

# Propofol attenuates mast cell degranulation via inhibiting the miR-221/PI3K/Akt/Ca<sup>2+</sup> pathway

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**Abstract.** The aim of the present study was to investigate the effect of propofol on immunoglobulin (Ig)E-activated mast cell degranulation and explore the underlying mechanisms responsible. RBL-2H3 cells were treated with propofol for at a variety of concentrations and different amounts of time. Cell viability was assessed using an MTT assay and microRNA (miR)-221 expression was quantified using reverse transcription-quantitative polymerase chain reaction. RBL-2H3 cells were transfected with miR-221 mimic or a negative control and degranulation, including the release of  $\beta$ -hexosaminidase and histamine, was evaluated using an ELISA kit. The effect of miR-221 overexpression on the phosphorylation of protein kinase B (Akt) was detected using western blotting and extracellular Ca<sup>2+</sup> influx was measured via a fura-2 assay. The phosphoinositide 3-kinase (PI3K) inhibitor LY294002 was used to investigate the association between PI3K/Akt signaling and Ca<sup>2+</sup> influx in the presence of propofol. The results demonstrated that propofol treatment suppressed RBL-2H3 cell proliferation in a dose- and time-dependent manner. Propofol inhibited miR-221 expression in a dose-dependent manner compared with the control group; however, the inhibitive effect was significantly abrogated following transfection with miR-221 mimics. Furthermore,  $\beta$ -hexosaminidase and histamine release, PI3K/Akt signaling and Ca<sup>2+</sup> influx were decreased following propofol application.

miR-221 overexpression markedly ameliorated the suppressive effect of propofol. Treatment with LY294002 reversed the propofol-induced decrement of Ca<sup>2+</sup> influx on IgE-mediated RBL-2H3 cells, suggesting an association between PI3K/Akt signaling and Ca<sup>2+</sup> influx. In conclusion, the results of the present study suggest that propofol treatment attenuates mast cell degranulation via inhibiting the miR-221/PI3K/Akt/Ca<sup>2+</sup> pathway. These results indicate that propofol may have a potential therapeutic effect as a treatment for allergic diseases.

## Introduction

Mast cells are widely distributed in sites around the body, including the skin, airways, gastrointestinal tract and mucosa, and are able to quickly respond to internal and external stimuli (1). Mast cells secrete a number of bioactive mediators, including proteases, cytokines, chemokines,  $\beta$ -hexosaminidase and histamine, which are associated with the regulation of innate and acquired immune responses (2). Mast cells serve a crucial role in allergic disorders, including asthma, atopic dermatitis and hypersensitivity (3), and are associated with the allergy-induced inflammatory responses (4). Mast cells present the high-affinity immunoglobulin E (IgE) receptor (Fc $\epsilon$ RI) on their surface, which is able to bind IgE and induce mast cell degranulation following repeated-allergen stimulation (5). During the degranulation process, mast cell-induced mediators are controlled by Fc $\epsilon$ RI-dependent signaling pathways, including phosphoinositide 3-kinase (PI3K) family members (6). However, the underlying mechanisms responsible for mast cell degranulation are complex and remain to be fully elucidated.

Propofol is a widely used intravenous anesthetic agent with rapid onset, short duration of action and rapid elimination (7). A number of pharmacological characteristics of propofol have been reported, including antipruritic, anti-convulsant, anti-oxidant and anti-inflammatory activities (8). Furthermore, propofol has been demonstrated to inhibit adhesion, proliferation, invasion and growth in various cancers, including ovarian cancer, hepatocellular carcinoma, pancreatic cancer, glioma and lung cancer (9-13). The anti-cancer effect of propofol is typically associated with an upregulation or downregulation

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of microRNA (miR or miRNA) (9,11,13). A previous study indicated that propofol treatment attenuated ischemia reperfusion injury in the small intestine via inhibiting oxidative stress and mast cell degranulation (14), suggesting that propofol may function by modulating mast cell activation. However, the underlying molecular mechanisms remain unclear.

The aim of the present study was to investigate the effect of propofol on IgE-activated mast cell degranulation and the underlying mechanisms. The results indicate that propofol acts via the miR-221/PI3K/protein kinase B (Akt)/Ca<sup>2+</sup> pathway to suppress mast cell activation, which suggests a possible target for treating allergic diseases via inhibiting IgE-mediated mast cell degranulation.

