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MicroRNA-1297 suppressed the Akt/GSK3β signaling pathway and stimulated neural apoptosis in an *in vivo* sevoflurane exposure model

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Abstract

Objective: Common inhalation anesthetics used for clinical anesthesia (such as sevoflurane) may induce nerve cell apoptosis during central nervous system development. Furthermore, anesthetics can produce cognitive impairments, such as learning and memory impairments, that continue into adulthood. However, the precise mechanism remains largely undefined. We aimed to determine the function of microRNA-1297 (miR-1297) in sevoflurane-induced neurotoxicity.

Methods: Reverse transcription-polymerase chain reaction assays were used to analyze miR-1297 expression in sevoflurane-exposed mice. MTT and lactate dehydrogenase (LDH) assays were used to measure cell growth, and neuronal apoptosis was analyzed using flow cytometry. Western blot analyses were used to measure PTEN, PI3K, Akt, and GSK3β protein expression.

Results: In sevoflurane-exposed mice, miR-1297 expression was up-regulated compared with the control group. MiR-1297 up-regulation led to neuronal apoptosis, inhibition of cell proliferation, and increased LDH activity in the *in vitro* model of sevoflurane exposure. MiR-1297 up-regulation also suppressed the Akt/GSK3β signaling pathway and induced PTEN protein expression in the *in vitro* model. PTEN inhibition (VO-Ohpic trihydrate) reduced PTEN protein expression and decreased the effects of miR-1297 down-regulation on neuronal apoptosis in the *in vitro* model.

Conclusion: Collectively, the results indicated that miR-1297 stimulates sevoflurane-induced neurotoxicity via the Akt/GSK3β signaling pathway by regulating PTEN expression.

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MicroRNA-1297, sevoflurane, PTEN, neurotoxicity, Akt, GSK3β, PI3K

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Introduction

Millions of children undergo surgeries each year, including infants aged less than 12 months.[1] Moreover, millions of patients receive surgical treatments every year, and pediatric patients account for a large portion of neurosurgery patients.[2] Thus, the use of anesthesia is an essential process in infants and young children.[3] The application of anesthetics can alleviate pain and anxiety, allow patients to maintain a fixed position, and provide an appropriate surgical condition.[4] Furthermore, anesthetics are required for numerous diagnostic processes to avoid the influence of random movements of children during diagnosis and reduce the potential risks of some interventional diagnoses.[2,3] Consequently, anesthetics have become an indispensable part of modern medicine, and great advancements have been made in recent years.[5]

The mechanisms of action of both intravenous anesthetics and inhalation anesthetics remain unclear.[6] Such drugs can act on the central nervous system and facilitate surgical procedures. However, their safety has increasingly become a focus of attention.[7] Particularly, determining whether anesthetics have adverse effects on neurodevelopment in infants and children is an urgent issue. Previous research concerning inhalation anesthetics has mainly concentrated on their protective effects on the nervous system.[8] Sevoflurane has been shown to promote cell survival in the cerebral ischemic region in rats.[7] Furthermore, it can alleviate cerebral reperfusion injury, and some scholars also consider that sevoflurane pretreatment can mitigate the impairment caused by hypoxia and ischemia in the rat brain.[9] These findings support the clinical safety of sevoflurane. However, an increasing number of studies have indicated that inhalation anesthetics such as sevoflurane have a certain degree of neurotoxicity.[10] In addition, they have a non-negligible effect on cognitive development in children.[11] As confirmed in numerous studies, inhalation anesthetics such as sevoflurane can induce cell apoptosis in many cerebral regions, including the cerebral cortex and hippocampus, in mice.[12] More importantly, exposure of young rats to sevoflurane will lead to remarkably decreased spatial cognitive ability in adulthood compared with that of normal rats.[13]MicroRNA (miRNA) is a type of endogenous, non-coding, single-strand, small molecular RNA approximately 18 to 25 nucleotides in length.[14] It is evolutionarily conserved and can inhibit target mRNA translation through complementary pairing with the 3′-untranslated region (UTR).[15] Complete or incomplete pairing and binding of miRNA with 3′-UTRs of its target mRNA can suppress mRNA translation or induce mRNA degradation.[16] Thus, miRNAs exert a silencing effect on the target gene at the post-transcriptional level.[16] Mammalian miRNA is involved in multiple processes, including embryogenesis, organogenesis, cell proliferation, cell apoptosis, stress responses, and tumorigenesis.[16] Wang et al.[17] showed that miR-1297 regulates glioma cell growth *in vivo* and *in vitro*. Bu et al.[18] showed that miRNA-1297

promotes cell proliferation of non-small cell lung cancer cells through the PTEN/Akt/Skp2 signaling pathway. Therefore, miRNA-1297 may participate in cell apoptosis caused by nerve injury. In this study, we aimed to examine the function of miRNA-1297 in sevoflurane-induced neurotoxicity and explore its molecular biological mechanisms.

Materials and methods

Mouse model

Male C57BL/6 mice (n = 20, 20–21 g, 6 weeks old) were purchased from the Animal Center of Shantou University and maintained in laminar flow rooms with exposure to 12-hour light and dark cycles (8:00–20:00) at a temperature of 22 to 24°C and 50% to 55% humidity with free access to food and water. The mice were randomly assigned to two groups: control and sevoflurane exposure model. Mice in the model group were treated with 3% sevoflurane.[19] Treatments were administered intraperitoneally at 2 hours prior to sevoflurane anesthesia, and mice were then sacrificed for other investigations. All experiments were approved by the institutional committee of the Clinical College of Ophthalmology, Tianjin Medical University, and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.[20]

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from cell and tissue samples (hippocampus) using the TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). mRNA (1 ng) was reverse transcribed into cDNA with a ReverTra Ace-α first strand cDNA synthesis kit (Toyobo, Osaka, Japan). The PCR reaction was performed using a qPCR instrument (ABI 7000; Applied Biosystems, Foster City, CA, USA) and the Express SYBR® GreenER™ miRNA qRT-PCR kit (Invitrogen Life Technologies) with the following PCR conditions: 95°C for 10 minutes; 40 cycles of 95°C for 10 s, 60°C for 20 s, and 72°C for 30 s; and 72°C for 10 minutes. MiRNA-1297 expression was determined using the $2-\Delta\Delta Ct$ method.

