PDCD4 silencing alleviates KA-induced neurotoxicity of HT22 cells by inhibiting endoplasmic reticulum stress via blocking the MAPK/NF-κB signaling pathway

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Abstract. Human programmed cell death 4 (PDCD4) has been reported to participate in multiple neurological diseases. However, the role of PDCD4 in epilepsy, as well as its underlying mechanism, remains unclear. To induce excitotoxicity, 100 μM kainic acid (KA) was applied for the stimulation of HT22 cells for 12 h. Initially, the mRNA and protein expression levels of PDCD4 were evaluated using reverse transcription-quantitative PCR and western blotting. A lactate dehydrogenase assay was performed to detect cell injury. Cell apoptosis was assessed using flow cytometry and western blotting was performed to determine the expression levels of apoptosis-related proteins. Oxidative stress was detected using dichlorodihydrofluorescein diacetate staining, and malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) assay kits. Furthermore, the expression levels of MAPK/NF-κB signaling-related proteins and endoplasmic reticulum (ER) stress-related proteins C/EBP homologous protein, glucose-regulated protein 78, activating transcription factor 4 and phosphorylated-eukaryotic initiation factor-2α were assessed by western blotting. It was revealed that PDCD4 expression was markedly elevated in KA-induced HT22 cells, whereas PDCD4 silencing alleviated KA-induced neurotoxicity of HT22 cells by alleviating cell injury and inhibiting apoptosis. In addition, PDCD4 silencing reduced the levels of reactive oxygen species and MDA, but elevated those of SOD and GSH-Px. PDCD4 silencing also suppressed ER stress by blocking the MAPK/NF-κB signaling pathway. By contrast, the MAPK agonist phorbol myristate acetate reversed the effects of PDCD4 silencing on KA-induced neurotoxicity and oxidative stress in HT22 cells. In conclusion, PDCD4 silencing alleviated KA-induced neurotoxicity and oxidative stress in HT22 cells by suppressing ER stress through the inhibition of the MAPK/NF-κB signaling pathway, which may provide novel insights into the treatment of epilepsy.

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Introduction

As a common neurological disorder, epilepsy is characterized by a lasting predisposition to generate spontaneous epileptic seizures (1). Abnormal excitatory or synchronized neuronal activity in the brain can lead to seizures (2). Identifying a suitable treatment is of great significance for reducing epilepsy-induced brain damage and combating the global burden of epilepsy (3). Notably, ~30% of patients suffer from intractable epilepsy, which is difficult to control with drugs (4). At present, epilepsy surgery is the most effective treatment method for long-term seizure relief in patients with partially drug-resistant focal epilepsy (5). However, epilepsy surgery is not widely used, due to its high risk and irreversible consequences (6). Upwards of 20 antiseizure drugs have been approved by the World Health Organization for the treatment of epilepsy; however, more than one-third of patients have seizures refractory to pharmacotherapy due to the complex underlying molecular mechanisms (7). Currently, molecular targeted therapy for epilepsy has attracted considerable attention in the academic field (8). The kainic acid (KA) model has been extensively used to investigate the molecular mechanisms and potential antiseizure targets of epilepsy for a few decades (9,10).

The occurrence of epilepsy is a complex pathological process, mainly involving neuronal death, endoplasmic reticulum (ER) stress and the inflammatory response (11). A previous study reported that seizure-induced brain insult may be attenuated by the inhibition of glucose-regulated protein 78 (GRP78) and C/EBP homologous protein (CHOP) (12). Moreover, salubrinal, an inhibitor of ER stress, has been reported to relieve ER stress and exert neuroprotective effects by reducing the protein levels of CHOP and caspase 3, suggesting that inhibition of the ER stress response can reduce the occurrence of apoptosis and suppress nerve cell death (13,14). Therefore, exploring the underlying molecular pathological mechanism of epilepsy is important for the development of new and more effective therapeutic targets.

Human programmed cell death 4 (PDCD4), which was initially identified as a nuclear antigen gene, is considered

to be a novel tumor suppressor due to its inhibitory effects on cell proliferation and invasion, as well as its inducive effects on apoptosis (15). PDCD4 is ubiquitously expressed in tissues, and is localized in the nucleus, cytoplasm or both, depending on the cell type (16). It is well-known that PDCD4 is a critical regulator in several neurological diseases. Notably, it has been reported that microRNA (miR)-499a-5p elevation alleviates cerebral ischemia/reperfusion injury by targeting PDCD4 (17). It has also been shown that PDCD4 regulates axonal growth through the translational suppression of neurite growth-associated genes (18). In addition, Peng *et al* (19) revealed that DGCR5 can attenuate neuropathic pain by sponging miR-330-3p and regulating PDCD4 in chronic sciatic nerve injury rat models. However, the role and potential mechanism of PDCD4 in epilepsy have not been comprehensively elucidated. Therefore, the present study aimed to explore the functional role of PDCD4 in HT22 cells induced by KA, which is an analog of excitotoxic glutamate (20), and to determine the mechanism by which PDCD4 influences KA-treated HT22 cells.

Materials and methods

Cell culture and treatment. The HT22 mouse hippocampal cell line, provided by the Korean Cell Line Bank; Korean Cell Line Research Foundation, was incubated in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (HyClone; Cytiva) and 1% penicillin/streptomycin at 37˚C with 5% CO_2. After 5 days of incubation, cell confluence reached 80-90% and HT22 cells were maintained in DMEM containing 100 μM KA (Sigma-Aldrich; Merck KGaA) for 12 h at room temperature to induce excitotoxicity (9,21). In addition, cells were pretreated with 100 ng/ml phorbol myristate acetate (PMA; MAPK agonist; cat. no. HY-18739; MedChemExpress) for 2 h and then treated with 100 μM KA for 12 h at 37˚C.

