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Original Article

Overexpression of p58ipk protects neuroblastoma against paraquat-induced toxicity

Fengrui Li[1,2], Biao Ge[3], Alatangaole Damirin[1]

[1]*School of Life Sciences, Inner Mongolia University, Hohhot 010021, China;* [2]*Department of Forensic Medicine, Baotou Medical College, Baotou, China;* [3]*Department of Geriatrics, Baogang Hospital of Inner Mongolia, Baotou, China*

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Abstract: Background: Paraquat (PQ) is a powerful pathologic pesticide that contribute to the neurotoxicity, however, the pathogenic mechanism between them was unclear. The aims of this study were to explore the underlying mechanism of PQ-induced toxicity and then make potential contribute to such neuronal diseases therapy. Methods: Human cell line SH-SY5Y was pretreated with a set concentrations of PQ to detect the cell apoptosis and the expression of related genes and proteins. Next, pcDNA 3.1-p58ipk or si-p58ipk was transfected the PQ-induced cells to detect the cytotoxicity. Results: PQ significantly increased the cell apoptosis as well as the expression of p58ipk and CHOP, but decreased the expression of pAKT. p58ipk suppression resulted in an increase of cell apoptosis and CHOP expression, but the expression of pAKT was significantly decreased in PQ-induced SH-SY5Y cells. However, overexpressed p58ipk led to an opposite result. Conclusion: The results indicated that the expression of p58ipk was related to the toxicity level of PQ-induced cells and the mechanism between them was that p58ipk regulated the toxicity might through regulating the endoplasmic reticulum stress (ER-stress) and then regulating cell apoptosis. Further studies take emphasize on the effect of ER-stress on neuron system and explore ER-stress-related therapy are important on the treatment of neurodegenerative disease.

Keywords: Paraquat (PQ), neurotoxicity, p58ipk, signaling pathway, cell apoptosis

Introduction

Parkinson's disease (PD) is a kind of progressive, long-lasting neurologic disorder, with the feature of loss the neurons in the substantianigracompacta [1, 2]. So far, the cause of PD is still unclear, but the epidemiological studies suggested that environmental toxin, ageing, genetic susceptibility as well as oxidative stress are supposed to contribute to the PD [3]. According to the previous studies, the main pathological character of PD is highly selective nigrostriatal dopaminergic degeneration that resulting in selective loss of dopaminergic neurons, and finally promoting the neuron cells apoptosis [4-6]. 1-Methyl-4-phenylpyridinium (MPP+), a neurotoxin that derived from the metabolite of 1-Methyl-4-phenyl-2,3,6-tetrahydropyridine (MPTP) was considered to induce the neuron cells into toxicity. Now the MPP+ toxin has been widely used to establishment of PD models [7-9].

1,1'-dimethyl-4,4'-bipyridinium (Paraquat, PQ) is a kind of insecticide, with its chemical structure closely like MPP+ that researchers inferred that PQ might also damage the nigrostriatal dopaminergic system and lead to the neuropathy of PD [10]. Since then, several studies have reported that PQ increased risk of PD and could considered as a toxicant for PD [11-14]. Moreover, an investigation has announced that in the rice field areas with widely PQ sprayed, the PD attack ratio was higher than the people who did not exposed to the herbicide [10]. Moreover, PQ was also known as a kind of mitochondrial toxin, which could cause severely mitochondrial dysfunction, however, the mitochondrial dysfunction also plays an important role in the pathogenesis of PD disease. Beyond those supportive standpoints, whether PQ plays a vital role on neurotoxicity and the pathogenic mechanism remains unclear.

Endoplasmic reticulum (ER) is a major site for folding and maturation of secretory and trans-

membrane proteins. Recent studies have revealed that numerous factors as genetic and environmental attacks impede the cells to correctly fold the proteins in ER, which caused of unfolded protein response (UPR), a condition called ER-stress [15, 16]. The studies have asserted that ER-stressed cells need to rapidly store capacity to meet the demand of protein-folding if they are to survival [15, 17]. However, with the consecutive cellular homeostasis that ER-stress cannot be reversed, cellular functions deteriorate, and finally leading to cell death. A previous study proposed that ER stress could be considered as a possible molecular mechanism underlying several neurodegenerative diseases, such as PD [18]. Moreover, it swell known that ER-stress cooperatively with abnormal protein degradation by the ubiquitin proteasome system are considered to contribute to the PD pathogenesis [19]. Thus, the therapeutic drugs aimed at interfering ER-stress cycle would be useful for the treatment of such disease.

P58ipk, a ER-stress-regulated chaperone, which located on the endoplasmic reticulum lumen, belonging to the HSP40 family [20]. P58ipk was firstly discovered as a cytosolic protein that with the function of an inhibitor of double-strand RNA protein kinase [21, 22]. Subsequently, p58ipk was found to have diverse functional domains to determine the protein's various activities that depended on its different locations in the cell [23]. Researchers even found that p58ipk could protect retina cell from harm through slowing ER-stress [24]. CHOP, is known as a growth arrest and DNA damage gene, and play an important role in ER stress-mediated apoptosis and in several diseases including brain ischemia and neurodegenerative disease [25]. Previous studies have utilized the selective attenuation and the molecular chaperone p58ipk to study the mechanism of lung cancer disease [26] as well as the cause of β-cell failure [27]. However, this study we were aims to explore the mechanism between p58ipk and PQ-induced toxicity.

Thus, there is a need to explore the mechanism and develop new protective agents that prevent the onset or development of such neuronal disease. Therefore, human cells SH-SY5Y were pretreated by PQ in the present study, and our purpose was to investigate the role of PQ in SH-SY5Y cells.

Method

Cell cultures and treatment

Human neuroblastoma cell line SH-SY5Y was obtained from Nanjing COBIOER biotechnology company (Nanjing, China). Cells were cultured at 37°C with 5% CO_2 by using DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS and 100 U/ml penicillin as well as 100 μg/ml streptomycin. PQ was then added to SH-SY5Y cells and cultured for 24 hours.

Cell viability

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay was used to detect the viability of SH-SY5Y cells. A total of 1×10^5 cells were planted in a 96-well plate, and incubated at 37°C with 5% CO_2 for 24 h. Then, 10 uL of MTT was added and incubation for 4 h. Next, DMSO was added for dissolving the crystals. Finally, cell viability was quantified by measuring the absorbance value at 570 nm, the results were expressed as percentages of the control.

