

MicroRNA-214 suppresses propofol-induced neuroapoptosis through activation of phosphoinositide 3-kinase/protein kinase B signaling by targeting phosphatase and tensin homolog expression

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Abstract. The present study aimed to investigate the effects of microRNA (miR)-214 on neuroapoptosis induced by propofol and the possible mechanism of its anti-apoptotic effects. Initially, it was observed that miR-214 expression was upregulated in propofol-induced neuroapoptosis rats. Next, propofol-treated nerve cells were transfected with miR-214 mimics. The results revealed that miR-214 overexpression induced apoptosis, inhibited cell proliferation, inhibited cyclin D1 protein expression, promoted caspase-3 activity and B-cell lymphoma 2-associated X protein expression, and enhanced the levels of inflammation factors in nerve cells treated with propofol. In addition, miR-214 overexpression suppressed phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) signaling by targeting the activation of phosphatase and tensin homolog (PTEN) and nuclear factor- κ B expression in nerve cells treated with propofol. Treatment with a PTEN inhibitor successfully suppressed the PTEN protein expression and decreased the apoptosis of propofol-treated nerve cells subsequent to miR-214 overexpression through PI3K/Akt signaling. In conclusion, the present study data revealed that miR-214 suppressed propofol-induced neuroapoptosis through the activation of PI3K/Akt signaling by targeting PTEN expression.

Introduction

Propofol is one of the commonly used inhalation anesthetics in clinical practice, and it is extensively applied in pediatric anesthesia (1). Clinical studies and animal experiments demonstrated that propofol induced neuroapoptosis in developmental

hippocampus, thus resulting in learning and memory dysfunction (2,3). Clinical research identified that exposure to inhaled anesthetics (such as propofol) during the developmental stage can lead to developmental neurotoxicity (3). In addition, this exposure causes long-term learning and memory dysfunction. The main mechanisms through which propofol induces developmental neurotoxicity are considered to be as follows (1): Propofol increases the concentration of calcium ion in developmental neurons, interferes with mitochondrial function and thus induces cell apoptosis (4); however, propofol also inhibits neuronal synaptic transmission and induces abnormal neuronal synaptic function. Further analysis has indicated that propofol-induced developmental neuroapoptosis and synaptic dysfunction may be associated with propofol-induced inflammatory response (4). It has been previously observed that propofol is able to induce the release of pro-inflammatory factors in hippocampal neurons in fetal rats (4). The release of these neuronal pro-inflammatory factors induces increased production of neuronal glutamic acid; thus, it results in imbalanced hemostasis of calcium ion in neurons, promoting mitochondrial dysfunction and cell apoptosis (4). However, the expression of inducible nitric oxide synthase may also be promoted, thereby aggravating the synaptic dysfunction in developmental neurons. Thus, inflammatory response is considered to serve a vital role in propofol-induced developmental neurotoxicity (3).

Phosphatase and tensin homolog (PTEN) is highly expressed in the majority of neuronal regions in the brain tissues of rats and mice (5). This is particularly true in the cytoplasm and nucleus of Purkinje cells, olfactory neurons and cortical pyramidal cells (5). In addition, PTEN participates in the process of the pathological formation of glioma, while it can also regulate normal brain functions, including the migration and size of nerve cells (6). It is also involved in brain injury, including injury resulting from cerebral ischemia and drug addiction (5).

In vivo and *in vitro* experiments on ischemic-hypoxic injury demonstrated that, among the cultured hippocampal neurons, those overexpressing PTEN exhibited reduced protein kinase B (Akt) levels. By contrast, neurons with no expression of PTEN exhibited elevated Akt levels (7). Furthermore, an increased number of neurons with PTEN overexpression presented

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excitatory glutamic acid-induced neuronal death as compared with that in neurons with low or absent PTEN expression (6). This demonstrated that PTEN was able to regulate the excitatory glutamic acid through an Akt-dependent signaling pathway; thus, it can regulate neuronal survival and death (5).

MicroRNA (miRNA or miR) is an endogenous non-coding single-strand small RNA molecule with a length of 18-25 nucleotides (5). miRNA is evolutionally conserved and inhibits the translation of a target gene through complementary pairing with the 3'-untranslated region (UTR) of the target mRNA. Complete or incomplete pairing of miRNA with the 3'-UTR of target mRNA inhibits mRNA translation or induces mRNA degradation (8). Thus, miRNA can exert its silencing effect on target genes at the post-transcription level. In mammals, miRNAs are involved in multiple processes (8), including embryonic development, organogenesis, cell proliferation, apoptosis, stress response and tumorigenesis. Furthermore, miRNA expression may be a contributing factor in neurodegenerative disease (9).

The current study aimed to investigate the effects of miR-214 on neuroapoptosis induced by propofol treatment and the possible mechanism underlying its effects.

Materials and methods

Animals and experimental groups. Sprague Dawley rats (weight, 220-250 g; 8-9 weeks old) were obtained from the Laboratory Animal Center of Shantou University Medical College (Guangdong, China) and maintained under standard housing conditions (24±2°C; 55-60% humidity, 12-h, light/dark cycle), and had access to food and water *ad libitum*. A total of 20 rats were randomly assigned into two groups, including the control (n=10) and propofol model (n=10) groups. In the propofol model group, rats were intraperitoneally administered with 100 mg/kg propofol (Diprivan; AstraZeneca, Cambridge, UK). In the control group, rats were intraperitoneally administered with normal saline. After 1 day, rats were sacrificed via decapitation under 35 mg/kg pentobarbital sodium and the hippocampus was collected. This study was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College (Guangdong, China).

Cell cultivation and transfection. The HT22 hippocampal neuronal cell line was grown in Dulbecco's modified Eagle's medium (Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and 1% antibiotics (penicillin/streptomycin). Negative control mimics (5'-CCCCCCCCCCCCCCCCCCCC-3' and 5'-CCCCCCCCCCCCCCCCCCCC-3'), miR-214 mimics (5'-GCGCGGATCCTTTTCTCCCTTCCCTTACTCT-3' and 5'-CCGGAATTCGAGCCCTTCATTTGGTTGTAG-3') and anti-miR-214 mimics (inhibitor, 5'-GCGGACTGCGAGCACTTGACA-3' and 5'-TTCCAGTGCTGGTCCGAGG-3') were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). HT22 cells were transfected with 100 ng negative control mimics, 100 ng miR-214 mimics or 100 ng anti-miR-214 mimics using Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Following transfection for 6 h, HT22 cells were treated with 50 μM propofol. The control group constituted HT22 cells that were treated with 50 μM propofol and transfected with negative mimics. After 48 h following transfection, cells were employed for further analysis.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the hippocampus or HT22 transfected with negative control, miR-214 mimics or anti-miR-214 mimics using the RNeasy Mini kit (Qiagen GmbH, Hilden, Germany), and then RNA was measured using a plate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA) prior to RT and was reverse transcribed into cDNA using a QuantiTect Reverse Transcription kit (Qiagen GmbH) according to the manufacturer's protocol. qPCR analysis was performed using SYBR[®]-Green (Takara Bio, Dalian, China) by an ABI7900 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences used were as follows: miR-214 forward, 5'-ACAGCAGGCACAGACAGG-3', and reverse, 5'-GTGCAGGGTCCGAGGT-3'; U6 forward, 5'-CTCGCTTCGGCAGCAC-3', and reverse, 5'-AACGCTTCACGAATTTGCGT-3'. U6 serves as the internal control. The PCR amplification protocol was as follows: Initial amplification at 95°C for 30 sec, and 40 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. The quantified relative miR-214 expression was calculated using the 2^{-ΔΔC_q} method (10).