## Materials and methods

**Reagents.** Propofol, LY294002, dimethyl sulfoxide (DMSO), MTT, anti-2,4-dinitrophenyl (DNP) IgE and DNP-human serum albumin (HSA) were purchased from Sigma Aldrich (Merck KGaA, Darmstadt, Germany). Dulbecco's modified eagle medium (DMEM), TRIzol, Lipofectamine® 2000 and fura-2/AM were obtained from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Fetal bovine serum (FBS), penicillin and streptomycin were obtained from Gibco (Thermo Fisher Scientific, Inc.). The TaqMan MicroRNA Reverse Transcription kit and TaqMan MicroRNA Assay kit were acquired from Applied Biosystems (Thermo Fisher Scientific, Inc.). ELISA kits ( $\beta$ -hexosaminidase cat. no. KT-17547; histamine cat. no. KT-60094) were purchased from Kamiya Biomedical Co. (Tukwila, WA, USA). Lysis buffer was obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA) and the BCA Protein Assay kit was acquired from Pierce (Thermo Fisher Scientific, Inc.). Rabbit anti-rat antibodies against Akt (cat. no. SC-8312), phosphorylated (p)-Akt (s473) (cat. no. SC-33437), GAPDH (cat. no. SC-25778) and horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (cat. no. SC-2030) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Unless otherwise stated, other reagents were purchased from Sigma Aldrich (Merck KGaA).

**RBL-2H3 cell culture and propofol treatment.** Rat basophilic leukemia RBL-2H3 cells were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in DMEM containing with 10% heat-inactivated FBS, 100 IU/ml penicillin and 100  $\mu$ g/ml streptomycin at 37°C in a humidified incubator containing 5% CO<sub>2</sub>. Propofol was diluted with DMSO to obtain indicated concentrations (5, 10, 15 or 20  $\mu$ g/ml) and was added to the cells for the indicated time (12, 24, 36 or 48 h).

The original RBL-2H3 cells were cultured in 12-well plates at a density of 1x10<sup>6</sup> cells/well. After 24 h following propofol treatment, 2x10<sup>5</sup> RBL-2H3 cells from each treatment group were collected for further analysis. Cells without propofol treatment or IgE stimulation were used as the control (Con) group.

**MTT assay.** Cell viability was analyzed using an MTT assay. RBL-2H3 cells were seeded in 96-well plates and treated with propofol as described. A total of 5 mg/ml MTT was added to the plates and incubated at 37°C for 4 h. DMSO was added

to dissolve the formazan crystals. Optical density (OD) was measured at 590 nm using a microplate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The inhibitory rate of cell proliferation (%) was measured using following formula: (OD of control group-OD of test group) x100/OD of control group (Table I).

**Activation of mast cell degranulation.** RBL-2H3 cells were sensitized with 0.5  $\mu$ g/ml anti-DNP IgE overnight at 37°C to induce IgE-mediated allergic responses. Cells were subsequently stimulated with 100 ng/ml DNP-HSA at 37°C for 4 h to activate the degranulation of mast cells.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was extracted using TRIzol and RT was performed with a TaqMan MicroRNA Reverse Transcription kit according to the manufacturers' protocol (16°C for 30 min, 42°C for 30 min and 85°C for 5 min). To detect miR-221 expression, qPCR was performed using the 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Inc.) with TaqMan MicroRNA Assay kit. The thermocycling conditions were as follows: 95°C for 2 min followed by 40 cycles of 95°C for 15 sec, 55°C for 30 sec, and 60°C for 1 min. The following primers were purchased from Shanghai GenePharma Co., Ltd., (Shanghai, China): miR-221, forward 5'-CCCAGCATT TCTGACTGTTG-3' and reverse 5'-TGTGAGACCATTG GTGAA-3'; U6, forward 5'-CAAGGATGACACGCAAAT-3' and reverse 5'-TGGTGTCTGGAGTCG-3'. The expression of U6 was used as endogenous control for miRNAs analysis. Data were analyzed by the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (15).

**Transfection.** RBL-2H3 cells were transfected with miR-221 mimic (5'-AGCUACAUGUCUGCUGGGUUUC-3') and its negative control (5'-UCACAACCUCCUAGAAAGAGU AGA-3'; both from Shanghai GenePharma Co., Ltd.) using Lipofectamine® 2000 reagent according to the manufacturer's protocol. The final concentration of oligonucleotides was 50 nM. Cells were subjected to RNA/protein extraction or further experiments at 24 h following transfection.