Microarray samples

Total RNA was extracted from cell and tissue samples (hippocampus) using the TRIzol® reagent (Invitrogen Life Technologies). mRNA (1 ng) was amplified with a Low Input Quick-Amp Labeling Kit (Agilent Technologies, Santa Clara, CA, USA) and labeled with Cy3 (Agilent Technologies). Cy3-labled cRNAs were then hybridized onto Agilent SurePrint G3 Mouse GE 8×60 K Microarray Chips (Agilent Technologies).

Cell culture and treatment

SH-SY5Y neuroblastoma cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences and cultured in Roswell Park Memorial Institute 1640 medium (GIBCO Life Technologies, Darmstadt, Germany) with 100 IU/L penicillin/streptomycin, 1% glutamate, and 10% fetal bovine serum (GIBCO Life Technologies) in 5% carbon dioxide at 37°C.

Plasmid transfection

SH-SY5Y cells were plated in six-well plates (1×10^5/well) and transfected with 100 nM miRNA-1297, anti-MiRNA-1297, or negative plasmid using lipofectamine 2000 (Invitrogen). After 24 hours of transfection, the neurons were treated with 2.4% sevoflurane.

Luciferase reporter

Fragments of the wild-type or mutant PTEN 3′-UTR were cloned and inserted into a pmiRGLO vector (PTEN500). SH-SY5Y cells were co-transfected with miRNA-1297, anti-miRNA-1297, or negative plasmid with the wild-type or mutant PTEN 3'-UTR using Lipofectamine 2000 (Invitrogen). After incubation for 48 hours, luciferase activity was measured using the Promega Dual-luciferase Reporter Assay kit (Promega, Madison, WI, USA).

MTT and lactate dehydrogenase (LDH) release assays

SH-SY5Y cells were plated in 96-well plates (10,000/well) for 48 hours, and 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, 5 mg/mL, 20 μL) was added to the cells and incubated for 4 hours at 37°C. The medium was removed, and dimethyl sulfoxide (150 μL) was added to the cells for 20 minutes at 37°C. The optical density (OD) values were measured with an automatic microplate reader (Bio-Tek, Winooski, VA, USA) at 492 nm. LDH activity was measured using LDH release assay kits, and the OD values were measured with an automatic microplate reader (Bio-Tek) at 450 nm.

Assessment of neuronal apoptosis

SH-SY5Y cells were plated in six-well plates (1×10^6/well) for 48 hours and washed with phosphate-buffered saline (PBS). The cells were resuspended in binding buffer (KeyGen Biotech Co., Ltd., Nanjing, China) and stained with Annexin V-fluorescein isothiocyanate (5 μL) and propidium iodide (5 μL) for 15 minutes or until they became dark. The samples were analyzed using a Beckman Coulter flow cytometer (Beckman Coulter, Fullerton, CA, USA).

Transfected cells were lysed with radioimmunoprecipitation assay (RIPA) buffer at 72 hours after transfection, and the protein concentration was determined using a Pierce BCA protein assay reagent kit (Pierce, Rockford, IL, USA). Caspase-3 and caspase-9 activity was analyzed in protein samples (5 μg) using caspase-3 or caspase-9 activity kits (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China). OD values were measured with an automatic microplate reader (Bio-Tek) at 405 nm.

To evaluate the effect of PI3K or PTEN on the sevoflurane-induced neurotoxicity or neuronal apoptosis mediated by miR-1297, cells were pre-incubated with a PI3K inhibitor (LY294002, 100 nM, 48 hours, Beijing Baiaolaibo Technology Co., Ltd., Beijing, China) or PTEN inhibitor (VO-Ohpic trihydrate, 15 nM, 48 hours, Nanjing Beiyu Biotechnology Co., Ltd., Nanjing, China) before treatment. The inhibitor was diluted in dimethyl sulfoxide (DMSO), and control cells were pre-incubated with equivalent amounts of DMSO alone.

Western blot analysis

Transfected cells were lysed with RIPA buffer at 72 hours after transfection, and the protein concentration was determined using a Pierce BCA protein assay reagent kit (Pierce). Proteins (30 μg) were separated by electrophoresis on 8% to 10% sodium dodecyl sulfate polyacrylamide gels and transferred to a polyvinylidene difluoride membrane. The membrane was blocked with 5% milk in tris-buffered saline with Tween 20 (TBST) at room temperature for 30 minutes and incubated with the appropriate primary antibody as follows: Bax (Santa Cruz Biotechnology, Santa Cruz, CA, USA), PI3K (Santa Cruz Biotechnology), p-Akt (Santa Cruz Biotechnology), p-GSK3β (Santa Cruz Biotechnology), or GAPDH (Santa Cruz Biotechnology) at 4°C overnight. The membranes were

washed with TBST and incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology) for 1 hour at room temperature. The protein blank was detected using enhanced chemiluminescence (ECL Plus™; GE Healthcare, Little Chalfont, UK).

Immunofluorescence

The cells were fixed with 4% wt/vol paraformaldehyde in PBS for 20 minutes and blocked with 0.25% Triton X-100 and 5% bovine serum albumin in PBS for 1 hour at room temperature. The cells were then incubated with a PTEN primary antibody (1:100, Santa Cruz Biotechnology) at 4°C overnight. After washing with PBS, the cells were incubated with Alexa 594-conjugated anti-rabbit immunoglobulin G (Beijing Baizhi Biotechnology Co., Ltd., Beijing, China) and captured with an LSM 780 NLO confocal microscope (Carl Zeiss, Germany).

Statistical analysis

The results are expressed as the mean ± standard deviation. Differences between groups were assessed using analysis of variance with Tukey's post-hoc test or Student's t test. A value of $p < 0.05$ was considered statistically significant.

Results

MiR-1297 expression levels in sevoflurane-exposed mice

To investigate the role of miR-1297 in sevoflurane-exposed mice, changes in miRNA levels were first assessed in normal and sevoflurane-exposed mice. In the present study, a gene chip assay showed that miR-1297 was up-regulated in sevoflurane-exposed mice compared with normal mice (Figure 1a). Furthermore, RT-PCR also revealed that miR-1297 was up-regulated in sevoflurane-exposed mice compared with normal mice (Figure 1b). In the *in vitro* model, incubation of SH-SY5Y cells with sevoflurane for 72 hours reduced cell growth and increased LDH activity, the apoptosis rate, and caspase-3/9 activity compared with the control group (Figure 1c–h).