Cell apoptosis

Flow cytometry was carried out for detecting the cell apoptosis. After centrifugation, SH-SY5Y cells were separately collected and washed by PBS and then stained by Annexin V-FITC and PI. Stained cells were collected and analyzed using a FACScalibur (Becton Dickinson, Mountain View, CA, USA) instrument.

Western blot

Proteins were isolated from SH-SY5Y cells by BIA method and quantified by BCA. 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was used to separate the proteins. After concentration, the proteins was quantified by the method of Bradford, the separated protein were then transferred onto the poly vinylidene fluoride (PVDF) membrane. Primary antibodies were added and incubated with the blot at room temperature for one night, then secondary antibodies were added and incubated at 37°C for 1 hour. Power Opti-ECL kit (4.6pg-4.7ng) was used for visualizing the bands and the NIH ImageJ software was used for quantifying the protein with β-actin as the internal control, blots are representative of 3 independent experiments.

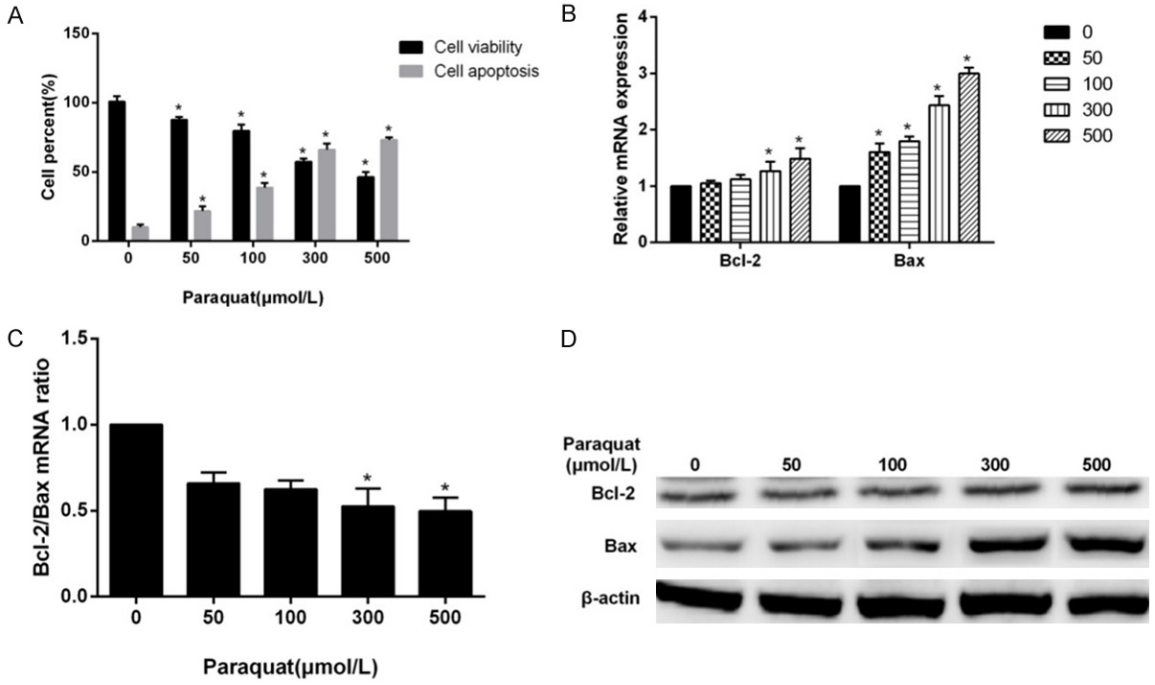

Figure 1. PQ induced neurotoxicity in SH-SY5Y cells. A: Cell viability and apoptosis following concentration-dependent treatments. A significant reduce of cell viability and increased cell apoptosis along with the increase of the PQ concentration (0, 50 μM, 100 μM, 300 μM, 500 μM). B: A significant increase of Bcl-2 was observed upon concentration treatment with 300 μM and 500 μM, and a significantly increase of Bax was observed upon all concentration comparing with the non-treated of PQ group. C: A significant reduction of the ratio of Bcl-2/Bax upon treatment with 300 Mm and 500 μM of PQ. D: Weston blot was used to detect the protein expression with the results that Bcl-2 was significantly increased and Bax was significantly increased. The experiments were repeated for three individual times. β-Actin was considered as internal control. *$P < 0.05$, results expressed as mean ± SD.

Real-time PCR

RNA was extracted from SH-SY5Y cells according to the manufacturer's protocols by using Trizol reagent (Invitrogen, USA). RNA quality was quantified by Nanodrop (Invitrogen, USA). Superscript III Reverse Transcriptase (Invitrogen) was used for reverse Transcripting the RNA into cDNA. Applied Biosystems (Thermo Fisher, USA) was used for Real time PCR according to the supplier's protocol. The data were repeated for three times. β-actin was considered as internal control, all data were performed for at least three times.

Cell transfection

After PQ was added to the SH-SY5Y cells for 24 hours, pcDNA 3.1 -p58ipk or si-p58ipk () was transfected the cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were planted on a six-well plates at 37°C for 8 h. The transfected cells were collected and real-time PCR was used to determine the transfection efficiency. The pcDNA 3.1 -p58ipk and si-p58ipk were obtained from Shanghai GenePharma Co., Ltd. (China).

Data analysis

All data were analyzed using a SPSS software. One-Way and Two-Way ANOVA was used to examine the parameters of different treatments. Data were presented as means ± SD. Different significance was defined as $P < 0.05$.