Hematoxylin and eosin staining. Hippocampal tissue was acquired and washed with PBS. Samples were fixed using 4% paraformaldehyde for 24 h at room temperature and was then dehydrated, embedded in paraffin, and sliced to 5 μm-thick sections. Sections were stained with hematoxylin and eosin, each for 5 min at room temperature and visualized using fluorescence microscopy (Olympus Corporation, Tokyo, Japan).

Cell survival assay. Following transfection for 6 h, HT22 cells were treated with 50 μM propofol and the survival of transfected HT22 cells was measured using a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay for 0, 1, 2 and 3 days. Briefly, 0.5 mg/ml MTT was added to each well (96-well plate) and incubated for 4 h. Subsequent to removing the media, 150 μl dimethyl sulfoxide was added to each well and incubated for 20 min. The optical density (OD) of the solubilized formazan crystals was then measured using a plate reader (Bio-Rad Laboratories, Inc.) at a wavelength of 490 nm.

ELISA for protein detection. Following transfection for 2 days, the cellular supernatant was collected by centrifugation at 2,000 x g for 10 min at 4°C. Next, the samples were used to measure the levels of tumor necrosis factor (TNF)-α (ELISA kit, cat. no. H052), interleukin (IL)-1β (cat. no. H002), IL-6 (cat. no. H007) and IL-18 (cat. no. H015) using ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The OD of the solubilized formazan crystals was then measured using a plate reader (Bio-Rad Laboratories, Inc.) at 450 nm.

Quantification of apoptosis and caspase-3 activity. After transfection for 2 days, cells were harvested, washed with PBS,

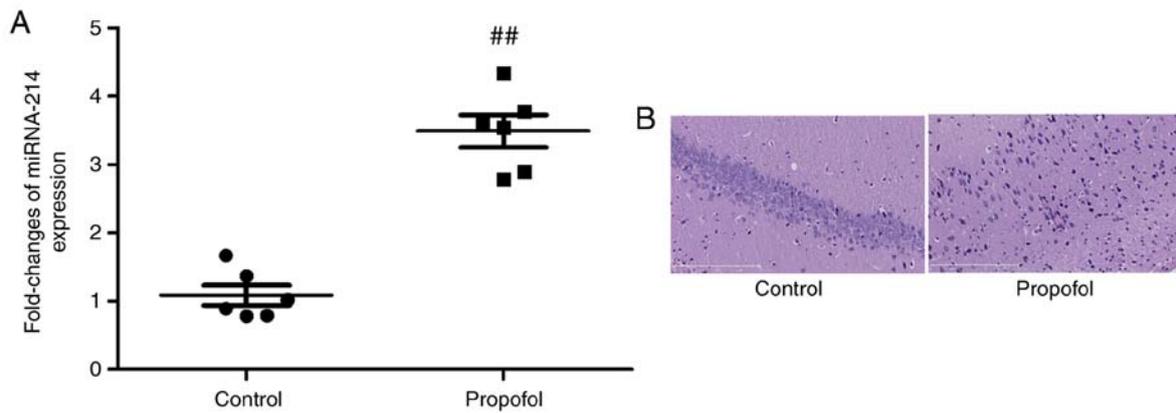


Figure 1. miR-214 expression in propofol-induced neuroapoptosis rats. (A) miR-214 expression and (B) hematoxylin and eosin staining (magnification, x10) in rats with propofol-induced neuroapoptosis. ##P<0.01 vs. the control group. miR, microRNA.

and then stained with 5 μ l Annexin V-FITC and 5 μ l propidium iodide solution (KeyGen Biotech Co., Ltd.) for 15 min in the dark. Subsequently, the apoptosis rate was analyzed by flow cytometry (Coulter Epics XL-MCL system; Beckman Coulter, Inc., Brea, CA, USA).

For caspase-3 activity detection, the protein was extracted from the cells after transfection for 2 days using radioimmuno-precipitation assay (RIPA) lysis buffer and quantified with a bicinchoninic acid (BCA) kit (both from Beyotime Institute of Biotechnology, Shanghai, China). Subsequently, 5 μ g protein was used to measure the caspase-3 activity using a caspase-3 activity kit (Beyotime Institute of Biotechnology). The OD was then measured using a plate reader (Bio-Rad Laboratories, Inc.) at 405 nm.

Western blot analysis. Total protein was extracted from the HT22 cells following transfection for 2 days using RIPA lysis buffer, and then the protein concentration was quantified by a BCA kit (both from Beyotime Institute of Biotechnology). Next, amount of protein (50 μ g) was loaded onto gels and subjected to SDS-PAGE (10% gel) prior to transfer to a polyvinylidene difluoride membrane (0.45 μ m; EMD Millipore, Bedford, MA, USA). The membranes were then blocked with 5% non-fat dry milk in Tris-buffered saline with Tween-20 (TBST) for 1 h at 37°C and probed with primary antibodies against B-cell lymphoma 2-associated X protein (Bax; cat. no. sc-6236; 1:2,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), phosphoinositide 3-kinase (PI3K, cat. no. sc-7174; 1:2,000; Santa Cruz Biotechnology), phosphorylated (p)-Akt (cat. no. sc-7985-R; 1:500; Santa Cruz Biotechnology), PTEN, nuclear factor (NF)- κ B (cat. no. sc-109; 1:500; Santa Cruz Biotechnology) and GAPDH (cat. no. 5174; 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) at 4°C overnight. The membranes were then washed three times with TBST and incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no. 7074; 1:5,000; Cell Signaling Technology, Inc.) at 37°C for 1 h. Protein bands were visualized using BeyoECL Star (Beyotime Institute of Biotechnology) and quantified by Image Lab 3.0 (Bio-Rad Laboratories, Inc.).

Immunohistochemical assay. Cells were washed with PBS following transfection for 2 day and washed with PBS. Next,

cells were fixed with 4% paraformaldehyde for 15 min at room temperature and immersed in 0.2% Triton X-100 with PBS for 15 min at room temperature. Subsequently, the cells were probed with PTEN antibody (1:500; Cell Signaling Technology, Inc.) at 4°C overnight. Following washing with PBS/0.1% Tween-20 for 15 min at room temperature, the samples were probed with Alexa Fluor fluorescent 568 secondary anti-rabbit antibodies (1:200; Thermo Fisher Scientific, Inc.) at 37°C for 1 h. Cells were then stained with DAPI for 30 min at room temperature and visualized using fluorescence microscopy (Olympus Corporation, Tokyo, Japan).

Statistical analysis. Data are expressed as the mean \pm standard error, and were analyzed with one-way analysis of variance, followed by Bonferroni post hoc analysis. A two-tailed P-value of <0.05 was considered to indicate a difference that was statistically significant.