Cell transfection. Short hairpin RNA (shRNA/sh) against PDCD4 or a non-targeting control sequence (sh-NC sense, 5'-AACAAGATGAAGAGCACCAA-3' and antisense, 5'-TTGGTGCTCTTCATCTTGTT-3') was inserted into the pGPU6/Neo plasmid (Shanghai GenePharma Co., Ltd.), and the recombined plasmids were referred to sh-PDCD4 (sh-PDCD4-1 sense, 5'-GGAGATGTTAAGAGACTTA-3' and antisense, 5'-TAAGTCTCTTAACATCTCC-3'; and sh-PDCD4-2 sense, 5'-GCATGGAGATACAAATGAA-3' and antisense, 5'-TTCATTTGTATCTCCATGC-3'). HT22 cells were seeded into 6-well plates at a density of $2x10^5$ cells/well and cultured until the cell confluence reached 80%. Using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), 100 nM shRNAs were transfected into HT22 cells for 48 h at 37˚C according to the manufacturer's instructions. A total of 48 h post-transfection, the cells were harvested for further experiments. The cells transfected with sh-NC or sh-PDCD4 were then treated with 100 μM KA for 12 h at room temperature.

Lactate dehydrogenase (LDH) activity measurement. Cell injury was determined using an LDH assay kit (cat. no. A020-2-2; Nanjing Jiancheng Bioengineering Institute). Following treatment, LDH activity in the supernatant of HT22 cells (20 μl) was detected using a microplate reader (Benchmark; Bio-Rad Laboratories, Inc.) at 530 nm.

Flow cytometry. The apoptosis of HT22 cells was assessed using a FITC Annexin V/PI Apoptosis Detection Kit I (Guangzhou RiboBio Co., Ltd.), according to the manufacturer's instructions. Briefly, cells ($1x10^6$ cells/ml) were washed with 300 μl PBS (cat. no. C0221A; Beyotime Institute of Biotechnology) and then resuspended in 500 μl binding buffer. Subsequently, the cells were incubated with 5 μl Annexin V-FITC at room temperature for 15 min, followed by incubation with 10 μl PI (10 mg/ml) in the dark at room temperature for 5 min. FlowJo vX.0.7 software (FlowJo, LLC) was used to analyze the early and late apoptosis.

Detection of caspase 3 levels. The content of caspase 3 in the supernatant of HT22 cells (100 μl) was determined using a caspase 3 assay kit (cat. no. ab39401; Abcam) according to the manufacturer's instructions. A microplate reader (BioTek Instruments, Inc.) was used for the detection of absorbance at 450 nm.

Dichlorodihydrofluorescein diacetate (DCFH-DA) staining. To investigate the effects of PDCD4 silencing on reactive oxygen species (ROS) generation, a ROS Assay Kit of DCFH-DA (Nanjing Jiancheng Bioengineering Institute) was used. HT22 cells ($1x10^5$ cells/well) were stained using DCFH-DA staining solution at 37˚C for 30 min in the dark. Fluorescence images were captured using a fluorescence microscope at an excitation wavelength of 485 nm and an emission wavelength of 520 nm.

Measurement of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels. HT22 cells were inoculated into six-well plates at a density of $1x10^5$ cells/well. Following the indicated treatments, the activities of MDA, SOD and GSH-Px were analyzed using MDA assay kits (cat. no. S0131S; Beyotime Institute of Biotechnology), SOD assay kits (cat. no. A001-3-2; Nanjing Jiancheng Bioengineering Institute) and GSH-Px assay kits (cat. no. A005-1-2; Nanjing Jiancheng Bioengineering Institute), respectively, according to the manufacturer's protocols. Subsequently, a microplate reader (Benchmark; Bio-Rad Laboratories, Inc.) was used to detect the absorbance at 532 nm.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from the cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was converted to cDNA using a First Strand cDNA Synthesis Kit (Beyotime Institute of Biotechnology), according to the manufacturer's instructions. Amplification of the cDNA was performed using the SYBR Green One-Step RT-qPCR Kit (Beyotime Institute of Biotechnology). The following thermocycling conditions were used for qPCR: 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 60 sec. The primer sequences for qPCR are as follows: PDCD4 forward, 5'-GGACAGAAGAGGAACCACCG-3',

reverse, 5'-AAAGAAAGGAGCGGCAGTCA-3'; and GAPDH forward, 5'-CAGGTTGTCTCCTGCGACTT-3' and reverse, 5'-CCCTAGGCCCCTCCTGTTAT-3'. GAPDH was used as an internal reference and the $2^{-\Delta\Delta Cq}$ method (22) was used to calculate relative gene expression.