**Detection of  $\beta$ -hexosaminidase release.** Following the activation of mast cell degranulation, the cells were separated by centrifugation at 150 x g for 5 min at 4°C and then the supernatants were incubated in citrate buffer with 1 mM 4-nitrophenyl N-acetyl- $\beta$ -D-glucosaminide at 37°C for 1 h. Cell pellets were lysed with Tyrode's buffer containing 1% Triton X-100 and the reaction was stopped by adding 150  $\mu$ l stop solution (0.1 M Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub>; pH=10). Absorbance was measured at 405 nm using the microplate reader (ELX808; BioTek Instruments, Inc., Winooski, VT, USA).

**Analysis of histamine release.** The levels of histamine in the supernatants of RBL-2H3 cells were measured using the ELISA kit according to the manufacturer's protocol.

**Western blotting.** RBL-2H3 cells were collected and lysed using lysis buffer containing protease and phosphatase inhibitors on ice. Following centrifugation at 400 x g for 5 min at 4°C, the supernatants were collected. The protein concentrations in the supernatants were measured using a BCA Protein Assay

Table I. Inhibitory rate of propofol on the proliferation of RBL-2H3 cells.

Propofol ( $\mu$ g/ml)	Optical density at 570 nm	Inhibitory rate (%)
0	0.943 $\pm$ 0.006	0
1	0.898 $\pm$ 0.012	4.416 $\pm$ 0.363
5	0.713 $\pm$ 0.014	23.038 $\pm$ 0.798
10	0.585 $\pm$ 0.018	36.846 $\pm$ 1.093
15	0.463 $\pm$ 0.015	49.675 $\pm$ 1.261
20	0.212 $\pm$ 0.011	76.035 $\pm$ 0.782

Optical density is presented as the mean  $\pm$  standard deviation.

kit. Proteins (20  $\mu$ g) were separated by electrophoresis with 10% SDS-PAGE gel and transferred onto polyvinylidene fluoride membranes. Membranes were blocked with 5% skim milk at 37°C for 2 h and incubated with the primary rabbit anti-rat antibodies against Akt, p-Akt (s473) and GAPDH overnight at 4°C. Membranes were then incubated with HRP-conjugated goat anti-rabbit secondary antibodies. Bands were visualized using enhanced chemiluminescence detection kit (ECL; GE Healthcare, Chicago, IL, USA) and scanned for analysis (Scanjet 7400C; Hewlett-Packard Co., Palo Alto, CA, USA).

**Cytosolic calcium ( $Ca^{2+}$ ) measurement.** The supernatants of RBL-2H3 cells were collected and incubated in Tyrode's buffer supplemented with fura-2/AM (2  $\mu$ M final concentration) at 37°C for 30 min. Absorbance was measured at 340 and 380 nm using a microplate fluorometer (Berthold Technologies GmbH & Co., Bad Wildbad, Germany) and the intracellular  $Ca^{2+}$  was shown as the 340/380 ratio.

**Statistical analysis.** Results are presented as the mean  $\pm$  standard deviation. All statistical analysis was performed using SPSS software (version 13.0; SPSS, Inc., Chicago, IL, USA). For multiple comparisons of quantitative data, one-way analysis of variance was used followed by Bonferroni's post hoc test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Propofol suppresses the proliferation of RBL-2H3 cells.** Following treatment with various concentrations of propofol, the proliferation of RBL-2H3 cells was significantly suppressed in a dose-dependent manner compared with the control (Fig. 1A). As the  $IC_{50}$  was 13.380  $\mu$ g/ml (Table I), 10  $\mu$ g/ml of propofol was selected as the highest concentration in further experiments. Propofol treatment also resulted in a time-dependent reduction in cell viability compared with the control (Fig. 1B). These results suggest that propofol inhibits RBL-2H3 cell proliferation in a dose- and time-dependent manner. Treatment duration of 24 h was selected for subsequent experiments.

**Propofol inhibits miR-221 expression in RBL-2H3 cells.** Treatment with 10  $\mu$ g/ml propofol significantly reduced

miR-221 expression in IgE-activated RBL-2H3 cells (Fig. 2A). The results indicate that propofol inhibits miR-221 expression in a dose-dependent manner. Because propofol was demonstrated to inhibit the proliferation of RBL-2H3 cells, miR-221 expression was also detected in the same number of RBL-2H3 cells obtained from a different group (Fig. 2B). The results suggest that miR-221 downregulation was independent of the decrease in RBL-2H3 cells following propofol application.