Results

miR-214 expression in propofol-induced neuroapoptosis rats. The present study analyzed the expression of miR-214 in a propofol-induced neuroapoptosis rat model using RT-qPCR. As demonstrated in Fig. 1A, the serum level of miR-214 expression was observed to be significantly upregulated in propofol-induced neuroapoptosis rats as compared with the control group. As demonstrated in Fig. 1B, the amount of neurocyte was reduced in propofol-induced neuroapoptosis rats compared with the control group. These results suggested that miR-214 may be involved in the propofol-induced neuroapoptosis.

miR-214 overexpression increases the neuroapoptosis induced by propofol treatment in cells. The potential mechanism underlying the role of miR-214 was subsequently explored. Initially, miR-214 expression was successfully promoted by miR-214 mimic transfection in HT22 cells, as observed in Fig. 2A. The overexpression of miR-214 via miR-214 mimic transfection suppressed the cell proliferation, as well as induced the apoptosis and caspase-3 activity in cells with propofol-induced neuroapoptosis, compared with the negative control group (Fig. 2B-D).

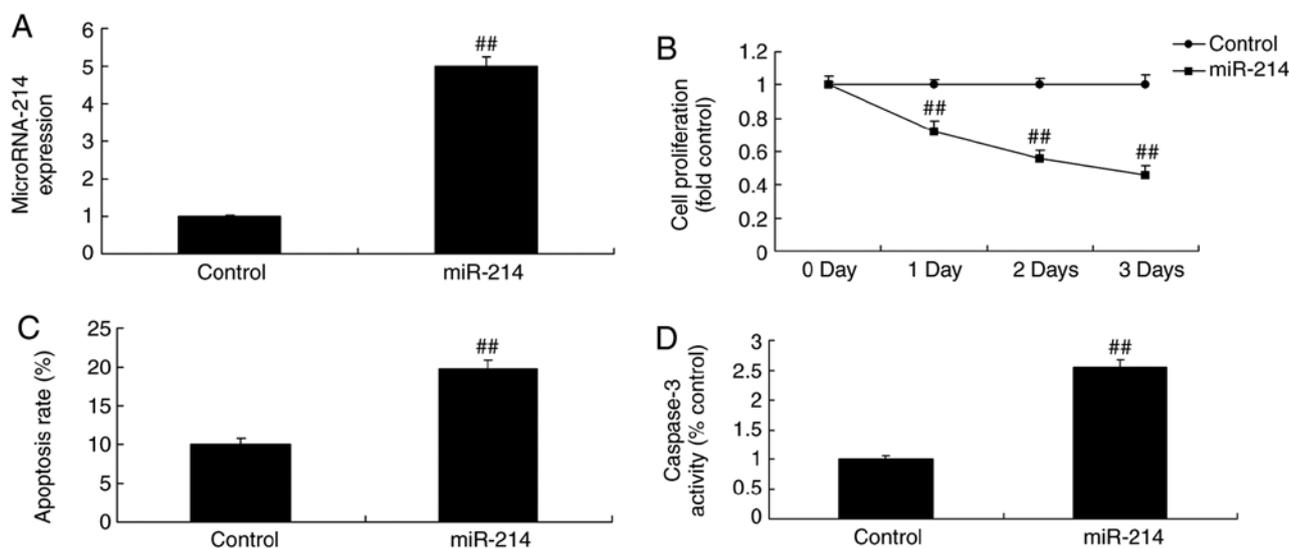


Figure 2. Overexpression of miR-214 induced apoptosis in a propofol-induced neuroapoptosis cell model. (A) miR-214 overexpression was successfully induced by mimic transfection. The (B) cell proliferation, (C) apoptosis rate and (D) caspase-3 activity following miR-214 overexpression are shown. The control group was transfected with negative control mimics, while the miR-214 group was transfected with miR-214 overexpression mimics. $^{##}P < 0.01$ vs. the control group. miR, microRNA.

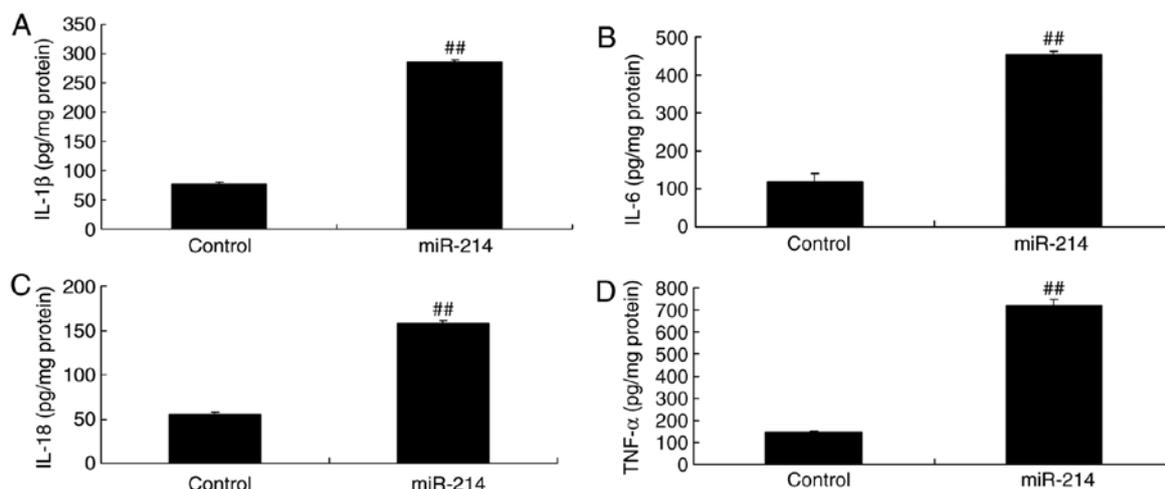


Figure 3. Overexpression of miR-214 increased the levels of pro-inflammatory cytokines in a propofol-induced neuroapoptosis cell model. (A) IL-1 β , (B) IL-6, (C) IL-18 and (D) TNF- α expression levels were examined using ELISA. $^{##}P < 0.01$ vs. the control group. miR, microRNA; IL, interleukin; TNF, tumor necrosis factor.

Overexpression of miR-214 increases inflammation in a propofol-induced neuroapoptosis cell model. The effect of miR-214 overexpression via miR-214 mimic transfection on the levels of various cytokines was then examined by ELISA. It was observed that overexpression of miR-214 significantly increased the IL-1 β , IL-6, IL-18 and TNF- α levels in cells with propofol-induced neuroapoptosis, as compared with the control group (Fig. 3). The results of the present study indicated that miR-214 may increase inflammation and apoptosis in propofol treated-cells.

Overexpression of miR-214 influences Bax, cyclin D1 and NF- κ B protein expression levels in a propofol-induced neuroapoptosis cell model. The results revealed that Bax and NF- κ B protein expression levels in cells with miR-214 overexpression via miR-214 mimic transfection were markedly higher when

compared with those of the control group. However, cyclin D1 protein expression in cells with miR-214 overexpression was significantly lower in comparison with that of the control group in the propofol-induced neuroapoptosis model *in vitro* (Fig. 4).