MiR-1297 led to neuronal apoptosis in the sevoflurane exposure model

On the basis of these results, we investigated the effects of miR-1297 on neuronal apoptosis in the sevoflurane exposure model. As shown in Figure 2a–e, miR-1297 significantly increased miR-1297 expression, LDH activity, and neuronal apoptosis and reduced cell proliferation in the sevoflurane exposure model *in vitro*. As shown in Figure 2f–j, anti-miR-1297 effectively decreased miR-1297 expression in the sevoflurane exposure model *in vitro*, which reduced neuronal apoptosis and LDH activity and promoted cell proliferation of SH-SY5Y cells.

MiRNA-1297 suppressed the Akt/GSK3β signaling pathway

To determine the mechanism of miR-1297 in sevoflurane-induced neurotoxicity, p-Akt and p-GSK3β protein expression levels were measured after transfection. Consequently, western blot analysis demonstrated that up-regulation of miR-1297 suppressed p-Akt and p-GSK3β protein expression, induced Bax protein expression, and increased caspase-3/9 activity in sevoflurane-exposed SH-SY5Y cells compared with the control group (Figure 3). Moreover, down-regulation of miR-1297 induced p-Akt and p-GSK3β protein expression and suppressed Bax protein expression and caspase-3/9 activity in

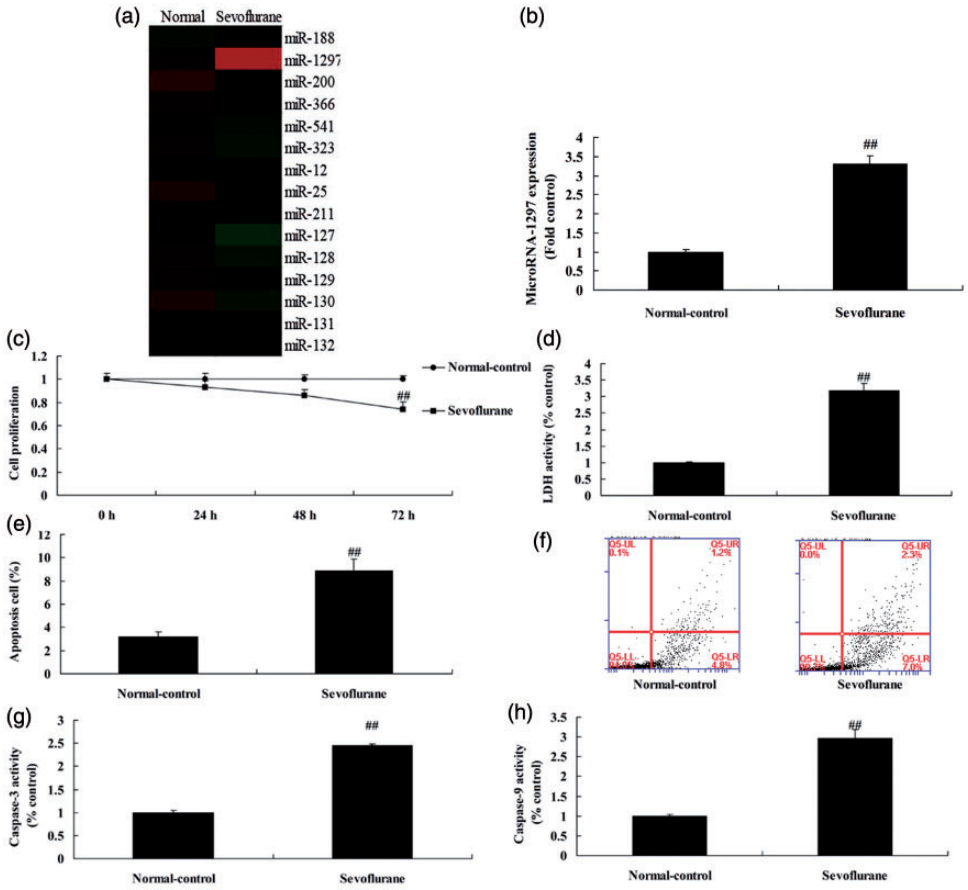

Figure 1. MicroRNA-1297 expression levels in sevoflurane-exposed mice. Heat map of a gene chip including microRNA-1297 (a), microRNA-1297 expression determined by reverse transcription-polymerase chain reaction (b), cell proliferation (c), lactate dehydrogenase (LDH) activity (d), apoptosis rate (e and f), and caspase-3/9 activity levels (g and h).
$^{\#\#}p < 0.01$ compared with control mice or the SH-SY5Y cell group.
Normal, control mouse group; Sevoflurane, sevoflurane-exposed mice or SH-SY5Y cell group; Normal-control, SH-SY5Y cell group.

sevoflurane-exposed SH-SY5Y cells compared with the control group (Figure 4).

MiR-1297 induced PTEN protein expression

As shown in Figure 5a–b, miR-1297 bound to several sites on the 3′-UTR of PTEN. Immunofluorescence showed that up-regulation of miR-1297 decreased PTEN protein expression in sevoflurane-exposed SH-SY5Y cells (Figure 5c). An analysis of protein expression showed that down-regulation of miR-1297 induced PTEN protein expression, while up-regulation of miR-1297 suppressed PTEN protein expression in sevoflurane-exposed SH-SY5Y cells compared with the control group (Figure 5d–g). These results showed that miR-1297 regulates the PTEN/Akt/GSK3β signaling pathway in sevoflurane-exposed SH-SY5Y cells.