Results

PQ induced neuronal cells death

To detect the dysregulation of PQ-induced toxicity, a set concentrations (0, 50 μM, 100 μM, 300 μM, 500 μM) of PQ were separately pretreated to human cells SH-SY5Y for 24 hours. According to **Figure 1A**, PQ induced cytotoxicity showed a concentration-dependent manner, and along with the increase of PQ concentration, the cell viability was significantly decreased and the cell apoptosis was significantly increased. Subsequently, the mRNA expression of apoptotic-related factors Bcl-2, Bax and the

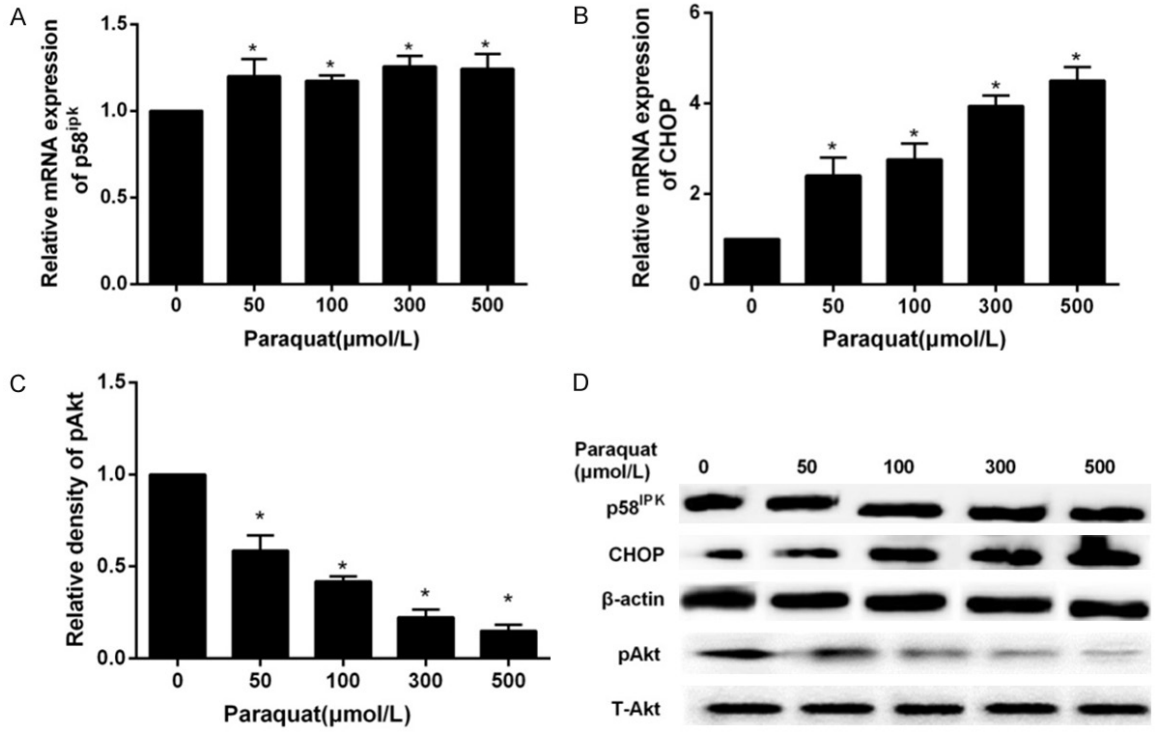

Figure 2. PQ treated SH-SY5Y cells displayed alterations in p58ipk, CHOP and pAkt. 50 μM, 100 μM, 300 μM, 500 μM PQ were treated with SH-SY5Y cells, and the mRNA expression level of CHOP was significantly increased comparing with non-treated PQ (B), but a significantly decreased in pAkt (C). However, the mRNA expression of p58ipk showed slowly increasing (A). (D) PQ-induced cells showed significantly increased protein expression of CHOP and decreased protein expression of pAkt, but s similar protein expression level of p58ipk along with the increase of PQ concentration. The experiments were repeated for three individual times. β-Actin was considered as internal control. *$P < 0.05$, results expressed as mean ± SD.

ratio between them were assayed by RT-qPCR and Western blot analysis. As shown in **Figure 1B**, a significant increase of Bcl-2 mRNA expression was observed upon the treatment with 300 μM and 500 μM of PQ, but not 50 μM or 100 μM. At the same time, the mRNA expression level of Bax also displayed a dose-dependent increase trends. However, the ratio of Bcl-2/Bax was significantly reduced with 300 μM and 500 Mm of PQ (**Figure 1C**). The proteins expression of Bcl-2 and Bax at different concentrations with PQ treatments were analyzed on **Figure 1D**, and the proteins expression showed an increasingly trends along with the increase concentration of PQ. Collectively, these data indicated that PQ induced a dose-dependent neuronal cells death.

PQ increased CHOP expression but decreased pAkt density

Cell apoptosis and viability are regulated by multistep intracellular signaling cascades. Thus, several major signaling proteins were selected in this study to examine the effects of PQ on cell apoptosis in SH-SY5Y cells. As is shown on **Figure 2**, all markers showed a concentration-dependent manner, and a significant increased mRNA expression of CHOP (**Figure 2B**), but the density of pAkt was significantly decreased (**Figure 2C**) along with the increase concentration of PQ comparing with the untreated control. However, the mRNA expression of p58ipk showed a slight increase with the increase concentration of PQ (**Figure 2A**). The proteins expression of P58ipk, CHOP and pAkt with different concentrations of PQ were analyzed by using SDS-PAGE. The expression of CHOP was increased and the expression of pAkt was decreased along with the increase concentration of PQ, however, the expression of p58ipk showed similar level with different PQ-treatments. (**Figure 2D**).

Low-expression of p58ipk accelerated PQ-induced neurotoxicity

In order to evaluate possible effects of p58ipk in neuron, a set of PQ-induced SH-SY5Y cells

