTT assay under various MPP⁺ concentration. With increase in MPP⁺ concentration, the relative survival rate of SH-SY5Y cells decreased ($$P<0.01, $$P<0.0001, comparison was between cell survival rate in 0 μM and 800μM MPP+ environment). With treatment of Cur, the survival rate was significantly improved. Significance between lines was marked by asterisk (*P<0.05, **P<0.01). 2way-ANOVA: F (4, 20) = 38.05, P<0.0001 for factor- MMP⁺, F (1, 20) =15.82, P<0.001 for factor-Cur and F (4, 20) =9.7, P<0.001 for interaction. (C) DA concentration was determined by Enzyme-linked immunosorbent assay (ELISA). MPP⁺ decreased the concentration of DA, which was recovered by treatment of Cur. 1way-ANOVA: F (2, 6) = 16.76, P<0.05. (D) Western blot was performed to check cell apoptosis level. While MPP⁺ increased cleaved caspase 3 and Bax but decreased Bcl-xl, the addition of Cur relieved the apoptosis rate by reversing these changes on apoptotic protein levels. 1way-ANOVA: F (4, 18) = 32.25, P<0.0001 (cleaved caspase 3), F (4, 18) = 23.71, P<0.0001 (Bax) and F (4, 18) = 56, P<0.0001 (Bcl-xl). (E) Western blot was performed to check dopaminergic neuron markers TH and DAT. MPP⁺ attenuated the level of TH and DAT whereas Cur relieved this suppression. 1way-ANOVA: F (2, 12) = 27.21, P<0.0001 (TH relative to β-actin), F (2, 12) = 116.1, P<0.0001 (DAT relative to β-actin), F (2, 12) = 14.09, P<0.001 (TH relative to tubulin) and F (2, 12) = 100.3, P<0.0001 (DAT relative to tubulin). PD means Parkinson model induced with MPP+ 400 μmol/L and PD cure means Cur 40 μmol/L treated cells after PD-induced. *P<0.05 compared to normal group, # P<0.05 compared to PD group.