**miR-221 has no significant effect on RBL-2H3 cell proliferation.** To investigate whether miR-221 is associated with the action of propofol in RBL-2H3 cells, an miR-221 overexpression model was constructed via transfection with miR-221 mimics. miR-221 notably abrogated the inhibitive effect of propofol in IgE-activated RBL-2H3 cells (Fig. 3A). However, no significant difference was observed compared with the NC group. Furthermore, following transfection with miR-221, cell viability was markedly reduced compared with the control. No statistical differences were observed in the presence of propofol, suggesting that miR-221 has no effect on RBL-2H3 cell proliferation (Fig. 3B).

**miR-221 and the effect of propofol on mast cell degranulation.**  $\beta$ -hexosaminidase and histamine release are typically used as indicators of mast cell granulation (16). In the present study,  $\beta$ -hexosaminidase and histamine release were significantly increased in the miR-221 overexpression group compared with the IgE stimulated group (Fig. 4). Following propofol treatment,  $\beta$ -hexosaminidase release was obviously decreased. However, miR-221 overexpression markedly restored the suppressive effect of propofol on  $\beta$ -hexosaminidase in IgE-stimulated mast cell degranulation (Fig. 4A). Similar results were observed for histamine (Fig. 4B).

**miR-221-induced PI3K/Akt signaling is associated with the suppressive effect of propofol.** Compared with the control group, Akt phosphorylation was significantly increased in IgE-activated RBL-2H3 cells. However, treatment with propofol significantly reduced Akt phosphorylation compared with cells treated with IgE alone (Fig. 5). miR-221 overexpression significantly reversed the suppressive effect of propofol on PI3K/Akt signaling, suggesting that miR-221 inhibits propofol via targeting its mechanistic pathway.

**Decreased  $Ca^{2+}$  influx regulates the effects of propofol in mast cell degranulation.** As  $Ca^{2+}$  is associated with mast cell degranulation, fura-2 induced changes in  $Ca^{2+}$  were measured.  $Ca^{2+}$  influx was significantly upregulated in IgE-activated RBL-2H3 cells. However, propofol treatment significantly inhibited  $Ca^{2+}$  influx (Fig. 6A). Furthermore, miR-221 treatment was able to reverse the suppressive effect of propofol (Fig. 6B), suggesting that  $Ca^{2+}$  participates in the regulation of propofol in mast cell degranulation.

**Propofol attenuates mast cell degranulation via inhibiting the miR-221/PI3K/Akt/ $Ca^{2+}$  pathway.** The association between PI3K/Akt signaling and  $Ca^{2+}$  influx was investigated in propofol-treated IgE-mediated mast cell activation (Fig. 7). Treatment with propofol reduced  $Ca^{2+}$  influx in activated RBL-2H3 cells compared with the control cells. LY294002, a

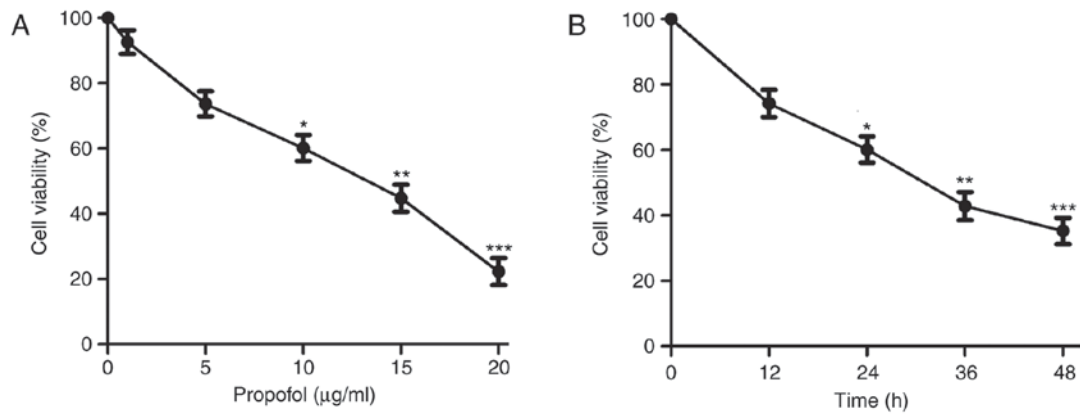


Figure 1. Effect of propofol on RBL-2H3 cell proliferation. (A) RBL-2H3 cells were treated with 0, 5, 10, 15 or 20  $\mu\text{g/ml}$  propofol for 24 h or (B) 10  $\mu\text{g/ml}$  propofol for 0, 12, 24, 36 or 48 h. Cell viability was determined using an MTT assay. \* $P<0.05$ , \*\* $P<0.01$  and \*\*\* $P<0.001$  vs. control.