Overexpression of miR-214 influences PTEN/PI3K/Akt signaling in cells with propofol-induced neuroapoptosis. The present study subsequently explored the potential mechanism of the effect of miR-214 in the propofol-induced neuroapoptosis cell model. As presented in Fig. 5, overexpression of miR-214 significantly induced PTEN protein expression, whereas it suppressed the PI3K and p-Akt protein expression level in a propofol-induced neuroapoptosis model *in vitro*, compared with the control group. Therefore, miR-214 regulates PTEN/PI3K/Akt signaling in cells with propofol-induced neuroapoptosis.

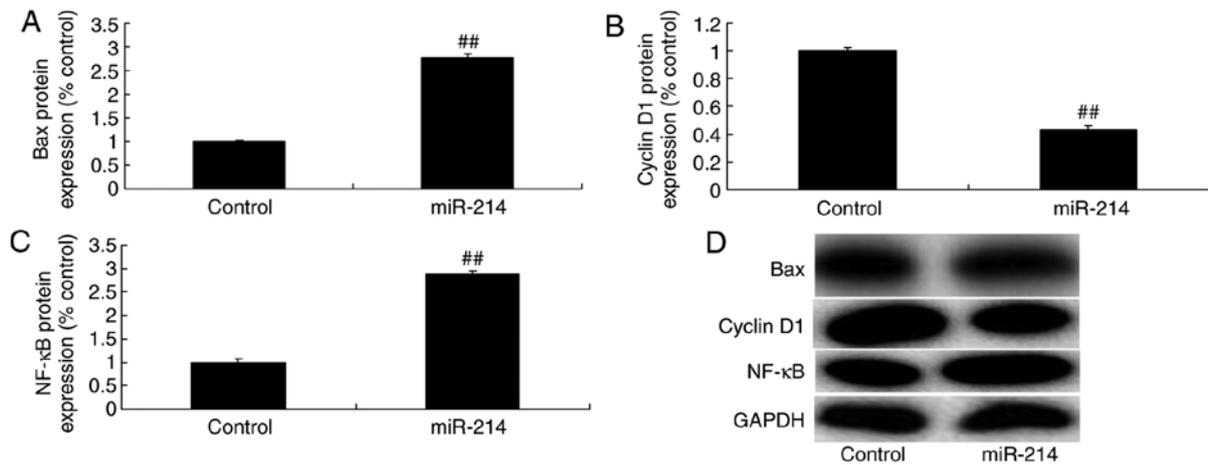


Figure 4. Overexpression of miR-214 influenced (A) Bax, (B) cyclin D1 and (C) NF-κB protein expression levels in a propofol-induced neuroapoptosis cell model. (D) Western blots of Bax, cyclin D1 and NF-κB are shown. ^{##}P<0.01 vs. the control group. miR, microRNA; Bax, B-cell lymphoma 2-associated X protein; NF, nuclear factor.

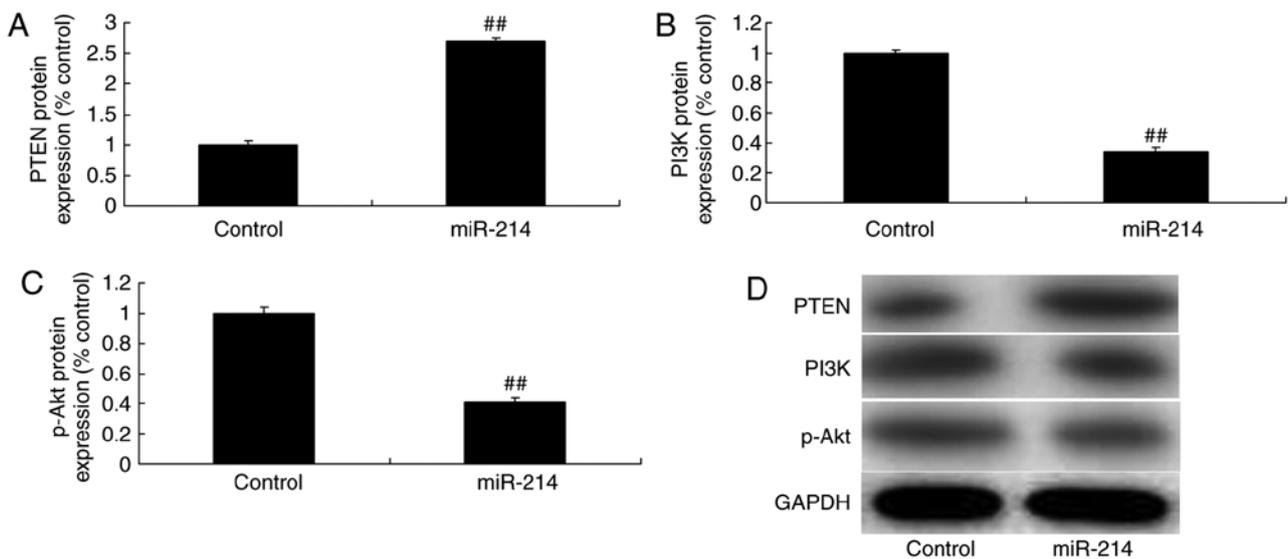


Figure 5. Overexpression of miR-214 influenced PTEN/PI3K/Akt signaling in a propofol-induced neuroapoptosis cell model. (A) PTEN, (B) PI3K and (C) p-Akt protein expression levels were quantified, and the (D) western blots are shown. ^{##}P<0.01 vs. the control group. miR, microRNA; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B.

Downregulation of miR-214 inhibits the neuroapoptosis induced by propofol treatment in cells. The present study also evaluated the potential therapeutic effect of inhibiting miR-214 on propofol-induced neuroapoptosis. It was observed that miR-214 expression was successfully reduced by anti-miR-214 mimic transfection (Fig. 6A). This downregulation of miR-214 significantly increased the cell proliferation on days 2 and 3 after transfection (Fig. 6B), whereas it inhibited the cell apoptosis rate (Fig. 6C) and caspase-3 activity (Fig. 6D) in a propofol-induced neuroapoptosis model *in vitro*, compared with the control group.

Downregulation of miR-214 reduces inflammation in a propofol-induced neuroapoptosis cell model. The results demonstrated that the TNF- α , IL-1 β , IL-6 and IL-18 levels were significantly reduced by downregulation of miR-214 within propofol-treated cells, as compared with the control group (Fig. 7).

Downregulation of miR-214 influences Bax, cyclin D1 and NF-κB protein expression in a propofol-induced neuroapoptosis cell model. The downregulation of miR-214 suppressed the Bax and NF-κB protein expression levels. By contrast, this downregulation induced cyclin D1 protein expression in the propofol-induced neuroapoptosis cell model, as compared with the control group (Fig. 8).

Downregulation of miR-214 influences PTEN/PI3K/Akt signaling in a propofol-induced neuroapoptosis cell model. To determine whether PTEN/PI3K/Akt signaling participated in propofol-induced neuroapoptosis, the PTEN, PI3K and p-Akt protein expression levels were measured by western blot analysis. It was observed that downregulation of miR-214 suppressed PTEN protein expression, whereas it induced the PI3K and p-Akt protein expression levels in propofol-induced model, when compared with the control group (Fig. 9).