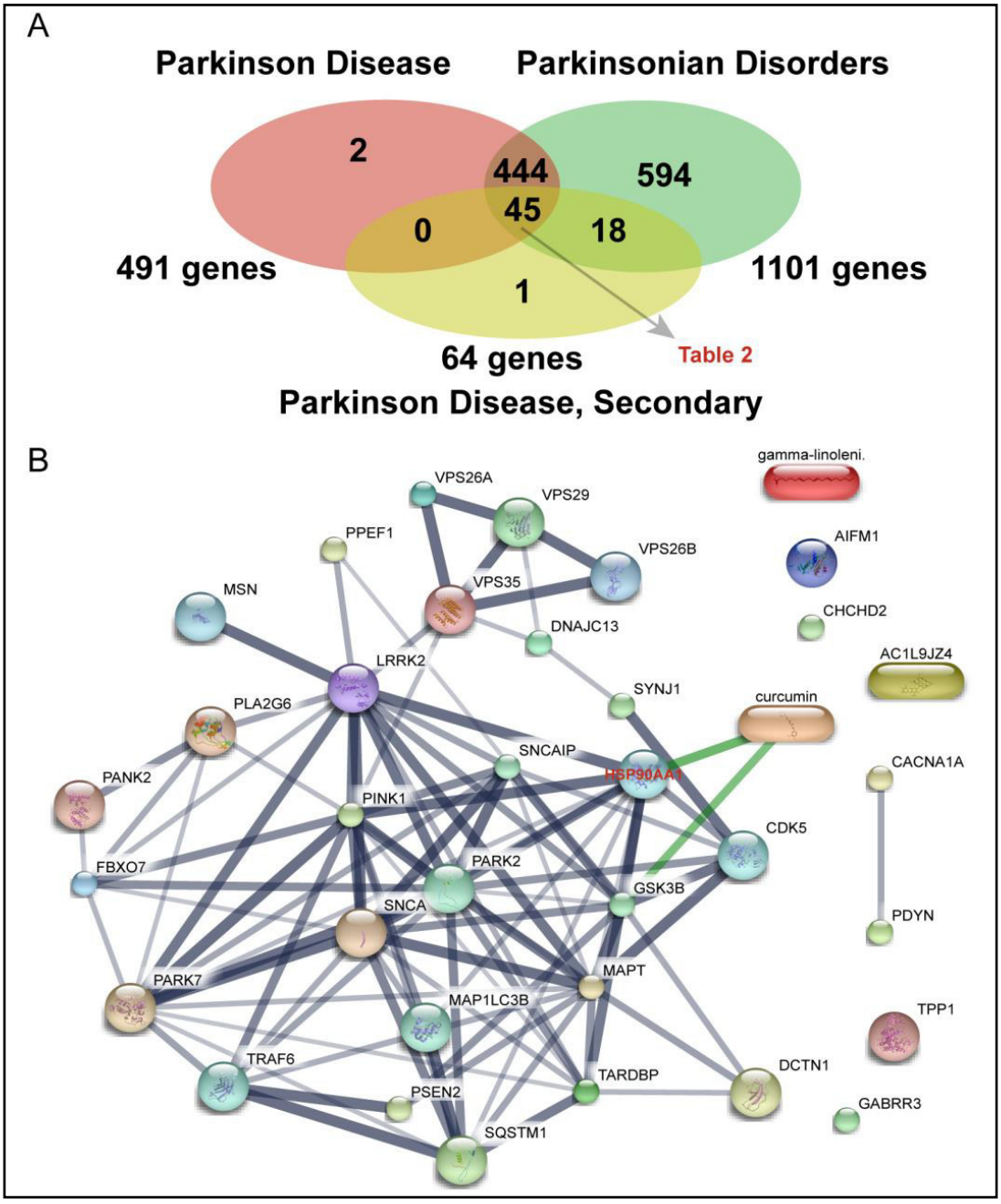

Fig. 3. HSP90 was identified as being closely related with Cur as a PD-correlated gene. (A) Genes related with keywords "Parkinson Disease", "Parkinson Disease, Secondary" or "Parkinsonian Disorders" were selected by DiGSeE, with the result illustrated by intersecting red, green-yellow and green circles. In total 45 co-existed genes appeared in the intersection of three set of genes. (B) The co-existed genes were analyzed by STRING with keyword "curcumin". HSP90 and GSK3β were the only two genes which had direct interaction with Cur, and since HSP90 appeared in a core position, it was selected for further analysis.

There were 45 co-existed genes in the intersection of three gene sets, which were listed in Table S2. The relationships between selected genes were visualized by Fig. 3A. The co-existed gene names were analyzed with keyword "curcumin" by STRING. Judging from the correlation network presented in Fig. 3B, *HSP90* and *GSK3β* directly interacted with Cur, and *HSP90* appeared in a core position.

Cur alleviated MPP⁺-induced injury via HSP90

Following the bioinformatic analysis, western blotting was performed to explore the role of Cur on *HSP90* expression in PD. The results of western blot showed that the HSP90 protein level in SH-SY5Y cell models induced by MPP^+ (400 μM) was significantly higher compared with the control group ($P<0.05$), which indicated that MPP^+ promoted the expression of *HSP90*. The addition of Cur (40 μM) could significantly reduce this uplift, and therefore diminished the toxic effects induced by MPP^+ (Fig. 1A, $P<0.05$). Hence, we hypothesized that *HSP90* was involved in the manipulation of Cur on PD.

To evaluate the function of *HSP90* in the process of Cur treatment on PD cell models, we transfected SH-SY5Y cells incubated with or without Cur (40 μM) and MPP^+ (400 μM) with overexpression vector (HSP90+) and short interference RNA (siRNA) to upregulate and knockdown the protein level of HSP90 (HSP90-) (Fig. 1B and Fig. S1A, $P<0.05$). In an MTT assay, each group (HSP90- group and HSP90+ group) was compared with control group which transfected with nothing but transfection reagent and normalized as 100%. After downregulating *HSP90*, the rescue effect of Cur on PD-models with treatment of increasing concentrations of MPP^+ was not statistically significant as indicated by increasing survival rate. On the contrary, overexpressed *HSP90* facilitated Cur (40 μM) treatment as indicated by similar survival rate compared to control group (without MPP^+) (Fig. 1C, $^{****}P<0.0001$, ns = no significance). As presented in Fig. 1D, there was a significant increase in DA concentration in HSP90+ group, indicating that *HSP90* overexpression promoted the state of dopaminergic neuron ($P<0.05$). The level of apoptosis-related protein Cleaved-caspase 3 and Bax was increased by *HSP90* knockdown and decreased by *HSP90* overexpression, and that of Bcl-xl acted likewise yet with a reversed trend (Fig. 1E, $P<0.05$). Both implied that silencing of *HSP90* introduced cell apoptosis. The weakening effect in the HSP90- group was also reflected by the detection of decreases in dopaminergic neuron markers TH and DAT by western blot (Fig. 1F, $P<0.05$). However, regulating *HSP90* alone on PD-models with MPP^+ (400 μM) showed no significance difference on the expression of those proteins (Fig. S1). In a word, the combined treatment of Cur and *HSP90* overexpression played a better role in improving MPP^+-induced cytotoxicity in PD-cells (Fig. S2).