Western blot analysis. Total proteins were extracted from the cells using RIPA buffer (cat. no. P0013B; Beyotime Institute of Biotechnology) and were quantified with a BCA assay kit. Equal amounts of protein (40 μg/lane) were separated by SDS-PAGE on 10% gels (Bio-Rad Laboratories, Inc.) and were transferred onto PVDF membranes (MilliporeSigma). After blocking with 5% skimmed milk for 1 h at room temperature, the membranes were incubated with primary antibodies targeting PDCD4 (1:5,000; cat. no. ab79405; Abcam), Bcl-2 (1:2,000; cat. no. ab182858; Abcam), Bax (1:1,000; cat. no. ab32503; Abcam), cleaved-poly (ADP-ribose) polymerase (PARP; 1:1,000; cat. no. ab32064; Abcam), PARP (1:1,000; cat. no. ab191217; Abcam), phosphorylated (p)-ERK1/2 (1:1,000; cat. no. 4370; Cell Signaling Technology, Inc.), p-JNK (1:1,000; cat. no. ab307802; Abcam), p-p38 (1:1,000; cat. no. ab195049; Abcam), ERK1/2 (1:10,000; cat. no. ab184699; Abcam), JNK (1:2,500; cat. no. ab199380; Abcam), p38 (1:1,000; cat. no. ab170099; Abcam), p-NF-kBp65 (1:1,000; cat. no. ab76302; Abcam), NF-kBp65 (1:1,000; cat. no. ab32536; Abcam), CHOP (1:1,000; cat. no. 5554; Cell Signaling Technology, Inc.), GRP78 (1:1,000; cat. no. ab108615; Abcam), activating transcription factor 4 (ATF4; 1:1,000; cat. no. ab216839; Abcam), p-eukaryotic initiation factor-2α (eIF2α) (1:1,000; cat. no. ab32157; Abcam), eIF2α (1:1,000; cat. no. ab169528; Abcam) and β-actin (1:1,000; cat. no. ab8227; Abcam) at 4˚C overnight, followed by incubation with a HRP-conjugated secondary antibody (1:2,000; cat. no. ab6721; Abcam) for 1 h at room temperature. Finally, the protein bands were visualized with an enhanced chemiluminescence detection system (Beyotime Institute of Biotechnology), and densitometry (Quantity One 4.5.0 software; Bio-Rad Laboratories, Inc.) was used for semi-quantification.

Statistical analysis. All experiments were performed at least three times. All experimental data were analyzed using SPSS 17.0 software (SPSS, Inc.) and are presented as the mean ± SD. The differences between two groups were determined using unpaired two-tailed Student's t-test, whereas one-way ANOVA and Bonferroni post hoc multiple comparisons test was used to compare multiple groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

PDCD4 expression is increased in KA-induced HT22 cells. With the aim of investigating the role of PDCD4 in the development of epilepsy, the expression levels of PDCD4 were detected in KA-induced HT22 cells. As shown in Fig. 1A, the mRNA and protein expression levels of PDCD4 in KA-induced HT22 cells were markedly elevated compared with those in the control group. The results of RT-qPCR and western blotting demonstrated that the expression levels of PDCD4 were significantly decreased post-transfection with sh-PDCD4 compared with those in the control group and sh-NC group (Fig. 1B). Notably, PDCD4 expression was lowest in the sh-PDCD4-1 group. In view of this, sh-PDCD4-1 (subsequently referred to as sh-PDCD4) was selected for further experiments. In addition, the KA-induced increase in PDCD4 expression in HT22 cells was reversed by sh-PDCD4 transfection (Fig. 1C).

PDCD4 silencing ameliorates KA-induced neurotoxicity in HT22 cells. To explore the biological role of PDCD4 in KA-induced HT22 cells, the effects of PDCD4 silencing on HT22 cell neurotoxicity were assessed. As shown in Fig. 2A, KA induction markedly increased the release of LDH, whereas PDCD4 silencing inhibited the production of LDH in KA-induced HT22 cells. In addition, the apoptotic rate of KA-induced HT22 cells was significantly increased compared with that in the control group, but was suppressed by PDCD4 silencing (Fig. 2B). Similarly, the activity of cleaved caspase 3 was increased by KA induction, which was then decreased by PDCD4 depletion (Fig. 2C). Western blotting results revealed that KA stimulation decreased Bcl-2 expression, and increased Bax and cleaved-PARP expression in HT22 cells compared with those in the control group, an effect that was subsequently reversed by PDCD4 knockdown (Fig. 2D).

Knockdown of PDCD4 suppresses KA-induced oxidative stress in HT22 cells. As shown in Fig. 3A, the levels of ROS in HT22 cells were markedly increased following treatment with KA, whereas PDCD4 silencing reduced ROS levels in KA-induced HT22 cells. In addition, KA treatment significantly increased the MDA levels, and suppressed the generation of SOD and GSH-Px compared with those in the control group, whereas these effects were reversed by PDCD4 silencing (Fig. 3B).

PDCD4 knockdown inhibits ER stress by blocking the MAPK/NF-κB signaling pathway. Subsequently, the potential mechanism through which PDCD4 regulates KA-induced HT22 cells was explored. As shown in Fig. 4A, the expression levels of p-ERK1/2, p-JNK, p-p38 and p-NF-kBp65 were significantly increased by KA stimulation compared with those in the control group, whereas PDCD4 knockdown reversed the increased levels of these proteins in KA-induced cells. In addition, western blotting was used to detect ER stress-related proteins; the results revealed that KA treatment significantly enhanced the protein expression levels of CHOP, GRP78, ATF4 and p-eIF2α in HT22 cells compared with those in the control group, whereas the expression levels of these proteins were subsequently reduced following PDCD4 knockdown. Compared with in the KA + sh-PDCD4 group, PMA treatment partially increased the expression of these proteins (Fig. 4B).

PDCD4 knockdown mitigates KA-induced neurotoxicity and oxidative stress of HT22 cells by blocking the MAPK/NF-κB pathway. The role of the MAPK/NF-κB pathway in the regulation of PDCD4 knockdown in KA-induced HT22 cells was further investigated. As shown in Fig. 5A, compared with in the KA + sh-PDCD4 group, PMA treatment significantly elevated LDH levels. Flow cytometry showed that PMA treatment increased the rate of cell apoptosis compared with that in the KA + sh-PDCD4 group (Fig. 5B). In addition, PMA treatment increased the levels of cleaved caspase 3, and the expression levels of Bax and cleaved-PARP, but reduced Bcl-2 expression in KA-induced