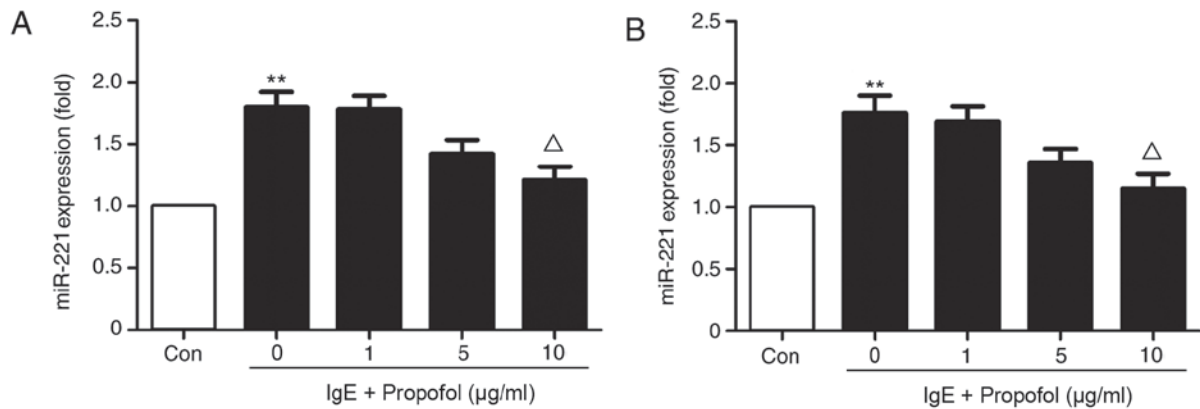


Figure 2. Effect of propofol on miR-221 expression in RBL-2H3 cells. RBL-2H3 cells were treated with 1, 5 or 10  $\mu\text{g/ml}$  propofol for 24 h. Cells were subsequently (A) sensitized with 0.5  $\mu\text{g/ml}$  anti-DNP IgE and stimulated with 100 ng/ml DNP-HSA, or (B) stimulated with anti-DNP IgE and DNP-HAS. The same number of cells were used from each group ( $2 \times 10^5$ ). miR-221 expression was assessed using reverse transcription-quantitative polymerase chain reaction. \*\* $P<0.01$  vs. Con  $\Delta$ P<0.01 vs. IgE-stimulated group without propofol treatment. miR, microRNA; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin; Con, control.