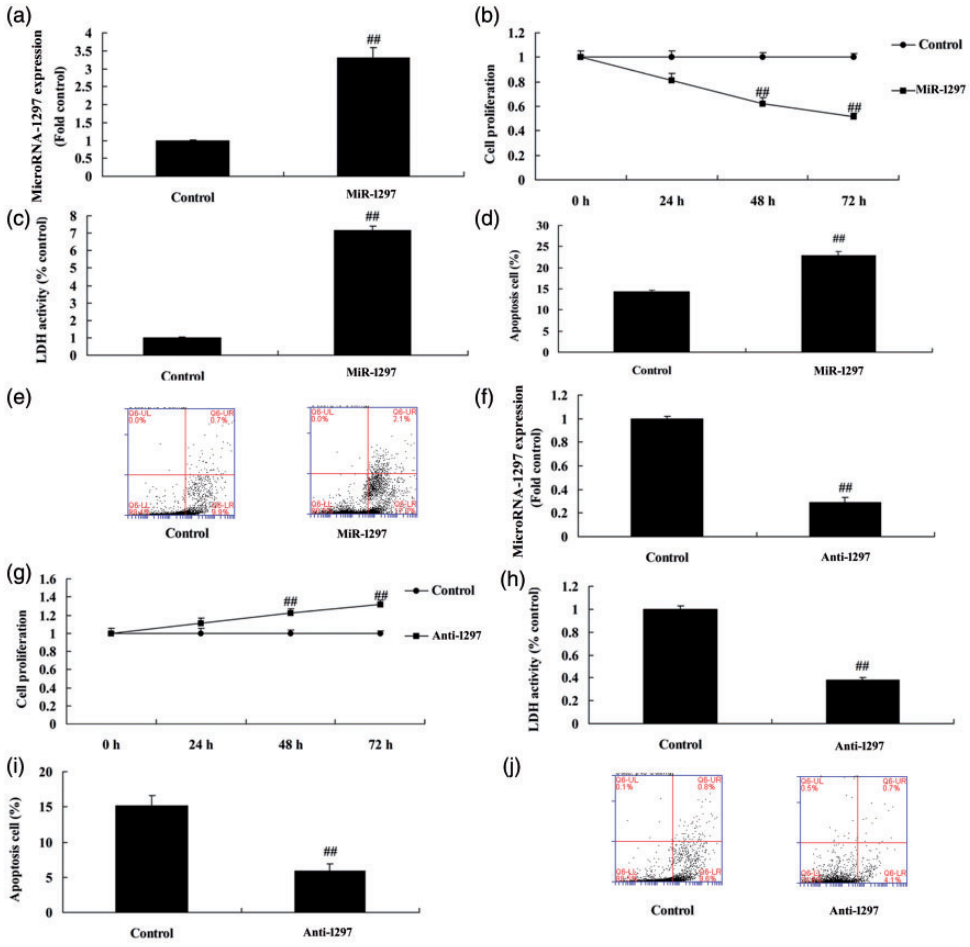

Figure 2. MicroRNA-1297 induces neuronal apoptosis in the sevoflurane exposure model. MicroRNA-1297 expression level (a and f), cell proliferation (b and g), lactate dehydrogenase (LDH) activity (c and h), and cell apoptosis (d and e/i and j).
$^{\#\#}p < 0.01$ compared with the control group.
Control, negative control group; Anti-1297, anti-miR-1297 group; MiR-1297, miR-1297 group.

PI3K inhibition decreased the effects of down-regulation of miR-1297 on neuronal apoptosis through the PI3K/Akt/GSK3β signaling pathway

To explore whether PI3K is involved in the effects of miR-1297 down-regulation on sevoflurane-induced neurotoxicity, a PI3K inhibitor (LY294002, 100 nM, 48 hours) was used to reduce PI3K expression in sevoflurane-exposed SH-SY5Y cells via down-regulation of miR-1297. As shown in Figure 6, PI3K inhibition suppressed PI3K protein expression, reduced p-Akt and p-GSK3β protein expression, and induced Bax protein expression in sevoflurane-exposed SH-SY5Y cells with miR-1297 down-regulation compared with

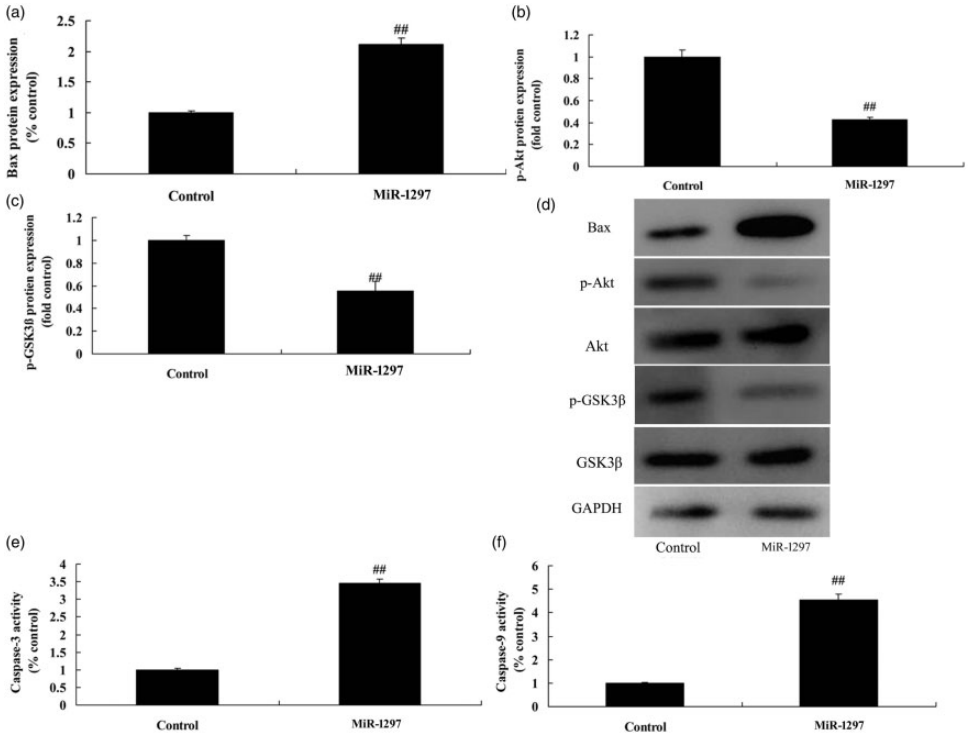

Figure 3. MicroRNA-1297 exposure leads to suppression of the Akt/GSK3β signaling pathway. Bax, p-Akt, and p-GSK3β protein expression levels (a, b, and c), western blotting analysis (d), and caspase-3/9 activity (e and f). ##$p < 0.01$ compared with the control group.
Control, negative control group; MiR-1297, miR-1297 group.

the miR-1297 down-regulation group. However, miR-1297 down-regulation did not affect PI3K protein expression in sevoflurane-exposed SH-SY5Y cells (Figure 6a, 6e). PI3K inhibition significantly decreased the effects of miR-1297 down-regulation on the inhibition of neuronal apoptosis and LDH activity and the promotion of cell proliferation in sevoflurane-exposed SH-SY5Y cells compared with the miR-1297 down-regulation group (Figure 7a–d). As shown in Figure 7e–f, PI3K inhibition decreased the effects of miR-1297 down-regulation on suppression of caspase-3/9 activity levels in the *in vitro* sevoflurane exposure model compared with the miR-1297 down-regulation group.