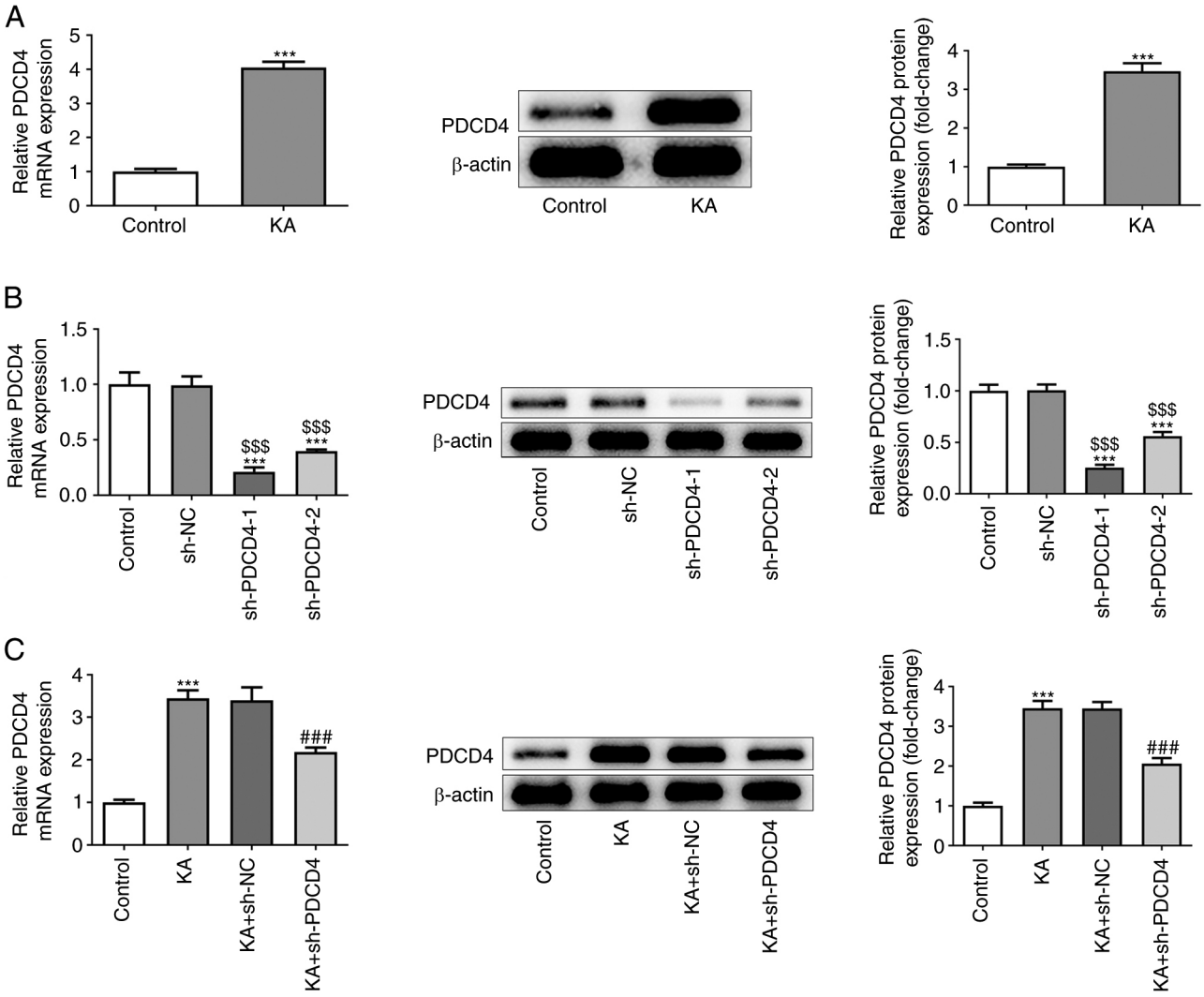

Figure 1. PDCD4 expression is upregulated in HT22 cells treated with KA. mRNA and protein expression levels of PDCD4 in (A) KA-induced HT22 cells, (B) HT22 cells transfected with sh-PDCD4 and (C) KA-induced HT22 cells transfected with sh-PDCD4 were detected by reverse transcription-quantitative PCR and western blotting. Data are presented as the means ± SD. ***P<0.001 vs. Control; $^{\$\$\$}$P<0.001 vs. sh-NC; $^{\#\#\#}$P<0.001 vs. KA + sh-NC. KA, kainic acid; NC, negative control; PDCD4, human programmed cell death 4; sh, short hairpin.

HT22 cells transfected with sh-PDCD4 (Fig. 5C and D). Furthermore, treatment with PMA stimulated ROS and MDA levels, and decreased the SOD and GSH-Px activity compared with those in the KA + sh-PDCD4 group (Fig. 6A and B).

Discussion

Epilepsy is one of the most common serious brain conditions that results in recurrent and unprovoked focal seizures due to an acute neurologic or systemic insult (23). Epilepsy is characterized by repetitive and persistent seizures, accompanied with transient brain dysfunction caused by abnormal synchronized discharge of important regions of the brain, as well as emotional or cognitive dysfunction (24).

The PDCD4 protein is a tumor suppressor that inhibits translation through the mTOR-dependent initiation factor, and its inhibitory role has been investigated (25). Chen *et al* (26) reported that PDCD4 can promote microglia activation through a PDCD4/MAPK/NF-κB positive loop, and can facilitate neuron apoptosis during neuroinflammation, which may aggravate nerve damage. Wang and Chang (27) revealed that PDCD4 levels are markedly increased in the plasma of patients with Alzheimer's disease, as well as in Aβ25-35-treated SH-SY5Y and IMR-32 cells. miR-212 was shown to regulate the proliferation and apoptosis of Aβ25-35-treated SH-SY5Y and IMR-32 cells through the PI3K/AKT signaling pathway by modulating PDCD4. In another study, silencing of the long non-coding RNA metastasis-associated lung adenocarcinoma transcript-1 was shown to protect cells against bupivacaine-induced neurotoxicity by regulating the miR-101-3p/PDCD4 axis (28). In addition, tribbles pseudokinase 3 silencing protects against KA-induced neurotoxic injury in rats (29). In the present study, it was revealed that PDCD4 was upregulated in KA-induced HT22 cells, and PDCD4 silencing reduced KA-induced neurotoxicity and oxidative stress. Furthermore, PDCD4 silencing suppressed ER stress by inhibiting the expression levels of ER stress-related proteins, CHOP, GRP78, ATF4 and p-eIF2α, in KA-induced HT22 cells.