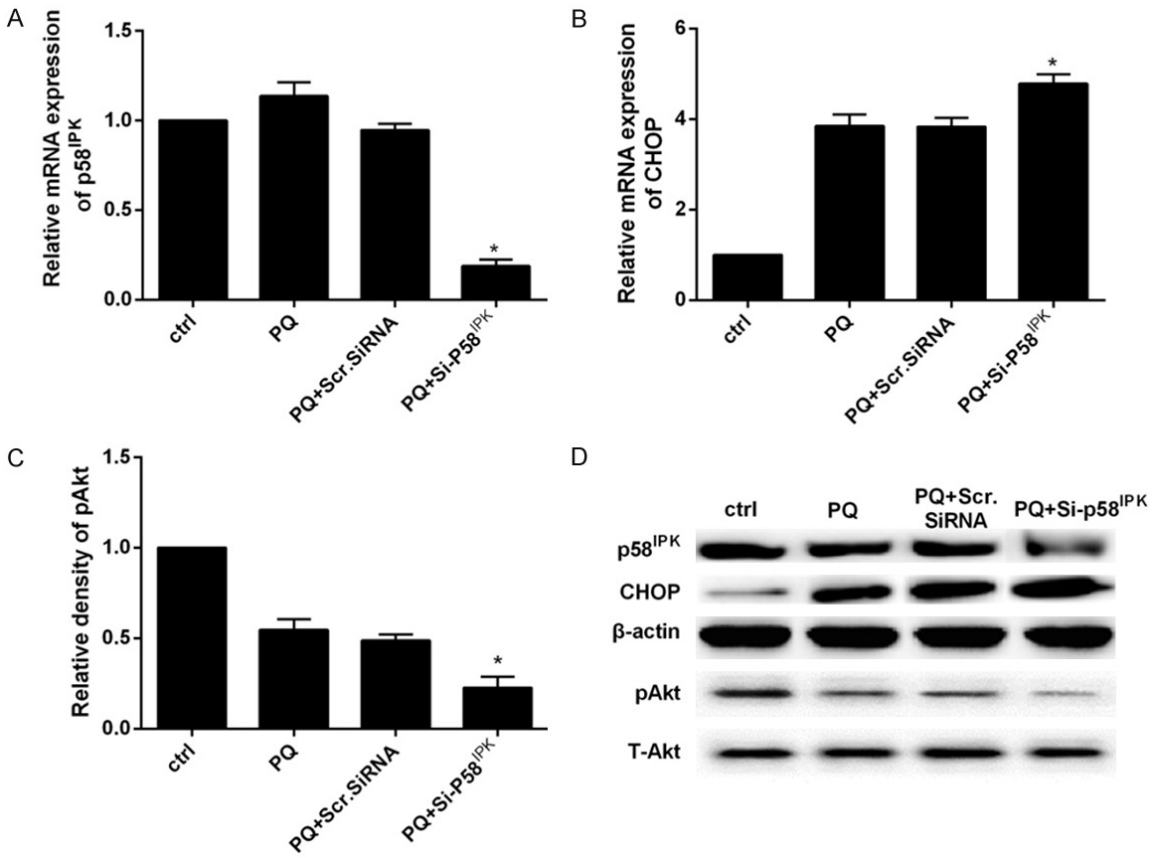

Figure 3. The effects of p58ipk knockdown on the expression of p58ipk, CHOP and pAkt. Comparing with the Scr. SiRNA (Scramble-siRNA) treated control, the mRNA expression of proteins by si-p58ipk treatment on PQ-induced cells was shown as following: A. The expression level of p58ipk was significantly decreased; B. The expression level of CHOP was significantly increased; C. The expression level of pAkt was significantly decreased. D. P58ipk knockdown decreased the protein expression of p58ipk and pAkt but increased the protein expression of CHOP. The experiments were repeated for three individual times. β-Actin was considered as internal control. $*P < 0.05$, results expressed as mean ± SD.

were transfected with si-p58ipk. According to **Figure 3**, comparing with the Scr.SiRNA (Scramble-siRNA) treated control, si-p58ipk treatment resulted in a significantly decreased mRNA expression of p58ipk (**Figure 3A**), and consistently attenuated the mRNA expression of pAkt (**Figure 3C**). Nevertheless, a significant increase level of CHOP expression was observed with the si-p58ipk treatment (**Figure 3B**). The results might indicated that low-expression of p58ipk might accelerated the neurotoxicity. The protein expression level of P58ipk, CHOP and pAkt was quantified by SDS-PAGE with the results that low-expression of P58ipk increased the expression of CHOP but decreased the expression of pAkt (**Figure 3D**).

To examine the effects of p58ipk knockdown on cell viability and apoptosis of PQ-induced SH-SY5Y, the cells were treated with various treatments (control, PQ, PQ+Scr.SiRNA, PQ+si-p58ipk). From the **Figure 4A**, p58ipk knock-down significantly decreased the cell viability and increased the cell apoptosis of cells induced by PQ. The mRNA expression of Bcl-2 and Bax was also investigated, and the results revealed that p58ipk knockdown reversed the increased expression of Bcl-2 induced by PQ and increased the expression of Bax, compar-ing with PQ+Scr.siRNA (**Figure 4B**). Moreover, the ratio of Bcl-2/Bax was significantly reduced by low-expression of p58ipk (**Figure 4C**). SDS-PAGE was used to analyze the protein expres-sion of Bcl-2 and Bax on **Figure 4D**, results revealed that low-expression of p58ipk decre-ased the expression of Bcl-2 but increased the expression of Bax.