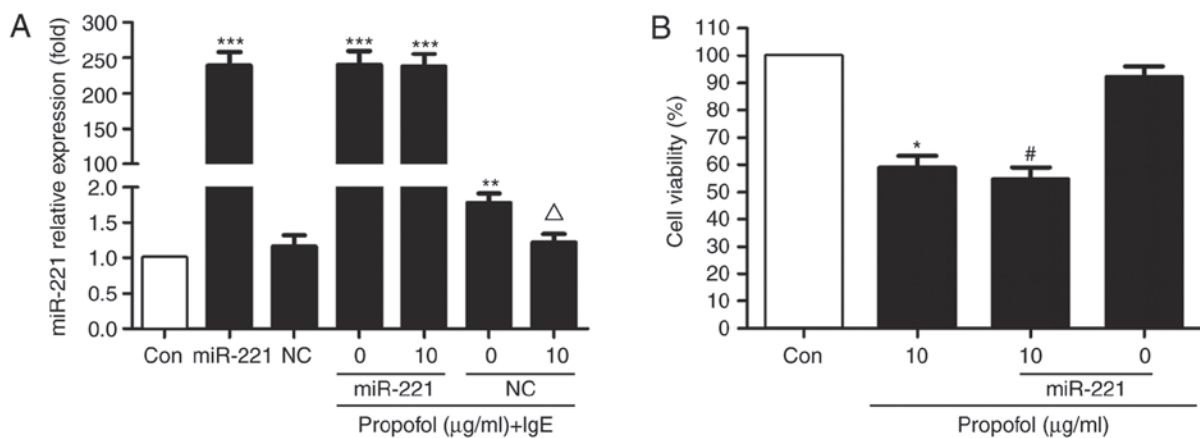


Figure 3. miR-221 overexpression in RBL-2H3 cells. RBL-2H3 cells were transfected with miR-221 mimic or NC miRNA for 24 h followed by treatment with 0 or 10  $\mu\text{g/ml}$  propofol. (A) Cells were activated with anti-DNP IgE and DNP-HSA and miRNA-221 expression was measured using reverse transcription-quantitative polymerase chain reaction. (B) Following propofol treatment, cell proliferation was measured using an MTT assay. \*\*\* $P<0.001$  vs. Con. \* $P<0.05$  vs. miR-221 + 0  $\mu\text{g/ml}$  propofol.  $\Delta$ P<0.05 vs. IgE-stimulated NC group + 0  $\mu\text{g/ml}$  propofol. miR and miRNA, microRNA; NC, negative control; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin.

specific PI3K inhibitor, reversed the propofol-induced reduction in  $\text{Ca}^{2+}$  influx, suggesting that propofol attenuates mast

cell degranulation via inhibiting the miR-221/PI3K/Akt/ $\text{Ca}^{2+}$  pathway (Fig. 7).



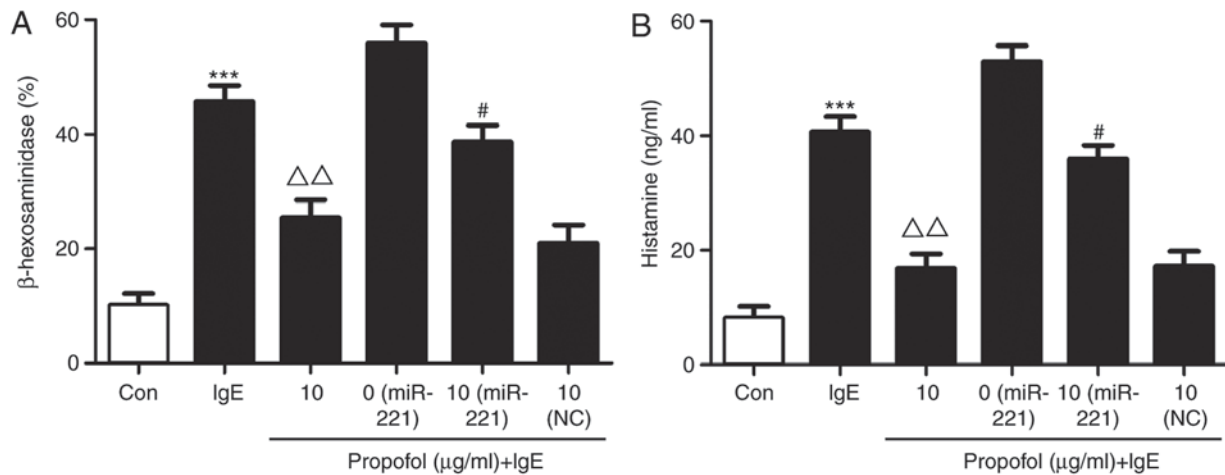


Figure 4. Effect of propofol on mast cell degranulation. RBL-2H3 cells were transfected with miR-221 mimic or NC miRNA and treated with 10  $\mu$ g/ml propofol for 24 h. Cells were sensitized with 0.5  $\mu$ g/ml anti-DNP IgE overnight and stimulated with 100 ng/ml DNP-HSA for 4 h. (A)  $\beta$ -hexosaminidase and (B) histamine release were detected. \*\*\*P<0.001 vs. Con. <sup>#</sup>P<0.05 vs. miR-221 + 0  $\mu$ g/ml propofol. <sup>△△</sup>P<0.01 vs. IgE + 0  $\mu$ g/ml propofol. miR and miRNA, microRNA; NC, negative control; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin; Con, control.