A previous study showed that PDCD4 silencing markedly inhibits the phosphorylation of MAPKs (p38, ERK and JNK), and suppresses NF-κB p65 phosphorylation and

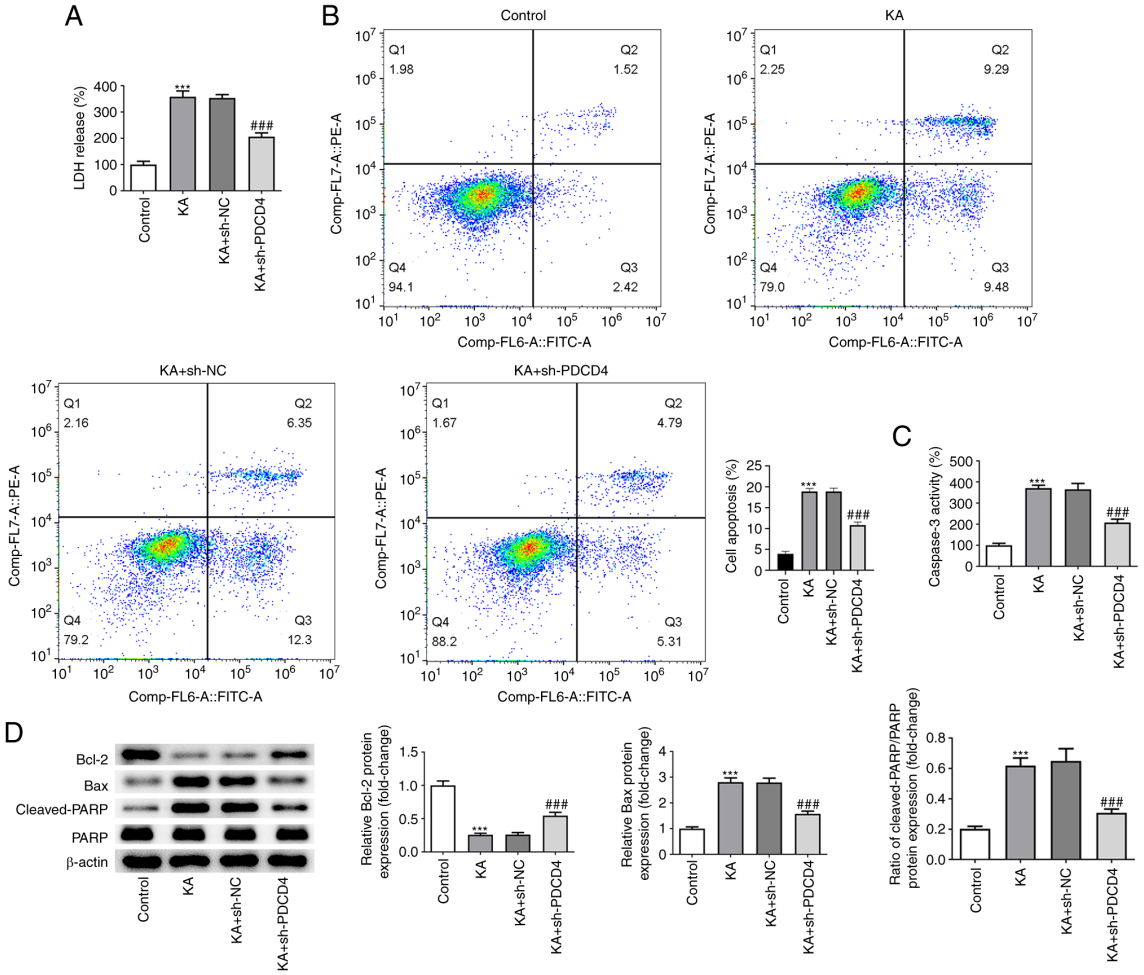

Figure 2. PDCD4 silencing ameliorates KA-induced neurotoxicity in HT22 cells. (A) Levels of LDH in KA-induced HT22 cells transfected with sh-PDCD4 were detected using an LDH assay. (B) Apoptosis was detected by flow cytometry. (C) Levels of caspase 3 were measured using the corresponding kit. (D) Western blot analysis was used to evaluate the expression levels of apoptosis-related proteins. Data are presented as the means ± SD. ***P<0.001 vs. Control; ###P<0.001 vs. KA + sh-NC. KA, kainic acid; LDH, lactate dehydrogenase; NC, negative control; PARP, poly (ADP-ribose) polymerase; PDCD4, human programmed cell death 4; sh, short hairpin.