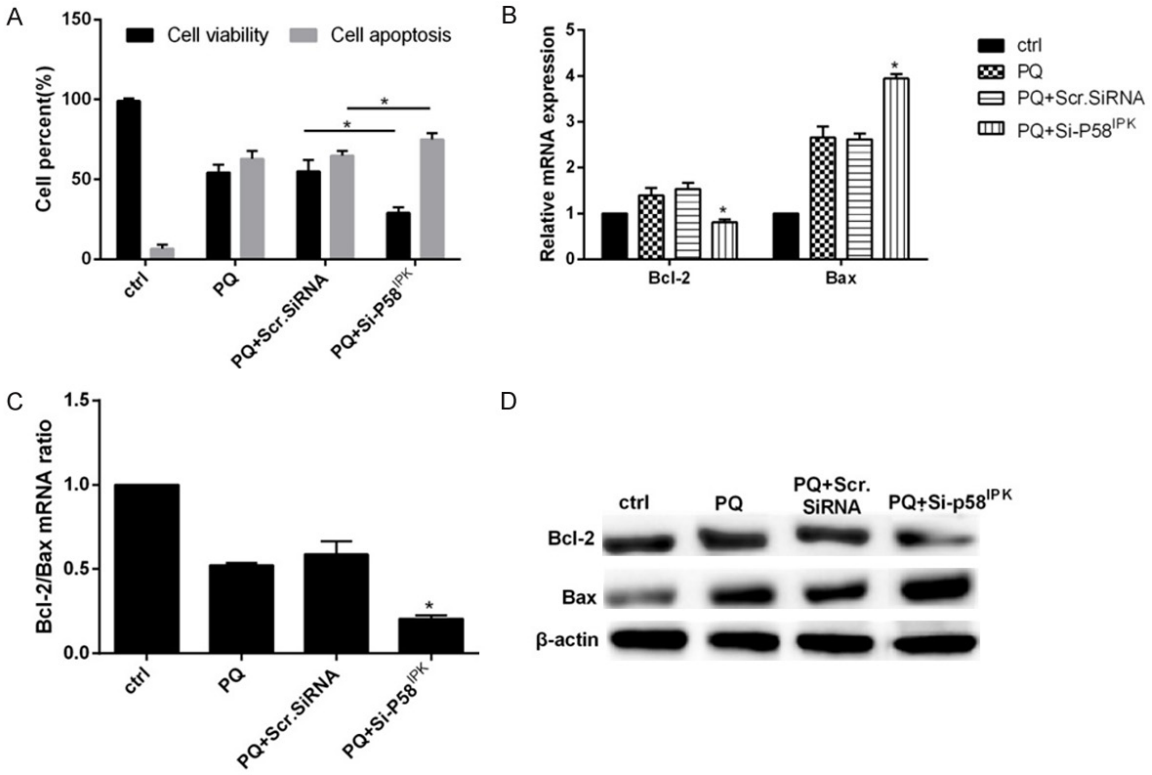

Figure 4. Low-expression of p58ipk enhanced the cell apoptosis of SH-SY5Y induced by PQ. Comparing with the PQ induced SH-SY5Y cells, p58ipk suppression significantly decreased cell viability and increased cell apoptosis (A), decreased the mRNA expression of Bcl-2 and increased the expression of Bax (B), decreased the ratio of Bcl-2/Bax (C), decreased the protein expression of Bcl-2 and increased the protein expression of Bax (D). The experiments were repeated for three individual times. β-Actin was considered as internal control. $*P < 0.05$, results expressed as mean ± SD.

Overexpression of p58ipk reduced the neurotoxicity induced by PQ

Whether overexpression of p58ipk was involved in the effects of CHOP, pAkt and P58 were evaluated. Firstly, pcDNA3.1-p58ipk was transfected on SH-SY5Y cells pretreated by PQ, then the mRNA expression of CHOP and pAkt was detected. According to **Figure 5A**, overexpression of p58ipk could significantly reverse the high expression of CHOP induced by PQ. Furthermore, overexpression of p58ipk also significantly reversed the low expression of pAkt induced by PQ (**Figure 5B**). The protein expression of CHOP, pAkt and P58 was quantified by SDS-PAGE. The results showed that overexpression of p58ipk decreased the exprression of CHOP, but increased the expression of pAkt induced by PQ (**Figure 5C**).

After pcDNA3.1-P58 was transfected on cells of SH-SY5Y pretreated by PQ, the viability and apoptosis were also detected. As presented in **Figure 6A**, overexpression of p58ipk could significantly reversed the decrease of cell viability and the increase of cell apoptosis induced by PQ. At the same time, overexpression of p58ipk reversed the increase of mRNA expression of Bax induced by PQ, but continually increased mRNA expression of Bcl-2 (**Figure 6B**). Then the ratio of Bcl-2/Bax was detected with the results thatoverexpression of p58ipk reversed the decrease induced by PQ (**Figure 6C**). The protein expression of Bcl-2 and Bax was analyzed by SDS-PAGE, however, the results showed that the same trends were obtained with mRNA expression, as overexpression of p58ipk increased the expression of Bcl-2, but decreased the expression of Bax induced by PQ (**Figure 6D**). Therefore, it appears that p58ipk alter the response of neuron cells to PQ and thus interference neurotoxicity induced by PQ.

Discussion

Several studies have reported that the possibility of environments chemicals, even including