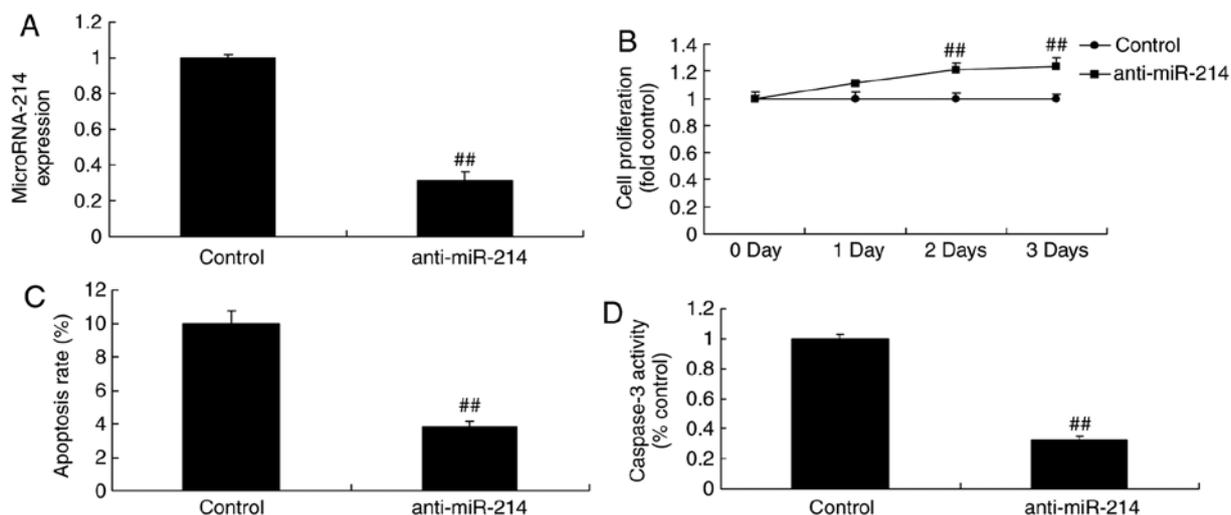


Figure 6. Downregulation of miR-214 inhibited apoptosis in a cell model of propofol-induced neuroapoptosis. (A) miR-214 expression, (B) cell proliferation, (C) apoptosis rate and (D) caspase-3 activity are displayed. The control group was transfected with negative control mimics, while the anti-miR-214 group was transfected with anti-miR-214 mimics for miR-214 downregulation. ## $P < 0.01$ vs. the control group. miR, microRNA.

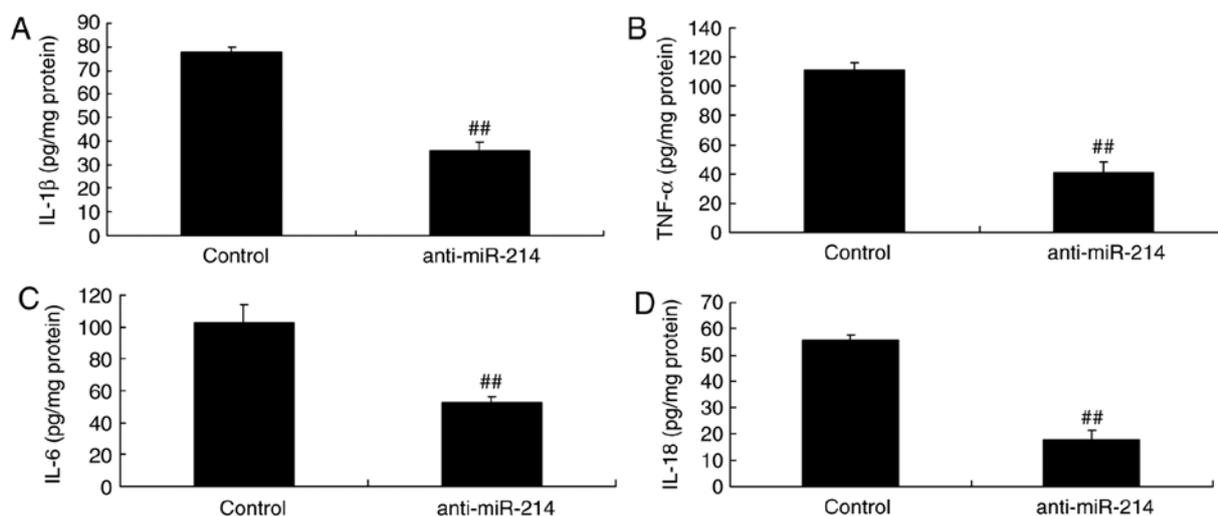


Figure 7. Downregulation of miR-214 reduced inflammation in a propofol-induced neuroapoptosis cell model. (A) IL-1 β , (B) TNF- α , (C) IL-6 and (D) IL-18 levels were reduced by miR-214 downregulation. ## $P < 0.01$ vs. the control group. miR, microRNA; IL, interleukin; TNF, tumor necrosis factor.

Inhibition of PTEN suppressed the PTEN/PI3K/Akt signaling induced by miR-214 overexpression in a propofol-induced neuroapoptosis cell model. Next, the role of PTEN in regulating PTEN/PI3K/Akt signaling in a propofol-induced neuroapoptosis cell model by miR-214 mimic was investigated. As shown in Fig. 10, treatment with a PTEN inhibitor (VO-OHpic trihydrate; 10 nM; 48 h) successfully suppressed PTEN protein expression, and induced PI3K and p-Akt protein expression levels in the propofol-induced neuroapoptosis cell model, as compared with the group with miR-214 overexpression alone. Immunofluorescence (Fig. 11) revealed that PTEN inhibition suppressed PTEN protein expression in the propofol-induced neuroapoptosis cell model, as compared with the group with miR-214 overexpression alone.

Inhibition of PTEN inhibits the neuroapoptosis induced by propofol treatment in cells by miR-214. The inhibition of PTEN promoted the miR-214 overexpression-suppressed cell prolif-

eration. Furthermore, PTEN inhibition significantly reduced the cell apoptosis rate and caspase-3 activity in the propofol-induced neuroapoptosis model *in vitro* compared with the group treated with miR-214 overexpression alone (Fig. 12).

Inhibition of PTEN inhibits the miR-214-induced inflammation in a propofol-induced neuroapoptosis. The results demonstrated that the TNF- α , IL-1 β , IL-6 and IL-18 levels induced by miR-214 overexpression were significantly decreased by PTEN inhibitor in the propofol-induced neuroapoptosis cell model, as compared with the miR-214 overexpression alone group (Fig. 13).