PTEN inhibition decreased the effects of miR-1297 up-regulation on neuronal apoptosis through the Akt/GSK3β *signaling pathway*

We further investigated the function of PTEN in the effects of down-regulation of miR-1297 on sevoflurane-induced neuronal apoptosis. Administration of a PTEN inhibitor, VO-Ohpic trihydrate (15 nM), for 48 hours significantly suppressed PTEN and Bax protein expression and induced p-Akt and p-GSK3β protein expression in the *in vitro* sevoflurane exposure model following miR-1297 up-regulation compared with the miR-1297 up-regulation group (Figure 8). PTEN inhibition significantly suppressed

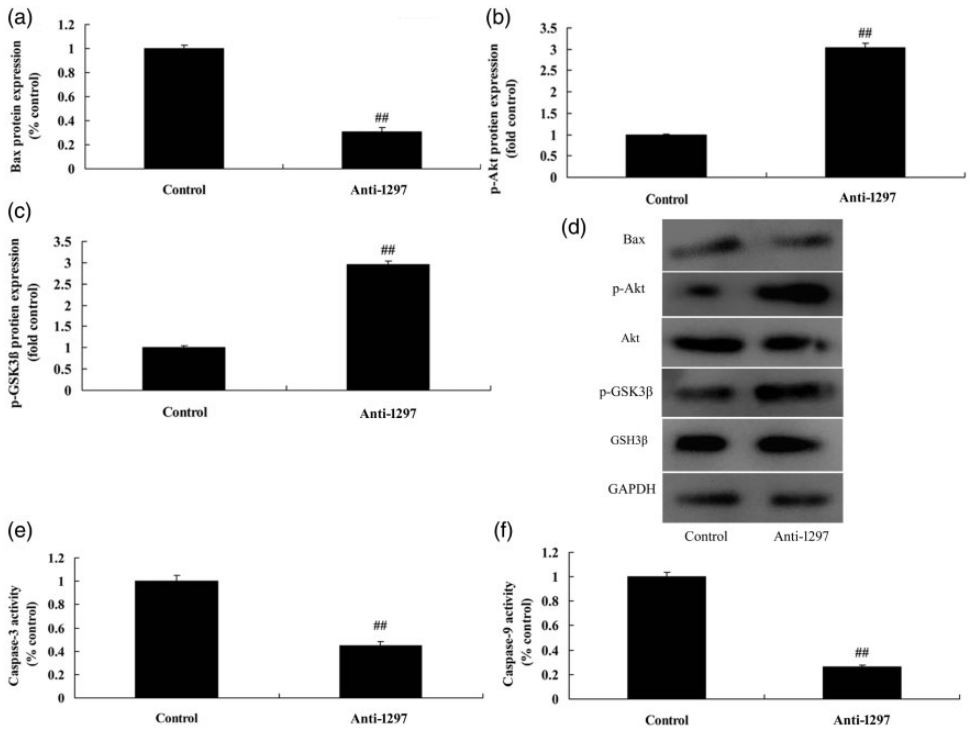

Figure 4. Anti-microRNA-1297 exposure leads to suppression of the Akt/GSK3β signaling pathway. Bax, p-Akt, and p-GSK3β protein expression levels (a, b, and c), western blotting analysis (d), and caspase-3/9 activity (e and f).
$^{\#\#}p < 0.01$ compared with the control group.
Control, negative control group; Anti-1297, anti-miR-1297 group.

caspase-3/9 activity levels, increased cell proliferation, and reduced neuronal apoptosis and LDH activity in the *in vitro* sevoflurane exposure model following miR-1297 up-regulation compared with the miR-1297 up-regulation group (Figure 9).

Discussion

Sevoflurane is a classical inhalation anesthetic that has been extensively applied for clinical anesthesia in recent years.[21] Its extensive use can be attributed to its superior characteristics such as its rapid effect, the ability of patients to easily recover, and the ease of controlling the anesthesia depth.[22] Furthermore, sevoflurane can be applied to patients in all age groups, such as the elderly, middle-aged, and young people as well as children. In particular, sevoflurane has significant advantages in general anesthesia induction and maintenance in children.[23] Therefore, children are an important group for sevoflurane application. MiRNA and transcription factors are two major trans-acting factors. They are closely related to each other in the regulatory network and can constitute feedback loops and pro-feedback loops to regulate gene expression. Furthermore, they play important roles in multiple biological processes in cells (cell differentiation) and diseases such as neurodegenerative diseases.[24] In this study, 3% sevoflurane was