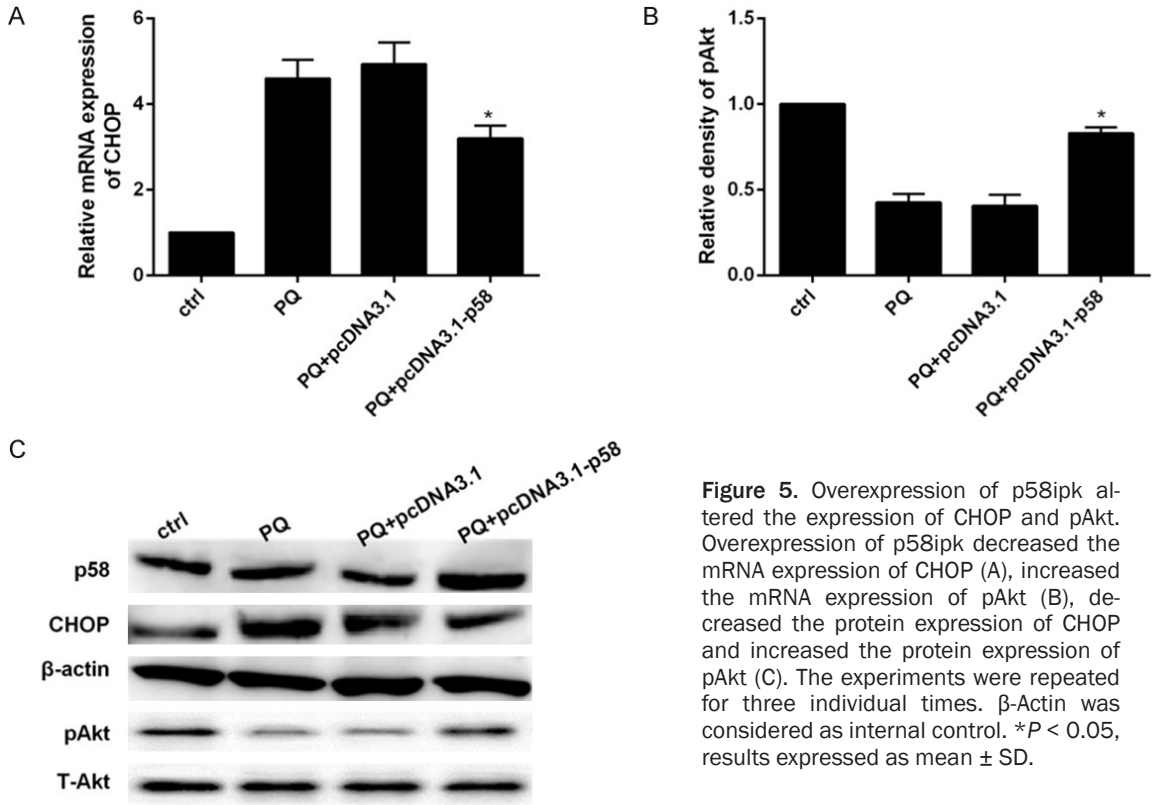

Figure 5. Overexpression of p58ipk altered the expression of CHOP and pAkt. Overexpression of p58ipk decreased the mRNA expression of CHOP (A), increased the mRNA expression of pAkt (B), decreased the protein expression of CHOP and increased the protein expression of pAkt (C). The experiments were repeated for three individual times. β-Actin was considered as internal control. *$P < 0.05$, results expressed as mean ± SD.

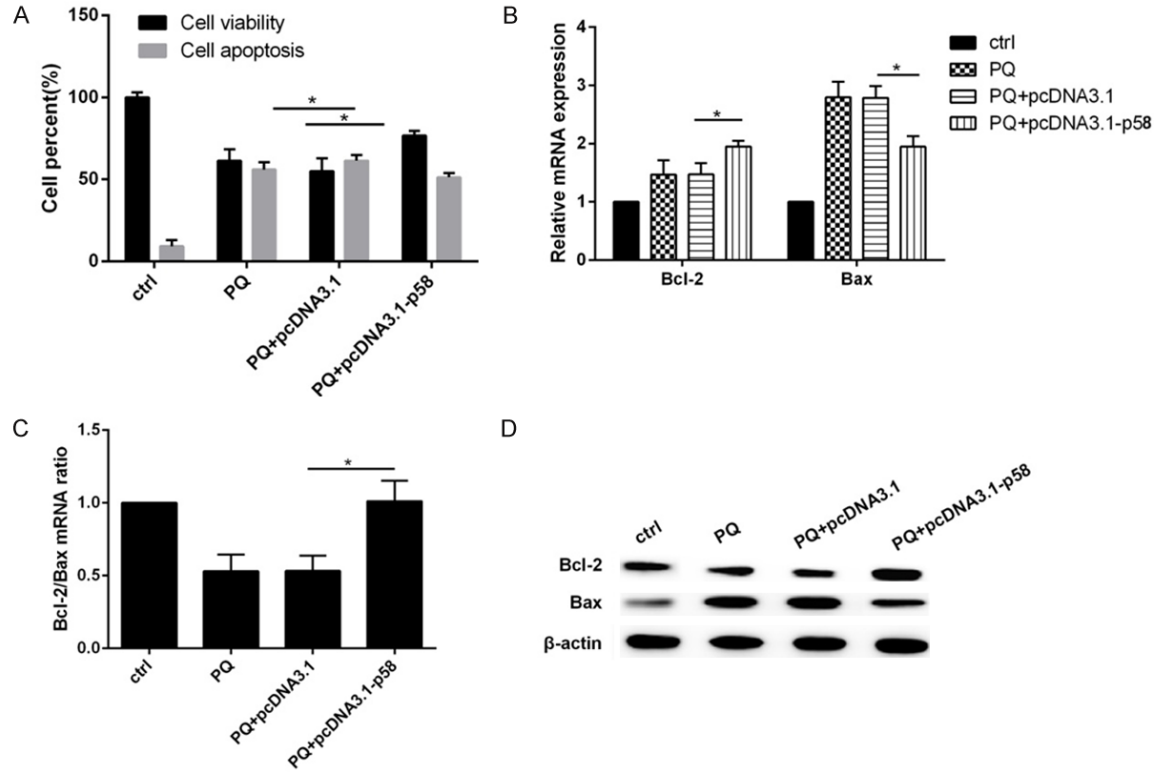

Figure 6. Overexpression of p58ipk attenuated PQ-induced apoptosis. Overexpression of p58ipk increased the cell viability and decreased cell apoptosis (A), increased the mRNA expression of Bcl-2 and decreased the mRNA expression of Bax (B), increased the ratio of Bcl-2/Bax (C), reversed the decreased protein expression of Bcl-2 and the increased protein expression of Bax (D). The experiments were repeated for three individual times. β-Actin was considered as internal control. *$P < 0.05$, results expressed as mean ± SD.

PQ may be related to the advance of PD, since that PQ appears to be promote the neuronal cell death [28-30]. In this study, we found that the degeneration changes and the significant cell loss induced by herbicide and the results demonstrated that PQ cause evident neurotoxic effects. However, the toxicity level showed a concentration-dependent manner, with the high concentration of PQ resulting in sever toxicity. These observations might be of valuable for the use of PQ-induced mice as a useful model to study the mechanism of neuron cell death as well as to develop neuroprotective drugs.

Bcl-2 plays an important role on disincentive for cell apoptosis and Bax contribute to promote the cell apoptosis. Bcl-2 and Baxfamily numbers and the ratio of Bcl-2/Bax were usually used as a ruler to measure the cell apoptosis [31-33]. Previous studies based on detecting the ratio of Bcl-2/Bax that induced by PQ have involved in many disease, for example, Huang et al found that PQ toxicity could significantly decreased the Bcl-2 expression as well as the ration of Bcl-2/Bax in lung injury, however, Lysine acetylsalicylate ameliorated the injury induced by PQ [34]. Another study found that PQ-induced toxicity in kidney was associated with oxidative stress, and the toxicity level could be revealed by the regulation of Bcl-2 and Bax [35]. From this study, after the cells were treated with PQ, the expression of the two proteins were significantly increased, but the ratio of Bcl-2/Bax was decreased. Then we found that the expression of Bcl-2 and Bax was affected by the expression of p58ipk, with the low-expression of p58ipk that decreased the expression of Bcl-2, whereas, the expression of Bax was increased. However, with the overexpression of p58ipk, we obtained the opposite results. Interestingly, the ratio of Bcl-2/Bax was found to keep in consistent with the cell apoptosis. These results suggested that PQ shift cell apoptosis, and low-expression of p58ipk exacerbated the imbalance, but overexpression of p58ipk prevents this alteration. Based on the results, we hypothesized that the effects of p58ipk on PQ induced apoptosis may be partly mediated by regulating the expression of Bcl-2 and Bax.