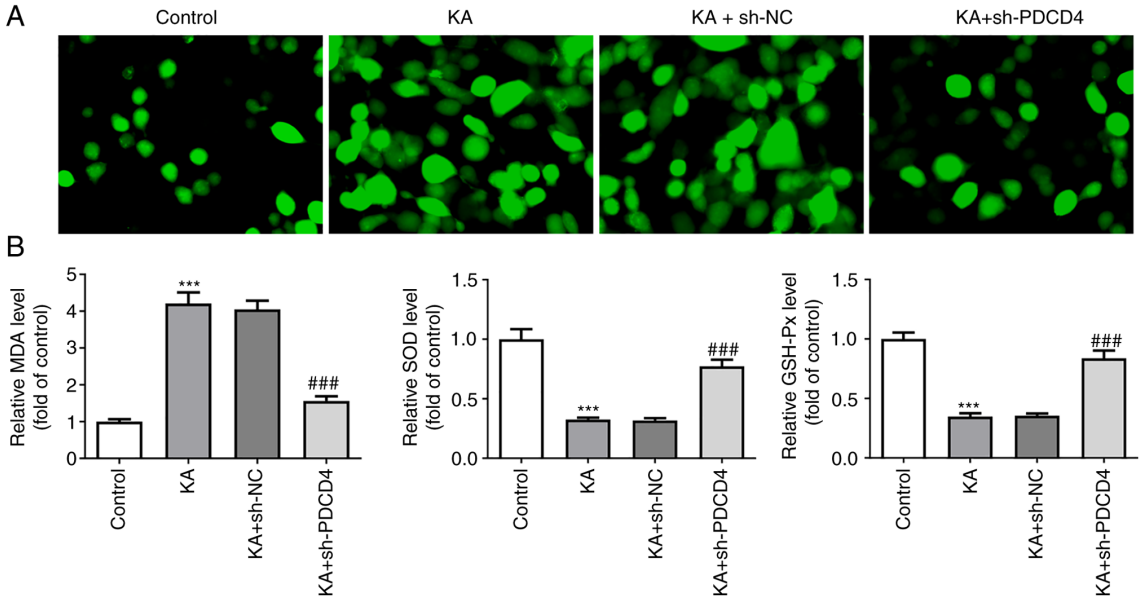

Figure 3. Knockdown of PDCD4 suppresses KA-induced oxidative stress in HT22 cells. (A) Dichlorodihydrofluorescein diacetate staining was used to assess reactive oxygen species levels. Original magnification, x200. (B) Levels of MDA, SOD and GSH-Px were detected using kits. Data are presented as the means ± SD. ***P<0.001 vs. Control; ###P<0.001 vs. KA + sh-NC. GSH-Px, glutathione peroxidase; KA, kainic acid; MDA, malondialdehyde; NC, negative control; PDCD4, human programmed cell death 4; sh, short hairpin; SOD, superoxide dismutase.