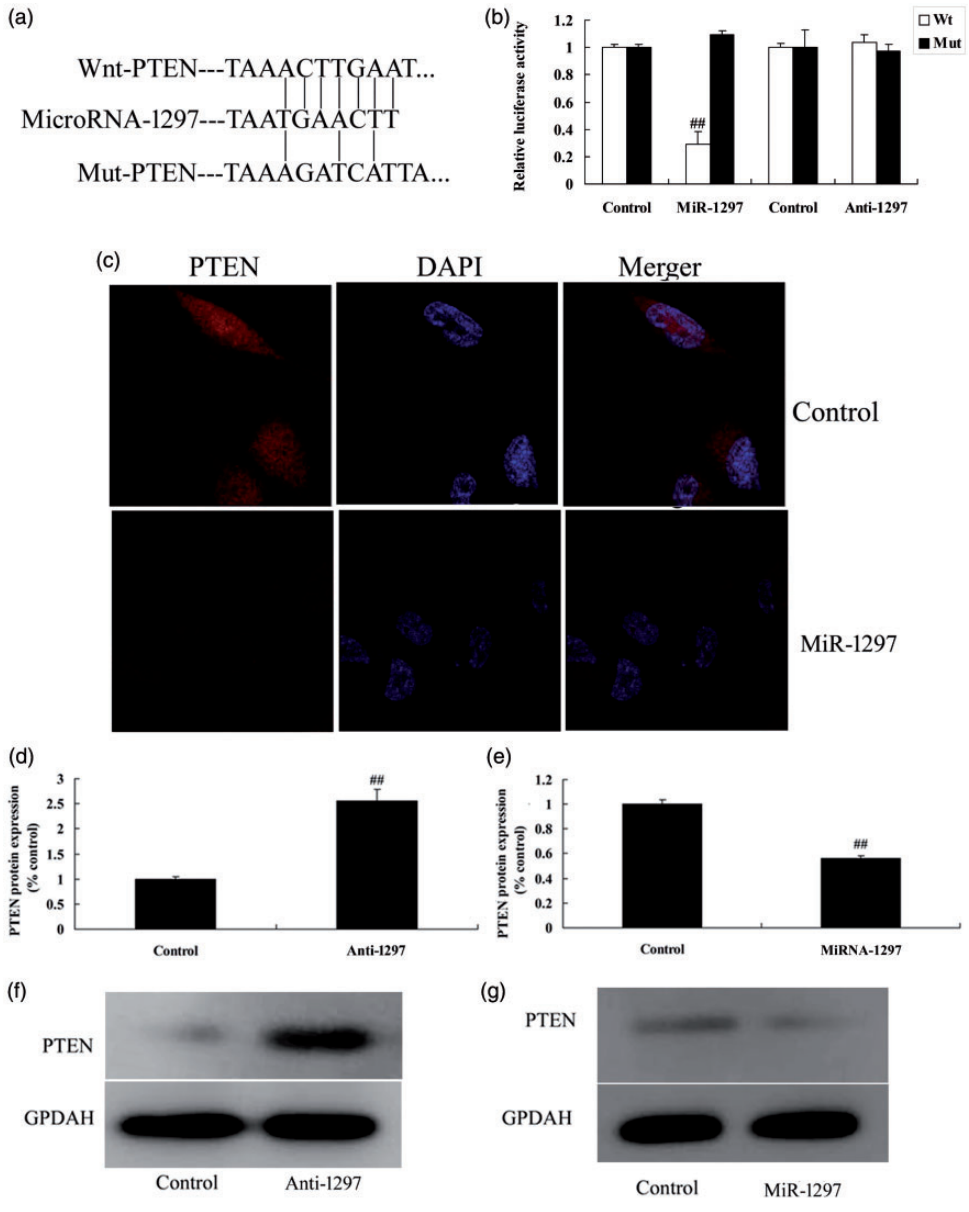

Figure 5. MicroRNA-1297-induced PTEN protein expression. Binding sites of microRNA-1297 on the 3′-untranslated region of PTEN (a), the relative luciferase activity (b), immunofluorescence showing PTEN protein expression (c), PTEN protein expression levels (d and e), and western blotting analysis (f and g)
$^{##}p < 0.01$ compared with the control group.
Control, negative control group; Anti-1297, anti-miR-1297 group; MiR-1297, miR-1297 group.

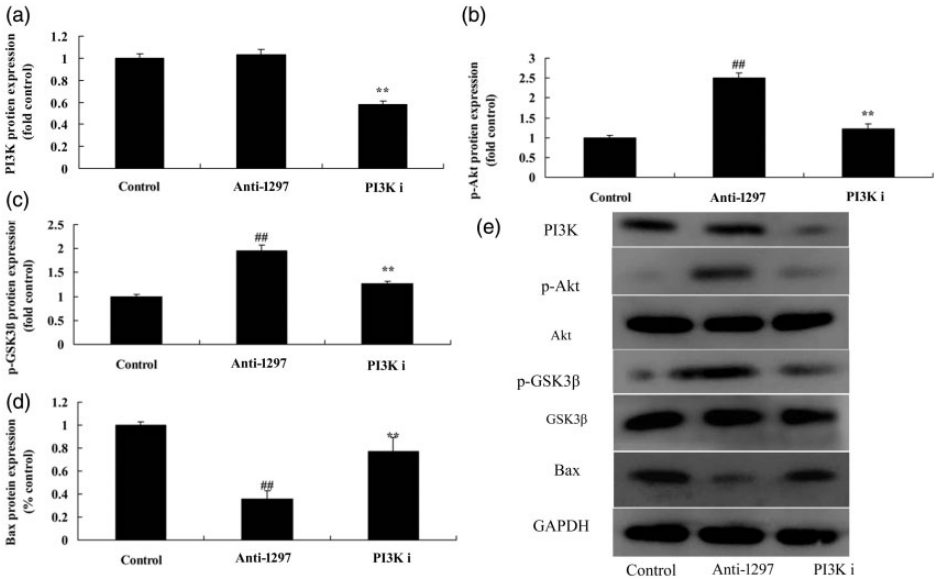

Figure 6. PI3K inhibition decreased the effects of down-regulation of microRNA-1297 on the PI3K/Akt/GSK3β signaling pathway. PI3K, p-Akt, p-GSK3β, and Bax protein expression levels (a, b, c, and d) and western blotting analysis (e).
$^{\#\#}p < 0.01$ compared with the control group, $^{**}p < 0.01$ compared with the miR-1297 group.
Control, negative control group; MiR-1297, miR-1297 group; PI3K i, miR-1297 and PI3K inhibitor group.

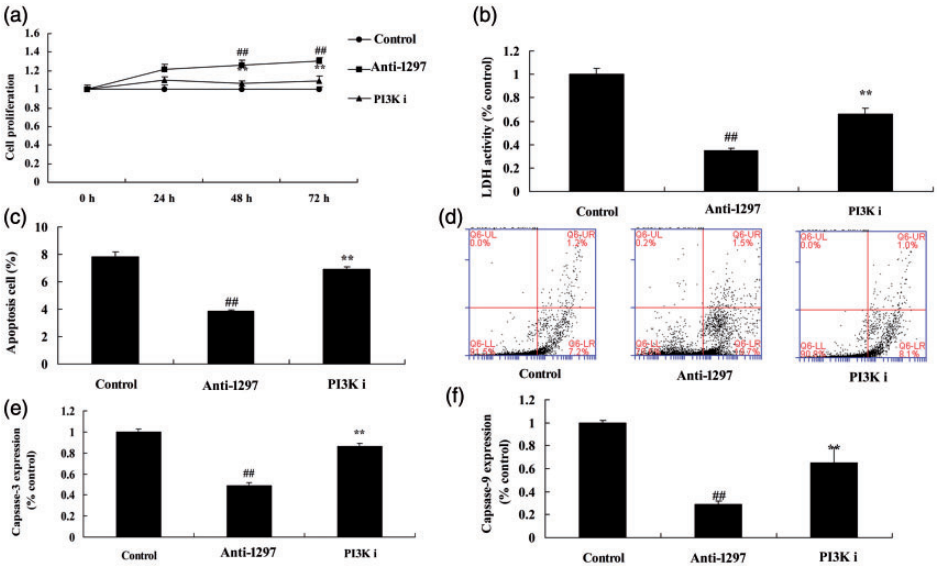

Figure 7. PI3K inhibition decreased the effects of down-regulation of microRNA-1297 on neuronal apoptosis via the PI3K/Akt/GSK3β signaling pathway. Cell proliferation (a), lactate dehydrogenase (LDH) activity (b), cell apoptosis (c and d), and caspase-3/9 activity (e and f).
$^{\#\#}p < 0.01$ compared with the control group, $^{**}p < 0.01$ compared with the miR-1297 group.
Control, negative control group; Anti-1297, anti-miR-1297 group; PI3K i, miR-1297 and PI3K inhibitor group.