Phosphorylated AKT (pAKT) is a major contributor to radioresistance, and considered as a significant target gene in cell apoptosis [36]. Moreover, pAkt is the important marker of PI3K/Akt signaling pathway, which could modulate celluar activities like cell proliferation, migration, etc. Saurabh found that PI3K/Akt signaling pathways protect the neurons from the detrimental effects of the p38 MAPK activation and promote neuron survival [36]; And the PI3K/Akt/mTOR is usually over-activated and enables cell over-proliferation in cancers and which made it the therapeutic targets, thus we thought that the PI3K/Akt pathway was singly regarded as the survival pathway in this article and inhibit cell apoptosis induced by paraquat. In this study, pAKT was investigated to determine whether it could be used to measure the neural cells apoptosis and as a signal for protecting the neurons system against injury-induced by PQ. In this study, a significantly lower density of pAkt in PQ-induced neurotoxicity, but increased in cells with overexpression of p58ipk. The results might indicated that p58ipk control the cell apoptosis might also via regulating the expression of pAKT.

ER-stress is a signal pathway that with the function of trigger apoptotic signals response in PD [37, 38]. However, accumulated oxidative stress and misfolded proteins that induced by ER-stress might play an important role on eliciting cellular responses, as well as impairing the protectiveness and ultimately resulting in neuronal cell death. P58ipk showed an effect on ER-stress by reducing protein synthesis and attenuating substrate into ER during the stress environment [39]. The absence of p58ipk would lead to overexpression of ER-stress inducible gene CHOP, which constantly leads to blocking of protein translation as well as aggravating of cell apoptosis [25, 40, 41]. Our results obtained that PQ-induced neurotoxicity could significantly increased the expression of p58ipk and CHOP, then we can speculate that there will be also a change of ER-stress. Next, we also evaluated whether overexpression or low-expression of p58ipk could influence the expression of CHOP. Among them, Overexpression of p58ipk inhibited expression of CHOP, and low-expression of p58ipk showed the opposite results. Noteworthy, low-expression of p58ipk accelerated the toxicity might through accelerating the ER-stress that increased the cell apoptosis, and overexpression of p58ipk reduced the toxicity seemed to through reducing the ER-stress that decreased the cell apoptosis. Previous

study of EvgeniiBoriushkin also found that the p58ipk increased as the ER stress, and it should exist one possibilities that p58ipk increased to inhibit CHOP to protect cell survival [42]. Based on our observations, thus, we inferred that p58ipk regulate the PQ-induced cell toxicity might partly through regulating the ER-stress and further regulating the cell apoptosis.

Taken together, our study suggested that PQ could cause severe neurotoxicity. Overexpression of p58ipk protected SH-SY5Y cells against PQ-induced toxicity through modulating the ER-stress that then regulated the cell apoptosis. In future, it will be important to emphasize the effect of ER-stress, and test if they can support neurons therapy on neurotoxins.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Alatangaole Damirin, School of Life Sciences, Inner Mongolia University, 235 University West Road, Hohhot 010021, China. Tel: +86-0472-7167910; E-mail: Damirin@163.com

References

[1] Dauer W and Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003; 39: 889-909.

[2] Coune PG, Schneider BL, Aebischer P. Parkinson's disease: gene therapies. Cold Spring Harb Perspect Med 2012; 2: a009431.

[3] Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: a mutation update. Hum Mutat 2010; 31: 763-80.

[4] Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 2000; 3: 1301-6.

[5] Li DW, Li GR, Lu Y, Liu ZQ, Chang M, Yao M, Cheng W, Hu LS. a-lipoic acid protects dopaminergic neurons against MPP+-induced apoptosis by attenuating reactive oxygen species formation. Int J Mol Med 2013; 32: 108-14.

[6] Hu HI, Chang HH, Sun DS. Differential regulation of caspase-2 in MPP(+)-induced apoptosis in primary cortical neurons. Exp Cell Res 2015; 332: 60-6.

[7] Liu S, Cui B, Dai ZX, Shi PK, Wang ZH, Guo YY. Long non-coding RNA Hotair promotes Parkinson's disease induced by MPTP through up-regulating the expression of LRRK2. Curr Neurovasc Res 2016; 13: 115-20.

[8] Peng J, Liu Q, Rao MS, Zeng X. Using human pluripotent stem cell-derived dopaminergic neurons to evaluate candidate Parkinson's disease therapeutic agents in MPP+ and rotenone models. J Biomol Screen 2013; 18: 522-33.

[9] Keane H, Ryan BJ, Jackson B, Whitmore A, Wade-Martins R. Protein-protein interaction networks identify targets which rescue the MPP+ cellular model of Parkinson's disease. Sci Rep 2015; 5: 17004.

[10] McCormack AL, Thiruchelvam M, Manning-Bog AB, Thiffault C, Langston JW, Cory-Slechta DA, Di Monte DA. Environmental risk factors and Parkinson's disease: selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat. Neurobiol Dis 2002; 10: 119-27.

[11] Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect 2011; 119: 866-72.

[12] Thiruchelvam M, Richfield EK, Baggs RB, Tank AW, Cory-Slechta DA. The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. J Neurosci 2000; 20: 9207-14.

[13] Dinis-Oliveira RJ, Remião F, Carmo H, Duarte JA, Navarro AS, Bastos ML, Carvalho F. Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology 2006; 27: 1110-22.

[14] Uversky VN. Neurotoxicant-induced animal models of Parkinson's disease: understanding the role of rotenone, maneb and paraquat in neurodegeneration. Cell Tissue Res 2004; 318: 225-41.

[15] Oakes SA and Papa FR. The role of endoplasmic reticulum stress in human pathology. Annu Rev Pathol 2015; 10: 173-94.

[16] Gomez JA, Tyra HM, DeZwaan-McCabe D, Olivier AK, Rutkowski DT. Synthetic embryonic lethality upon deletion of the ER cochaperone p58(IPK) and the ER stress sensor ATF6alpha. Biochem Biophys Res Commun 2014; 443: 115-9.