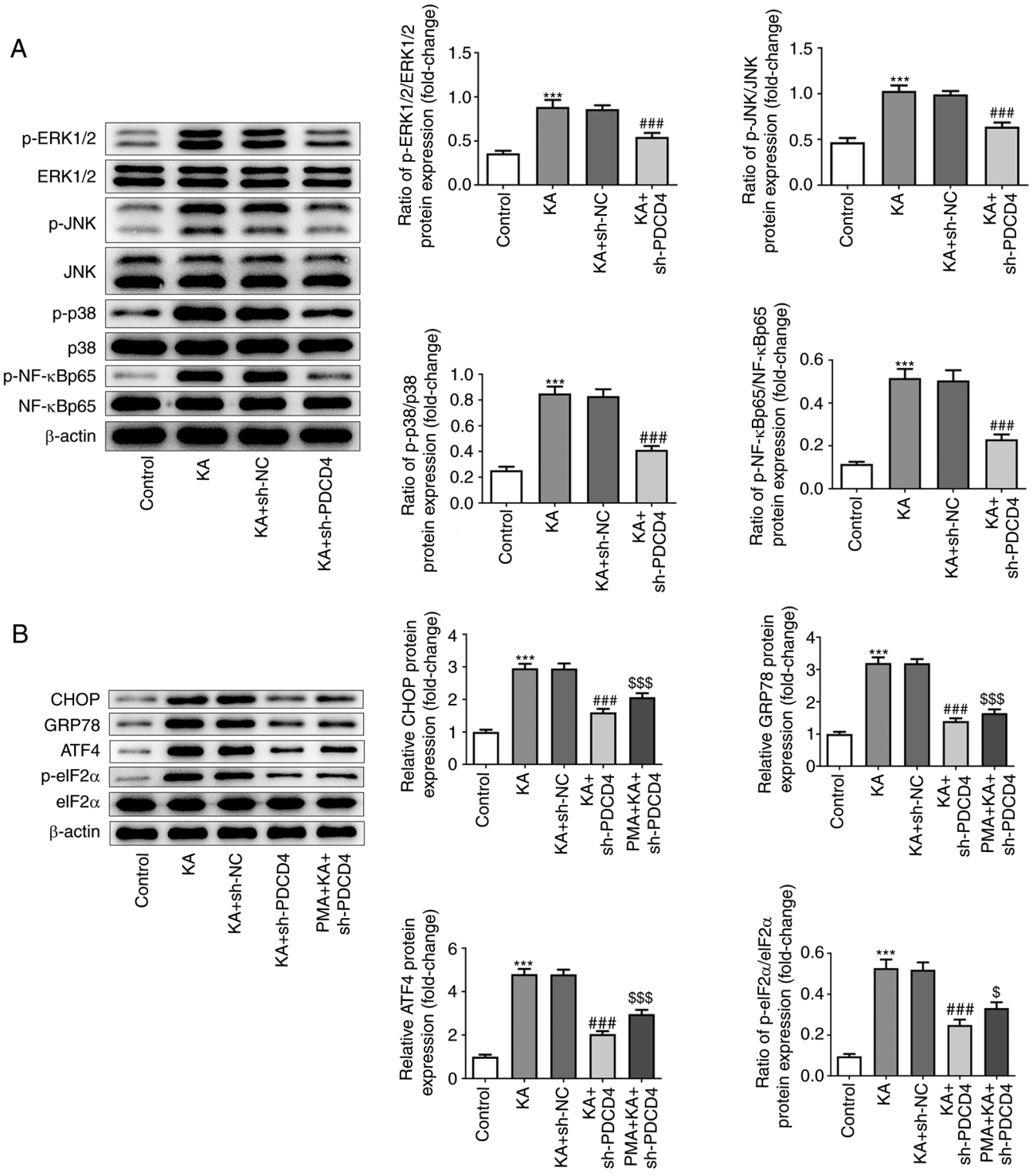

Figure 4. PDCD4 knockdown inhibits endoplasmic reticulum stress by blocking the MAPK/NF-κB signaling pathway. Protein expression levels of (A) ERK1/2, JNK, p38, NF-kBp65, p-ERK1/2, p-JNK, p-p38 and p-NF-kBp65, and (B) CHOP, GRP78, ATF4, eIF2α and p-eIF2α in KA-induced HT22 cells transfected with sh-PDCD4 with or without PMA treatment were detected by western blot analysis. Data are presented as the means ± SD. ***P<0.001 vs. Control; $^{###}$P<0.001 vs. KA + sh-NC; $^{\$}$P<0.05 and $^{\$\$\$}$P<0.001 vs. KA + sh-PDCD4. ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor-2α; GRP78, glucose-regulated protein 78; KA, kainic acid; NC, negative control; p-, phosphorylated; PDCD4, human programmed cell death 4; PMA, phorbol myristate acetate; sh, short hairpin.

nuclear translocation, thereby inhibiting lipopolysaccharide (LPS)-induced inflammatory activation in microglia cells (26). Li *et al* (30) also demonstrated that the MAPK signaling pathway is activated in rats with epilepsy. Losmapimod was shown to exert protective effects on epilepsy by reducing neuron loss through the suppression of MAPK phosphorylation. Qi *et al* (31) demonstrated that miR-494 inhibits receptor-interacting serine/threonine-protein kinase 1 to