Inhibition of PTEN combined with miR-214 overexpression influences Bax, cyclin D1 and NF- κ B protein expression in a propofol-induced neuroapoptosis model. Finally, the current study demonstrated that the miR-214 overexpression-induced Bax and NF- κ B protein expression levels, as well as the

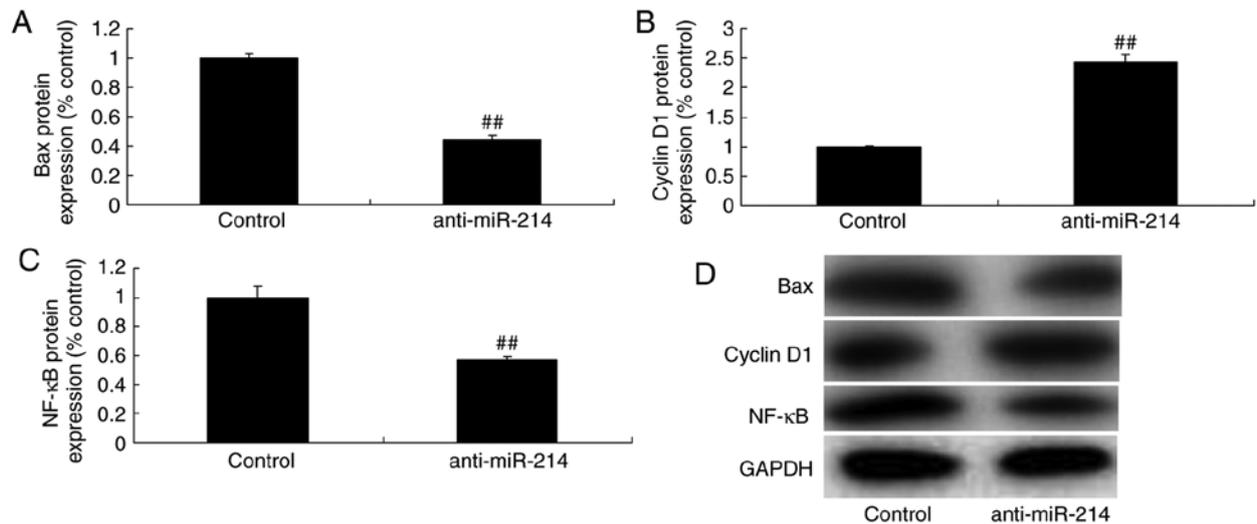


Figure 8. Downregulation of miR-214 influenced Bax, cyclin D1 and NF- κ B protein expression in a propofol-induced neuroapoptosis cell model. (A) Bax, (B) cyclin D1 and (C) NF- κ B protein expression levels were quantified following western blot analysis, and the (D) western blot images are shown. ##P<0.01 vs. the control group. miR, microRNA; Bax, B-cell lymphoma 2-associated X protein; NF, nuclear factor.

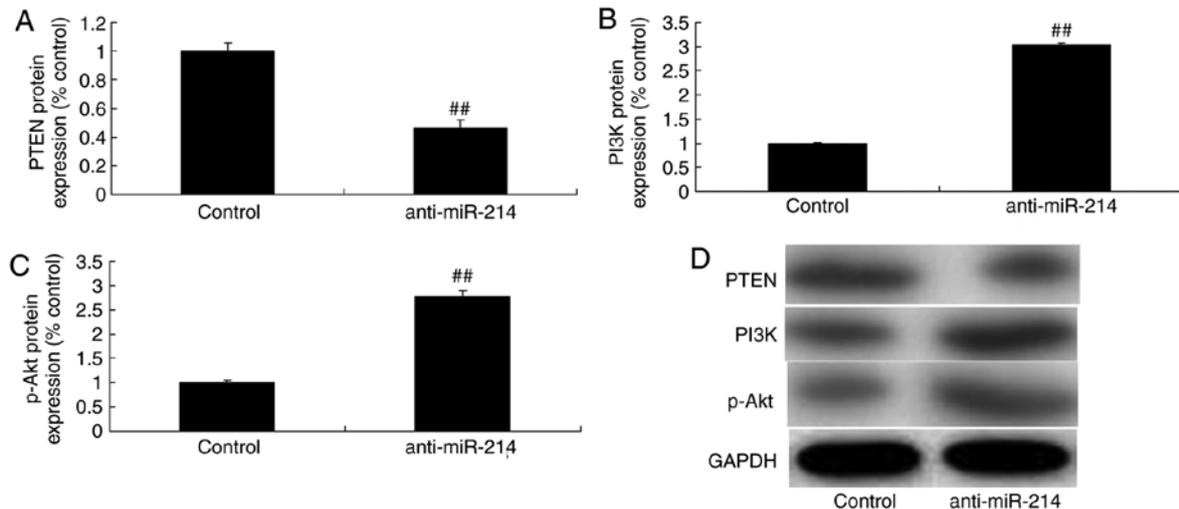


Figure 9. Downregulation of miR-214 influenced PTEN/PI3K/Akt signaling in a propofol-induced neuroapoptosis cell model. (A) PTEN, (B) PI3K and (C) p-Akt protein expression levels were quantified following western blot analysis, and the (D) western blot images are shown. ##P<0.01 vs. the control group. miR, microRNA; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B.

inhibition of cyclin D1, were reversed upon the inhibition of PTEN in the propofol-induced neuroapoptosis cell model, as compared with the group with miR-214 overexpression alone (Fig. 14). Thus, we found that miR-214 regulates PI3K/Akt signaling in cells with propofol-induced neuroapoptosis by PTEN.

Discussion

Propofol is an essential drug used for implementing local anesthesia and analgesia in clinical practice (11). These agents are extensively applied in blocking the peripheral nerve, in epidural and spinal anesthesia, and in pain management. They can reversibly block the generation and transmission of sensory nerve impulse locally (12). Common local anesthetics are divided into three categories according to their chemical structure, including the ester local anesthetics (such

as tetracaine and procaine), amide local anesthetics (such as bupivacaine, lidocaine and ropivacaine) and general anesthetics (such as propofol, sevoflurane, desflurane, ketamine and propofol) (12). Epidemiological studies have suggested that long-term or high-dose application of local anesthetics may induce potential nerve injury (13).

miRNAs serve vital roles in multiple biological processes (including differentiation) and diseases, such as neurodegenerative disease (14). In the current study, the serum expression level of miR-214 was upregulated in propofol-induced neuroapoptosis rats, compared with that in the control group. A study by Wang *et al* (14) also revealed that miR-214 participates in the neuroprotective effect of resveratrol in MPTP-induced Parkinson's disease mice.