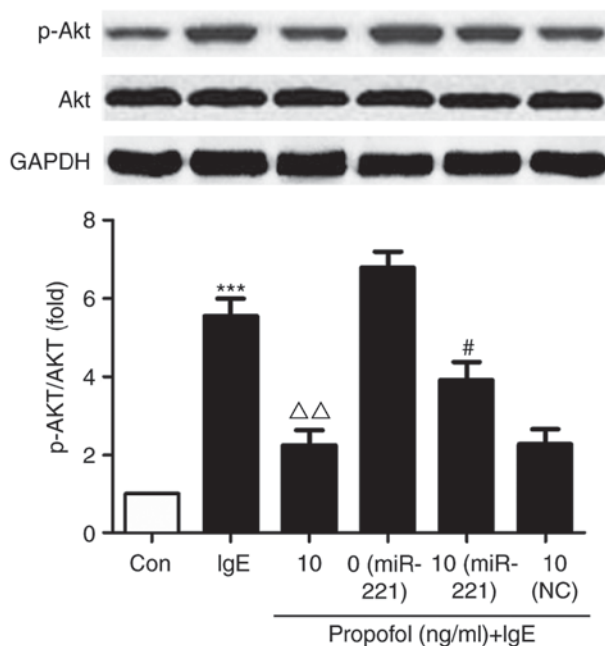


Figure 5. Effect of propofol on Akt phosphorylation. RBL-2H3 cells were transfected with miR-221 mimic or NC miRNA and treated with 10  $\mu$ g/ml propofol. Cells were activated with anti-DNP IgE and DNP-HSA, following which the expression of Akt and p-Akt was assessed using western blotting. \*\*\*P<0.001 vs. Con. <sup>#</sup>P<0.05 vs. miR-221 + 0  $\mu$ g/ml propofol. <sup>△△</sup>P<0.01 vs. IgE + 0  $\mu$ g/ml propofol. Akt, protein kinase B; miR or miRNA, microRNA; NC, negative control; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin; p, phosphorylated; Con, control.

## Discussion

Although propofol has been demonstrated to have anti-oxidant, anti-inflammatory and anti-tumor properties, few studies have investigated the effect of propofol on mast cell degranulation (8). The results of the present study demonstrate that propofol is able to affect RBL-2H3 cell proliferation, reduce miR-221 expression, suppress the release of  $\beta$ -hexosaminidase and histamine, limit activation of the PI3K/Akt signaling

pathway and decrease  $Ca^{2+}$  influx in mast cells. These findings suggest that propofol may have potential as an anesthetic for surgical procedures associated with allergic responses.

MicroRNAs (miRs) are a group of small, single-stranded non-coding RNAs that regulate gene expression at the post-transcriptional level (17). Through binding to the 3'-untranslated region of target mRNAs, miRs are able to induce mRNA degradation or translational inhibition (18). miR-221 has been reported to be associated with the modulation of mast cell degranulation (19). A previous study demonstrated that miR-221 influenced the cell cycle, the extent of degranulation, cytokine production and the actin cytoskeleton in activated bone marrow-derived mast cells (20). It has been indicated that miR-221, which was overexpressed in a murine asthma model, is able to stimulate interleukin-4 secretion in mast cells through a pathway involving phosphatase and tensin homolog, p38 and nuclear factor (NF)- $\kappa$ B (21). In line with these results, propofol treatment inhibited miR-221 expression in IgE-stimulated RBL-2H3 cells, suggesting that miR-221 may serve a role in the suppressive effect of propofol. These results were further confirmed by cell transfection with the miR-221 mimic.

The effect of PI3K/Akt signaling in mast cells has previously been investigated (22,23), as has the association between miR expression and PI3K/Akt signaling in mast cell degranulation (24,25). Although miR-223 expression was upregulated in IgE-mediated mast cells, miR-223 downregulation was demonstrated to promote mast cell degranulation and apoptosis via the PI3K/Akt pathway by targeting insulin-like growth factor 1 receptor in mast cells (25,26). *Helicobacter pylori* neutrophil-activating protein induced the release of histamine and interleukin-6 in human mast cell line-1 via the G protein-mediated mitogen-activated protein kinase (MAPK) and PI3K/Akt pathways (27). These studies indicated that PI3K/Akt signaling is associated with the regulation of mast cell activation, which may contribute to the inhibitive biological properties of propofol. The results of the present study also confirmed that propofol treatment restricted mast cell degranulation, as evidenced by the downregulation of