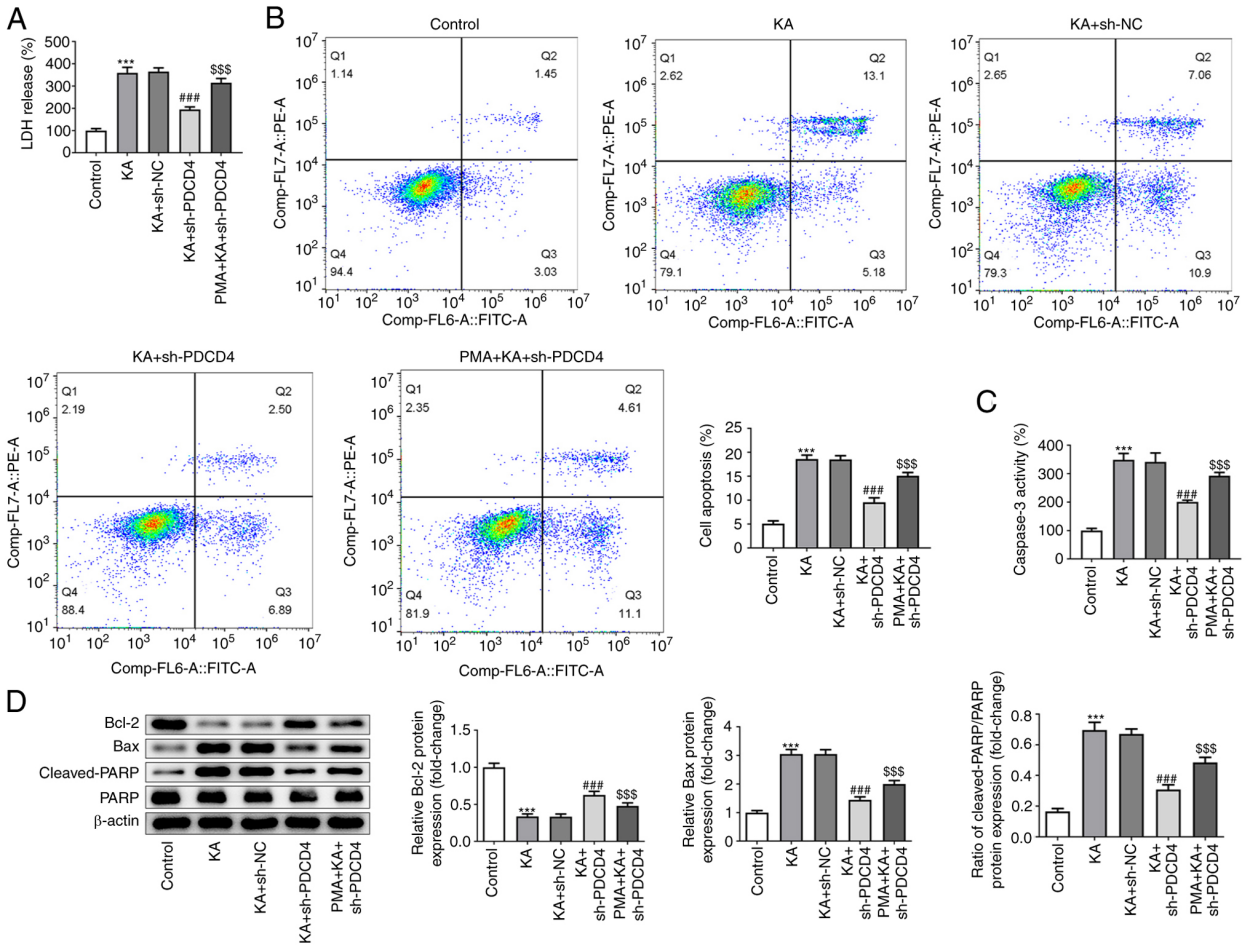

Figure 5. PDCD4 knockdown mitigates KA-induced neurotoxicity of HT22 cells by blocking the MAPK/NF-κB pathway. (A) Levels of LDH in KA-induced HT22 cells transfected with sh-PDCD4 with or without PMA treatment were detected using an LDH assay. (B) Apoptosis was detected by flow cytometry. (C) Levels of caspase 3 were measured using the corresponding kit. (D) Western blot analysis was used to evaluate the expression levels of apoptosis-related proteins. Data are presented as the means ± SD. ***P<0.001 vs. Control; $^{###}$P<0.001 vs. KA + sh-NC; $^{\$\$\$}$P<0.001 vs. KA + sh-PDCD4. KA, kainic acid; LDH, lactate dehydrogenase; NC, negative control; PARP, poly (ADP-ribose) polymerase; PDCD4, human programmed cell death 4; PMA, phorbol myristate acetate; sh, short hairpin.

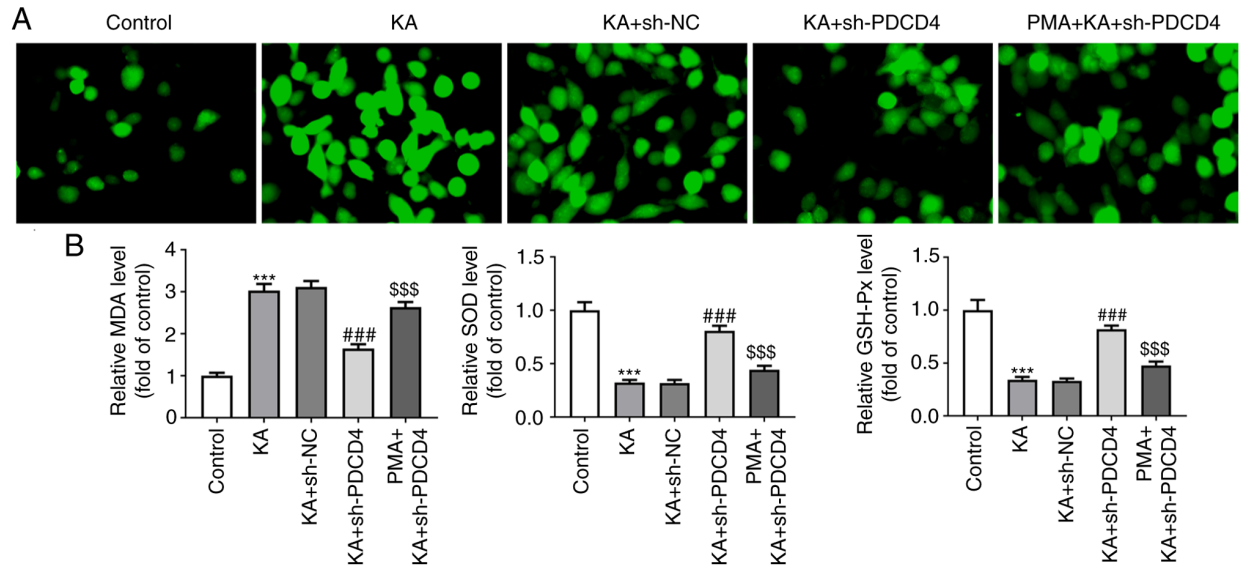

Figure 6. PDCD4 knockdown inhibits KA-induced oxidative stress of HT22 cells by blocking the MAPK/NF-κB pathway. (A) Dichlorodihydrofluorescein diacetate staining was used to assess reactive oxygen species levels. Original magnification, x200. (B) Levels of MDA, SOD and GSH-Px were detected using kits. Data are presented as the means ± SD. ***P<0.001 vs. Control; $^{###}$P<0.001 vs. KA + sh-NC; $^{\$\$\$}$P<0.001 vs. KA + sh-PDCD4. GSH-Px, glutathione peroxidase; KA, kainic acid; MDA, malondialdehyde; NC, negative control; PDCD4, human programmed cell death 4; PMA, phorbol myristate acetate; sh, short hairpin; SOD, superoxide dismutase.

suppress the apoptosis of hippocampal neurons and reduce the neuronal damage in epileptic rats by deactivating the NF-κB pathway. In addition, it has been reported that rehmannioside A attenuates depression by suppressing ER stress and apoptosis in the hippocampus of chronic unpredictable mild stress-induced rats by regulating MAPK signaling (32). Another study revealed that ulinastatin attenuates LPS-induced inflammation and inhibits ER stress-induced apoptosis through regulation of the TLR4/NF-κB and Nrf2/heme oxygenase-1 pathways in renal tubular epithelial cells (33). In the present study, PMA was used to activate the MAPK pathway, and it was revealed that PMA treatment activated ER stress-related proteins, including CHOP, GRP78, ATF4 and p-eIF2α. Furthermore, the activation of the MAPK pathway potentiated cell injury and apoptosis, as well as oxidative stress. Notably, there are several limitation in the present study. Only the KA-induced HT22 hippocampal cell line was used to explore the relationship between epilepsy and PDCD4 silencing, and we aim to explore the roles of PDCD4 in other cell lines of neurological diseases in further studies. In addition, further experiments, including animal and clinical studies, are required to confirm the present findings.

In conclusion, the present study indicated the inhibitory role of PDCD4 silencing in KA-induced neurotoxicity, cell apoptosis and oxidative stress by suppressing ER stress through inhibition of the MAPK/NF-κB pathway, thus suggesting that PDCD4 may be a promising targeted therapy for epilepsy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PL and GC designed the study, performed the experiments, drafted and revised the manuscript. PL and GC confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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