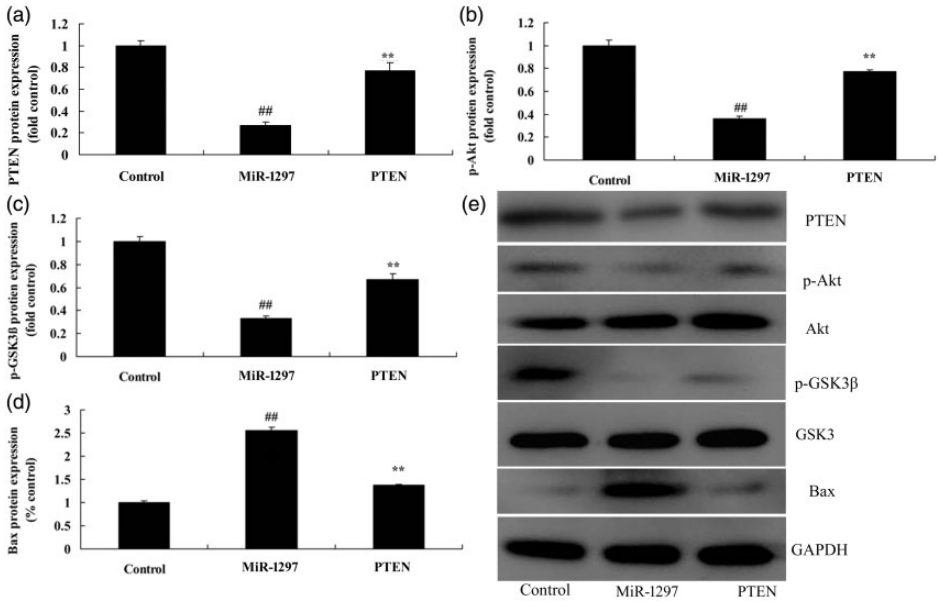

Figure 8. PTEN inhibition decreased the effects of up-regulation of microRNA-1297 on the PTEN/Akt/GSK3β signaling pathway. PTEN, p-Akt, p-GSK3β, and Bax protein expression levels (a, b, c, and d) and western blotting analysis (e).
##$p < 0.01$ compared with the control group, **$p < 0.01$ compared with the miR-1297 group.
Control, negative control group; MiR-1297, miR-1297 group; PETN group, anti-miR-1297 and PETN inhibitor group.

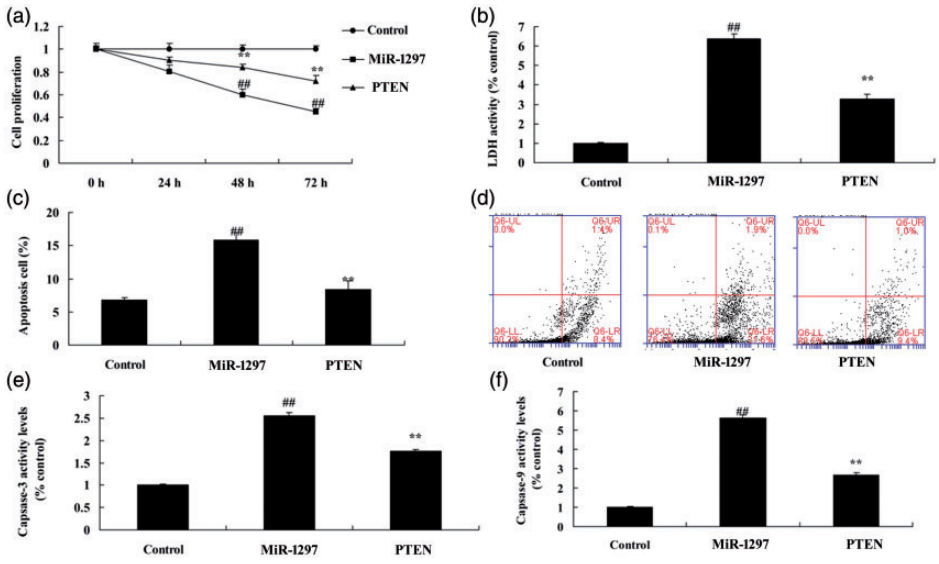

Figure 9. PTEN inhibition decreased the effects of up-regulation of microRNA-1297 on neuronal apoptosis through the PTEN/Akt/GSK3β signaling pathway. Cell proliferation (a), lactate dehydrogenase (LDH) activity (b), cell apoptosis (c and d), and caspase-3/9 activity (e and f).
##$p < 0.01$ compared with the control group, **$p < 0.01$ compared with the MiR-1297 group.
Control, negative control group; MiR-1297, anti-miR-1297 group; PETN group, anti-miR-1297 and PETN inhibitor group.

used to induce neural apoptosis, according to the findings of Lu et al.[19] The present study demonstrated that miRNA-1297 was up-regulated in sevoflurane-exposed mice compared with normal mice. Upregulation of miRNA-1297 leads to neuronal apoptosis, inhibition of cell proliferation, and increased LDH activity by sevoflurane *in vitro*. Wang et al.[17] showed that miR-1297 regulates glioma cell growth *in vivo* and *in vitro*. In this study, we only examined the molecular effects in a cell model. In further studies, we will analyze other models, such as a rat model and other cell lines.