Apoptosis or programmed death is a type of gene-regulated cell death that may occur under a normal state of the central nervous system (15). Certain neurons die prior to becoming

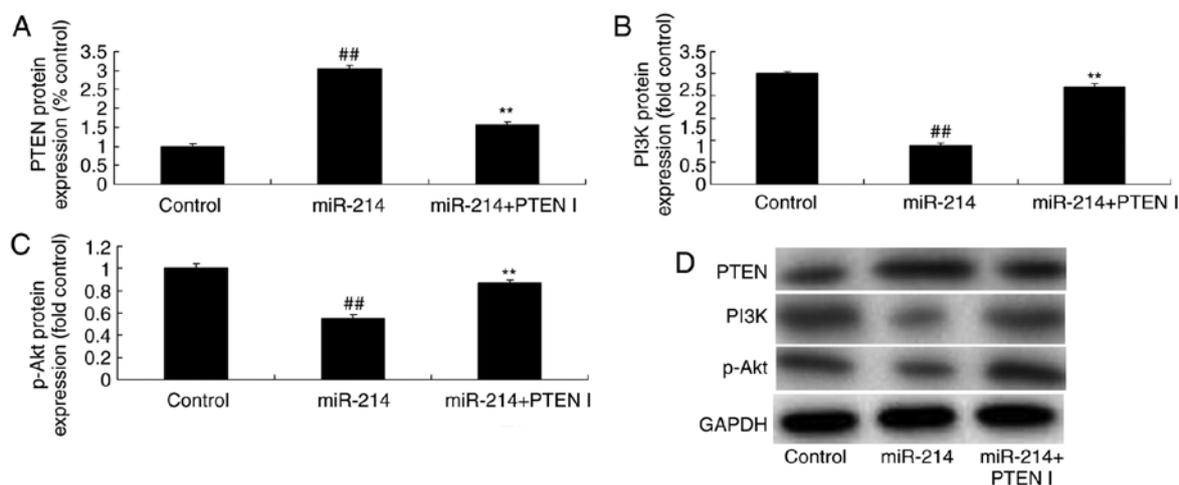


Figure 10. Inhibition of PTEN suppressed the PTEN/PI3K/Akt signaling in a propofol-induced neuroapoptosis cell model with miR-214 overexpression. (A) PTEN, (B) PI3K and (C) p-Akt protein expression levels were quantified following western blot analysis, and (D) western blots are shown. Negative control mimics were used in the control group, miR-214 overexpression was induced by mimic transfection in the miR-214 group, and PTEN inhibition was induced by VO-OHpic trihydrate combined with miR-214 overexpression in the PTEN I + miR-214 group. ^{##} $P < 0.01$ vs. the control group, ^{**} $P < 0.01$ vs. the miR-214 overexpression group. miR, microRNA; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PTEN I, PTEN inhibitor.

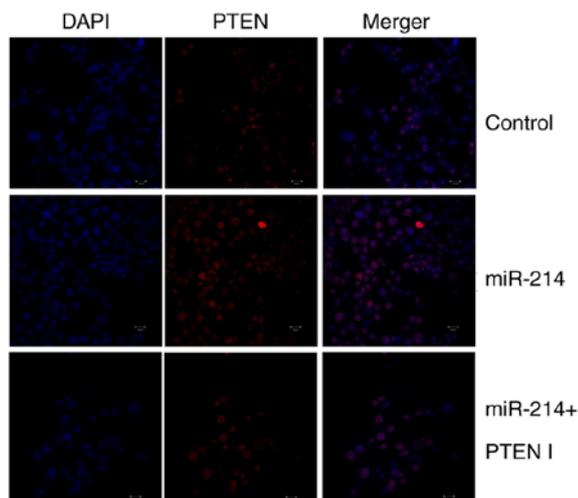


Figure 11. Inhibition of PTEN suppressed the PTEN protein expression in a propofol-induced neuroapoptosis cell model that was induced by miR-214 overexpression (immunohistochemical assay, magnification, $\times 10$). DAPI staining appears blue, while PTEN staining appears red. miR, microRNA; PTEN, phosphatase and tensin homolog; PTEN I, PTEN inhibitor.

stable mature neurons. Apoptosis is a suicidal behavior and eliminates superfluous cells in nervous system. However, destruction of the nervous system microenvironment will induce abnormal cell apoptosis (16,17), therefore leading to nervous system structural dysfunction. The results of the current study demonstrated that miR-214 overexpression induced apoptosis, inhibited cell proliferation and cyclin D1 protein expression, and promoted caspase-3 activity and Bax protein expression in nerve cells treated with propofol.

It has been observed that propofol-induced neurotoxicity is associated with the release of pro-inflammatory factors (18). A clinical study identified that exposure to inhalation anesthetics (including propofol) during the developmental stage may lead to developmental neurotoxicity, and long-term learning and memory dysfunction (19). Further studies have indicated that

propofol-induced developmental neuroapoptosis and synaptic dysfunction may be associated with propofol-induced inflammatory response (18,20). Propofol induces the release of neuron pro-inflammatory factors in the hippocampus of fetal rats, which may result in increased production of neuronal glutamic acid (19). Furthermore, it may induce imbalanced calcium ion homeostasis in neurons, promote mitochondrial dysfunction and thus increase apoptosis (20). In the present study, miR-214 overexpression enhanced the release of inflammation factors in nerve cells treated with propofol. Li *et al* (21) also observed that miR-214-3p inhibited osteoblastic bone formation and reduced inflammation, suggesting that the loss of miR-214-3p within propofol-induced neuroapoptosis may prevent any secondary action and untoward effects of operative anesthesia.

The PTEN protein is extensively expressed in the majority of the rat and mouse brain tissue regions, particularly in neurons (22). In particular, high PTEN expression is observed in the cytoplasm and nucleus of Purkinje cells, olfactory neurons and cortical pyramidal cells. In addition, PTEN is expressed in the cytoplasm and nucleus of neuroglial cells (16). Further roles of PTEN in the brain tissue have been preliminarily examined (16), and it was observed to regulate normal brain function, including the nervous migration and the size of nerve cells. Furthermore, PTEN is involved in brain injury, including cerebral ischemia, brain development and drug addiction. Research on β -amyloid peptide-induced neuron cytotoxic injury in cultured hippocampal brain tissues has recently been conducted, revealing that increasing PTEN protein expression in injured neurons leads to the phosphorylation and inactivation of Akt, consequently leading to neuronal death (16,23). The PI3K/Akt signaling pathway serves an important role in cell survival, and Akt is the major effective component of this pathway. PI3K-activated Akt can inhibit cell apoptosis through phosphorylating Bad, caspase-9 and NF- κ B. Therefore, Akt is an important anti-apoptotic regulatory factor (23). The present study demonstrated that miR-214 overexpression suppressed