Discussion

In the present study, we found a cell-protective effect of Cur in MPP^+-induced SH-SY5Y cell models of PD. Cur treatment restored cell morphological change, increased cell survival rate, inhibited apoptosis and relieved dopaminergic neuron loss. *HSP90* was detected as a core gene in correlation with both PD and Cur, and its protein level was upregulated in PD models. The rescuing effect of Cur was significantly attenuated by silencing *HSP90* and facilitated by *HSP90* overexpression. In a word, Cur protects against PD injury via *HSP90*.

Cur rehabilitated PD-cells in our observation, which is consistent with previous findings on similar therapeutic benefits. Yang *et al.* proposed that Cur reduced leucine-rich repeat kinase 2 (LRRK2) activity in LRRK2-stimulated PD cell models and promoted recovery of LRRK2 transgenic drosophilae [15]. Singh *et al.* figured out the biophysical mechanism of Cur on treatment for PD that Cur bound to preformed oligomers and fibrils to prevent aggregation of α-synuclein [16]. Although some researchers highlighted the low bioavailability of curcumin *in vivo*, which was attributed to limited absorption and metabolic instability [17, 18], stable analogs of Cur such as curcumin pyrazole and its derivative N-(3-Nitrophenylpyrazole) curcumin were utilized by Ahsan *et al.* to prevent neurotoxicity in PD along with other neurodegenerative diseases [17]. Ojha *et al.* reported that curcuminoid including curcumin, demethoxy curcumin and bis-demethoxy curcumin relieved dopaminergic neurodegeneration in MPTP-stimulated PD models [19]. In addition to naturally refined curcumin, semi-synthetic curcuminoid and its metal complexes were also potential therapeutic agents for PD treatment.

Among the 45 selected genes as PD relates, *HSP90* was identified as the core gene associated with Cur. Bioinformatics tools have been adopted in previous literature such as by Monti *et al.* to perform systematic analysis of MPP^+-induced PD models, which identified HSP60 proteinas the central hub in the protein-protein interaction network [20]. The HSP family plays a major role in activating against protein misfolding in diseases including PD [21]. As reviewed by Lackie *et al.*, early observation of Hsp90, Hsp70, Hsp60, Hsp40, and Hsp27 has provided a deeper insight into these chaperones [21]. For instance, Hsp60 was found to intervene in PD pathogenesis in 6-OHDA-lesioned rat models [22]. Hsp70 overexpression was reported to prevent α-synuclein accumulation and to reverse dopaminergic neuron loss [21]. Falsone *et al.* revealed that Hsp90 blocked α-synuclein aggregation in an *in vitro* model [23]. Hsp90 inhibition was a potential strategy to increase endogenous Hsp70 protein levels and further alleviated PD [4]. Hsp90 inhibitors may increase dopamine in PD, which could be potential therapeutic targets [24].

Our study proved that Cur could decrease *HSP90* expression in PD-cells, while overexpression of *HSP90* improved therapeutic effect of Cur on PD mode cells, indicating there was a positive intermediate role of the combination of *HSP90* and Cur. A growing body of researches has focused on Cur mechanism, such as activating the Akt/Nrf2 pathway, regulating nuclear factor kappa beta (NFκB) [8, 25]. The HSP-modulated Cur treatment of PD has rarely been touched. In our study, *HSP90* was upregulated in SH-SY5Y cells treated with 400 μM MPP^+. With treatment of Cur, *HSP90* expression was decrease and cell survival rate was significantly increased. Moreover, overexpression of *HSP90* was found to enhance the neuroprotective effect of Cur on PD models. However, overexpression of *HSP90* alone did not provide sufficient neuroprotection in our results. Accordingly, we encouraged more studies to investigate the potential of combination therapy that includes both Cur and regulation of *HSP90* protein in PD.

To our knowledge, it is the first time that the effect of combination therapy is implied in PD. However, some limitations exist in the current experiment. Thus, more experiments are required to verify the current point of view. For example, the underlying regulatory mechanism of *HSP90* remains to be investigated. An *in vivo* study using MPTP-conditioned animal models is necessary to illustrate the potential value for clinical application. Furthermore, an increased survival rate of *HSP90*-transfected SH-SY5Y cells with increased MPP^+ concentration (200 to 800 μmol/L) may provide valuable evidence for demonstrating the impact of *HSP90* on PD.

Conclusion

In summary, our research substantiated the neuroprotective role of Cur via *HSP90* against PD toxicity. Cur reversed morphological change, restored cell survival rate, attenuated apoptosis and controlled dopaminergic neuron loss. Hsp90 protein level was promoted by MPP^+-stimulation, and silencing of *HSP90* reversed the protective effect of Cur. Meanwhile, we also verified that, combination therapy of Cur and Hsp90 protein would yield a better efficacy for PD.

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Disclosure Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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