The PI3K/Akt signaling pathway is an important intracellular signal transduction pathway that is closely related to vital cellular activities.[25] It can mediate numerous external stimulus signals, induce cell growth, differentiation, and apoptosis, and play a vital role against sevoflurane toxicity.[25] Activation of this pathway can up-regulate p-Akt expression, which can influence the anti-apoptotic mechanism of myocardial cells through a variety of pathways and reduce cell apoptosis.[26] PI3K has many downstream effector molecules and proteases.[26] Of them, Akt and GSK3β, the major downstream targets of PI3K, can transmit important information.[27] As previously confirmed, the PI3K/Akt signaling pathway participates in the protective effects mediated by sevoflurane pretreatment or post-treatment.[27] GSK3β is a multi-function kinase consisting of more than 40 subunits.[27] It not only regulates carbohydrate metabolism, but also plays an important role in cell proliferation, growth, and death.[27]

The PI3K/Akt signaling pathway is closely associated with cell activity, inflammatory responses, and apoptosis.[28] In the case of sevoflurane toxicity, the PI3K/Akt signaling pathway is activated.[28] Subsequently, p-Akt can activate the anti-apoptotic protein BCL-2, improve mitochondrial energy synthesis, and maintain the stability of the outer mitochondrial membrane.[28] Furthermore, it can also reduce activation of the pro-apoptotic protein Bax and exert its anti-apoptotic effect.[26] Akt activated by PI3K can further activate or inhibit its downstream target protein through phosphorylation.[29] The PI3K/Akt-mediated signaling pathway exhibits extensive biological effects and can promote growth and proliferation.[30] Moreover, it can regulate cell survival, endothelial growth, and angiogenesis.[30] Research has shown that activating the PI3K/Akt pathway will increase its downstream GSK3β phosphorylation, inhibit mitochondrial permeability transition pore opening, and reduce neural apoptosis.[30] The present study revealed that up-regulation of miRNA-1297 suppressed the Akt/GSK3β signaling pathway via PTEN activation. A PTEN inhibitor was used to inhibit the Akt/GSK3β signaling pathway and decrease the toxicity of sevoflurane with regard to neuronal apoptosis via up-regulation of miRNA-1297 in an *in vitro* model. Bu et al.[18] showed that miRNA-1297 promotes cell proliferation of non-small cell lung cancer cells through the PTEN/Akt/Skp2 signaling pathway. However, we only examined a PTEN inhibitor in this study. We intend to use additional methods to analyze the mechanism of miR-1297 in further studies.

As a type of protein kinase, GSK3β is the rate-limiting enzyme and can promote inactivation of glycogen synthase during glycogen synthesis.[31] Thus, it can increase the blood glucose level. GSK3β is highly expressed in the brain.[31] Recent studies have shown that GSK3β can regulate cyclin, multiple transcription factors, Alzheimer's disease-associated proteins, and cytoskeletal proteins.[31] Furthermore, it plays an important role in neuronal development and neuron signaling pathways.[31] GSK3β is regulated by three signaling pathways, namely, Wnt, MAPK, and PI3K.[32]

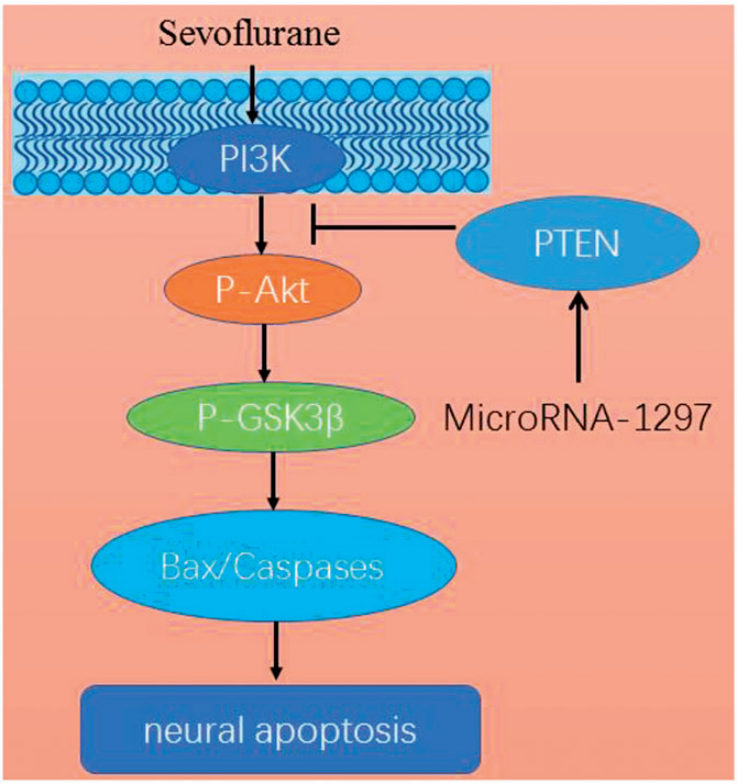

Figure 10. MicroRNA-1297 suppresses the Akt/GSK3β signaling pathway to stimulate neural apoptosis in the *in vivo* and *in vitro* sevoflurane exposure models by targeting the PTEN protein.

Insulin and nerve growth factor can activate the PI3K/Akt pathway, while p-Akt can further phosphorylate the Ser9 locus of GSK3β, thus inhibiting GSK3β activity.[32] Sevoflurane can reduce p-Akt expression through the BDNF neural apoptosis pathway.[27] Additionally, sevoflurane can activate the endogenous mitochondrial apoptosis pathway, thus inducing neuronal apoptosis.[27]

The PI3K-Akt-GSK3β pathway plays an important role in the development of neuron signaling pathways.[33] Current research has shown that GSK3β can control several cytoskeletal proteins that influence cell structure characteristics and changes in cell dynamics through phosphorylation.[33] For instance, the phosphorylated microtubule-associated proteins (MAP) MAP1B and MAP2 can regulate neuron structure and plasticity.[34] Meanwhile, the phosphorylated tau protein participates in the pathogenesis of Alzheimer's disease. The collapsin response mediator protein 2 family and APC can inhibit axon growth.[35] Thus, these proteins play an important regulatory role in neuron development and function.[31] In addition, GSK3β can also regulate multiple transcription factors associated with cell growth, differentiation, proliferation, and apoptosis.[36] Our study showed that down-regulation of miRNA-1297 induced the Akt/GSK3β signaling pathway via inactivation of PTEN.

In conclusion, we demonstrated that miRNA-1297 was up-regulated in

sevoflurane-exposed mice. MiRNA-1297 suppressed the Akt/GSK3β signaling pathway to stimulate neural apoptosis in *in vivo* and *in vitro* sevoflurane exposure models by targeting the PTEN protein (Figure 10). Thus, miRNA-1297 represents a key regulatory factor, diagnostic marker, and therapeutic target for sevoflurane-induced neurotoxicity.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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