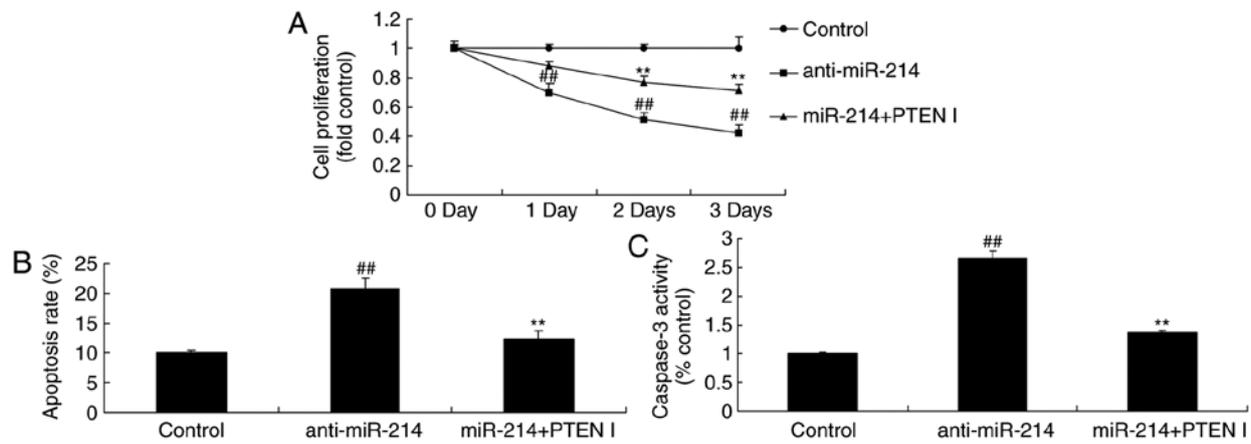


Figure 12. Inhibition of PTEN inhibited the apoptosis of propofol-induced neuroapoptosis cells that was induced by miR-214 overexpression. (A) Cell proliferation, (B) apoptosis rate and (C) caspase-3 activity are displayed. $^{##}P<0.01$ vs. the control group, $^{**}P<0.01$ vs. the miR-214 overexpression group. miR, microRNA; PTEN, phosphatase and tensin homolog; PTEN I, PTEN inhibitor.

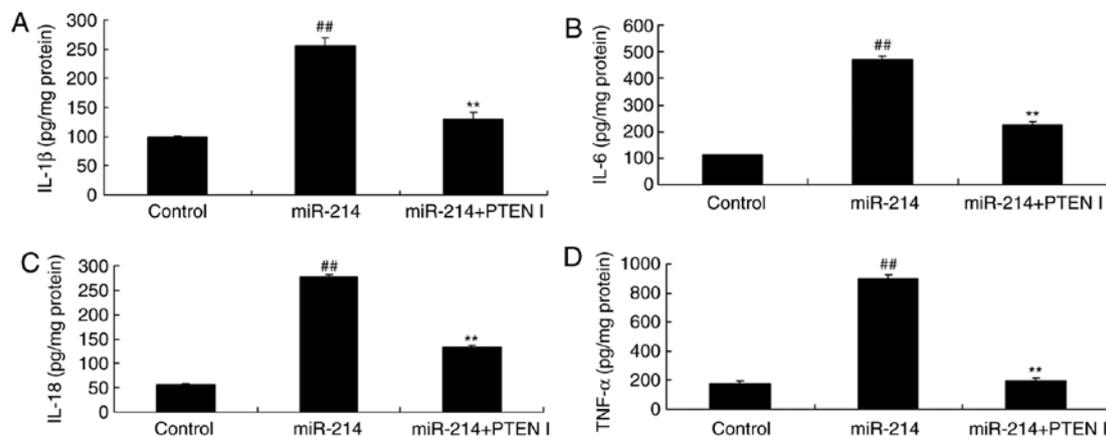


Figure 13. Inhibition of PTEN inhibited the miR-214 overexpression-induced inflammation in a propofol-induced neuroapoptosis cell model. (A) IL-1 β , (B) IL-6, (C) IL-18 and (D) TNF- α levels are presented. $^{##}P<0.01$ vs. the control group, $^{**}P<0.01$ vs. the miR-214 overexpression group. miR, microRNA; IL, interleukin; TNF, tumor necrosis factor; PTEN, phosphatase and tensin homolog; PTEN I, PTEN inhibitor.

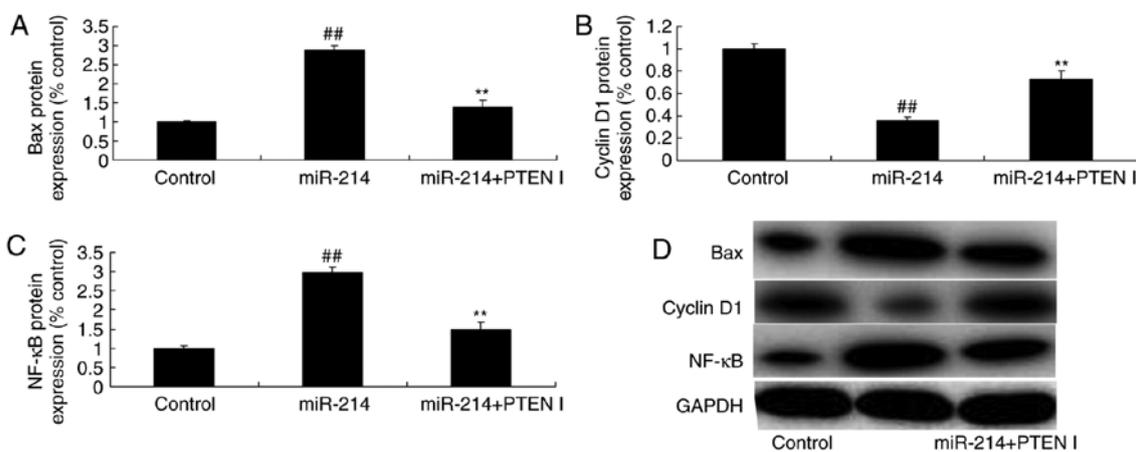


Figure 14. Inhibition of PTEN influenced Bax, cyclin D1 and NF- κ B protein expression in a propofol-induced neuroapoptosis cell model with miR-214 overexpression. (A) Bax, (B) cyclin D1 and (C) NF- κ B protein expression levels were quantified following western blot analysis, and the (D) western blot images are shown. $^{##}P<0.01$ vs. the control group, $^{**}P<0.01$ vs. the miR-214 overexpression group. miR, microRNA; Bax, B-cell lymphoma 2-associated X protein; NF, nuclear factor; PTEN, phosphatase and tensin homolog; PTEN I, PTEN inhibitor.

PI3K/Akt signaling by targeting the activation of PTEN and NF- κ B expression in nerve cells treated with propofol.

The study by Wang *et al* (24) revealed that miR-214 protects against hypoxia/reoxygenation-induced cell damage via the

suppression of PTEN/PI3K/Akt expression in myocardial ischemia/reperfusion injury. Additionally, Chu *et al* (25) observed that miR-214 suppressed the NF- κ B-mediated inflammatory response in fish. These aforementioned data suggest that therapeutic of miR-214-3p/PTEN/PI3K/Akt/NF- κ B attenuated the inflammatory response in propofol-induced neuroapoptosis.

Only the PTEN inhibitor VO-OHpic trihydrate was used in the current experiments, which may pose as a limitation of the present study. Further inhibitors or siRNA-mimics to regulate PTEN/PI3K/Akt signaling should be used in further studies.

In conclusion, the *in vitro* data of the present study suggested that, as observed by the inhibition of propofol-induced neuroapoptosis, miR-214 downregulation may be a potential therapeutic strategy against the secondary action and untoward effects of operative anesthesia. Collectively, miR-214 downregulation suppressed the propofol-induced neuroapoptosis and inflammation via activation of PI3K/Akt signaling by targeting PTEN expression.

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Availability of data and materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Authors' contributions

XJ made substantial contributions to the design of the present study; XG, MC, WK and YW performed experiments, including cell cultivation, transfection and the MTT assay; XJ analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the First Affiliated Hospital of Shantou University Medical College (Guangdong, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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