[17] Ron D and Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol 2007; 8: 519-29.

[18] Kaneko M, Koike H, Saito R, Kitamura Y, Okuma Y, Nomura Y. Loss of HRD1-mediated protein degradation causes amyloid precursor protein accumulation and amyloid-beta generation. J Neurosci 2010; 30: 3924-32.

[19] Cali T, Ottolini D, Brini M. Mitochondria, calcium, and endoplasmic reticulum stress in Parkinson's disease. Biofactors 2011; 37: 228-40.
[20] Boriushkin E, Wang JJ, Zhang SX. Role of p58IPK in endoplasmic reticulum stress-associated apoptosis and inflammation. J Ophthalmic Vis Res 2014; 9: 134-43.
[21] Polyak SJ, Tang N, Wambach M, Barber GN, Katze MG. The P58 cellular inhibitor complexes with the interferon-induced, double-stranded RNA-dependent protein kinase, PKR, to regulate its autophosphorylation and activity. J Biol Chem 1996; 271: 1702-7.
[22] Melville MW, Hansen WJ, Freeman BC, Welch WJ, Katze MG. The molecular chaperone hsp40 regulates the activity of P58IPK, the cellular inhibitor of PKR. Proc Natl Acad Sci U S A 1997; 94: 97-102.
[23] Rutkowski DT, Kang SW, Goodman AG, Garrison JL, Taunton J, Katze MG, Kaufman RJ, Hegde RS. The role of p58IPK in protecting the stressed endoplasmic reticulum. Mol Biol Cell 2007; 18: 3681-91.
[24] Hu WK, Liu R, Pei H, Li B. Endoplasmic reticulum stress-related factors protect against diabetic retinopathy. Exp Diabetes Res 2012; 2012: 507986.
[25] Oyadomari S Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 2004; 11: 381-9.
[26] Huber AL, Lebeau J, Guillaumot P, Pétrilli V, Malek M, Chilloux J, Fauvet F, Payen L, Kfoury A, Renno T, Chevet E, Manié SN. p58(IPK)-mediated attenuation of the proapoptotic PERK-CHOP pathway allows malignant progression upon low glucose. Mol Cell 2013; 49: 1049-59.
[27] Han J, Song B, Kim J, Kodali VK, Pottekat A, Wang M, Hassler J, Wang S, Pennathur S, Back SH, Katze MG, Kaufman RJ. Antioxidants complement the requirement for protein chaperone function to maintain beta-cell function and glucose homeostasis. Diabetes 2015; 64: 2892-904.
[28] Wang F, Franco R, Skotak M, Hu G, Chandra N. Mechanical stretch exacerbates the cell death in SH-SY5Y cells exposed to paraquat: mitochondrial dysfunction and oxidative stress. Neurotoxicology 2014; 41: 54-63.
[29] Omura T, Asari M, Yamamoto J, Oka K, Hoshina C, Maseda C, Awaya T, Tasaki Y, Shiono H, Yonezawa A, Masuda S, Matsubara K, Shimizu K. Sodium tauroursodeoxycholate prevents paraquat-induced cell death by suppressing endoplasmic reticulum stress responses in human lung epithelial A549 cells. Biochem Biophys Res Commun 2013; 432: 689-94.
[30] Pang YW, Sun YQ, Sun WJ, Du WH, Hao HS, Zhao SJ, Zhu HB. Melatonin inhibits paraquat-induced cell death in bovine preimplantation embryos. J Pineal Res 2016; 60: 155-66.
[31] Blum D, Torch S, Lambeng N, Nissou M, Benabid AL, Sadoul R, Verna JM. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. Prog Neurobiol 2001; 65: 135-72.
[32] Perier C, Bove J, Vila M. Mitochondria and programmed cell death in Parkinson's disease: apoptosis and beyond. Antioxid Redox Signal 2012; 16: 883-95.
[33] Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, Thenmozhi AJ, Selvaraju S, Guillemin GJ. Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. Oxid Med Cell Longev 2013; 2013: 102741.
[34] Huang WD, Wang JZ, Lu YQ, DI YM, Jiang JK, Zhang Q. Lysine acetylsalicylate ameliorates lung injury in rats acutely exposed to paraquat. Chin Med J (Engl) 2011; 124: 2496-501.
[35] Wei T, Tian W, Liu F, Xie G. Protective effects of exogenous beta-hydroxybutyrate on paraquat toxicity in rat kidney. Biochem Biophys Res Commun 2014; 447: 666-71.
[36] Jha SK, Jha NK, Kar R, Ambasta RK, Kumar P. p38 MAPK and PI3K/AKT Signalling Cascades inParkinson's Disease. Int J Mol Cell Med 2015; 4: 67-86.
[37] Fouillet A, Levet C, Virgone A, Robin M, Dourlen P, Rieusset J, Belaidi E, Ovize M, Touret M, Nataf S, Mollereau B. ER stress inhibits neuronal death by promoting autophagy. Autophagy 2012; 8: 915-26.
[38] Chinta SJ, Rane A, Poksay KS, Bredesen DE, Andersen JK, Rao RV. Coupling endoplasmic reticulum stress to the cell death program in dopaminergic cells: effect of paraquat. Neuromolecular Med 2008; 10: 333-42.
[39] van Huizen R, Martindale JL, Gorospe M, Holbrook NJ. P58IPK, a novel endoplasmic reticulum stress-inducible protein and potential negative regulator of eIF2alpha signaling. J Biol Chem 2003; 278: 15558-64.
[40] Malagelada C, Jin ZH, Greene LA. RTP801 is induced in Parkinson's disease and mediates neuron death by inhibiting Akt phosphorylation/activation. J Neurosci 2008; 28: 14363-71.
[41] Wang J, Cheng Q, Wang X, Zu B, Xu J, Xu Y, Zuo X, Shen Y, Wang J, Shen Y. Deficiency of IRE1 and PERK signal pathways in systemic lupus erythematosus. Am J Med Sci 2014; 348: 465-73.
[42] Boriushkin E, Wang JJ, Li J, Jing G, Seigel GM, Zhang SX. Identification of p58IPK as a novel neuroprotective factor for retinal neurons. Invest Ophthalmol Vis Sci 2015; 56: 1374-86.