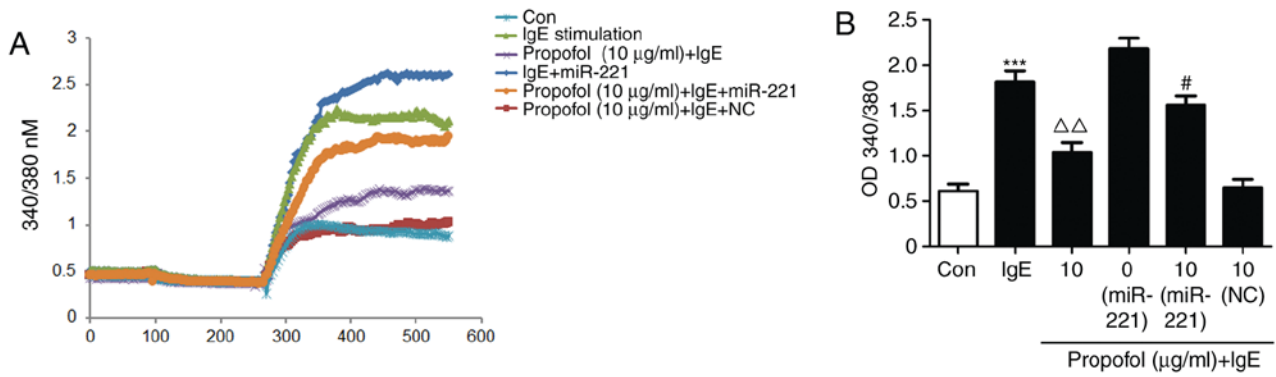


Figure 6. Effect of propofol on  $\text{Ca}^{2+}$  influx in RBL-2H3 cells. RBL-2H3 cells were transfected with miR-221 mimic or NC miRNA and treated with 10  $\mu\text{g/ml}$  propofol. Cells were activated with anti-DNP IgE and DNP-HSA, following which extracellular  $\text{Ca}^{2+}$  influx was (A) measured and (B) analyzed. \*\*\* $P < 0.001$  vs. Con. <sup>#</sup> $P < 0.05$  vs. miR-221 + 0  $\mu\text{g/ml}$  propofol. <sup>ΔΔ</sup> $P < 0.01$  vs. IgE + 0  $\mu\text{g/ml}$  propofol. miR or miRNA, microRNA; NC, negative control; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin; Con, control.

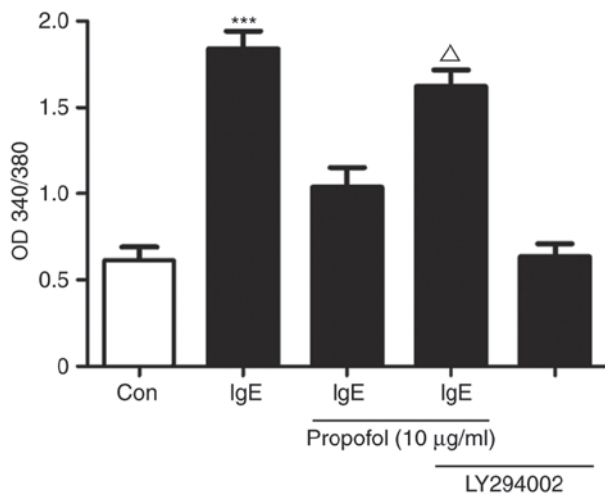


Figure 7. Effect of PI3K inhibitor (LY294002) on propofol-mediated  $\text{Ca}^{2+}$  influx. RBL-2H3 cells were pretreated with PI3K-inhibitor (LY294002) for 30 min prior to propofol administration (10  $\mu\text{g/ml}$ ). Cells were activated with anti-DNP IgE and DNP-HSA, following the extracellular  $\text{Ca}^{2+}$  influx was measured and statistically analyzed. \*\*\* $P < 0.001$  vs. Con. <sup>Δ</sup> $P < 0.05$  vs. 10  $\mu\text{g/ml}$  propofol without LY294002. PI3K, phosphoinositide 3-kinase; DNP, 2,4-dinitrophenyl; Ig, immunoglobulin; HSA, human serum albumin; Con, control; OD, optical density.

above findings, propofol treatment resulted in reduced  $\text{Ca}^{2+}$  influx, miR-221 and Akt phosphorylation, which were abrogated by the specific PI3K-inhibitor LY294002. This suggests that the miR-221/PI3K/Akt/ $\text{Ca}^{2+}$  pathway is responsible for the suppressive effect of propofol.

In conclusion, the results of the present study demonstrate that propofol attenuates the IgE-mediated activation of mast cell degranulation via inhibiting the miR-221/PI3K/Akt/ $\text{Ca}^{2+}$  pathway. Although the present study provides a novel insight into the biological effect of propofol and suggests a potential molecular target for the treatment of mast cell-associated allergic diseases. However, there were various limitations to the present study. Firstly, miR-221<sup>-/-</sup> derived from animal or bone marrow mast cells were not utilized. Use of these cells in future studies may provide results to support the conclusion of the present study. In addition, interactions with different signaling pathways including MAPK and NF- $\kappa\text{B}$ , or its involvement with miR-221-associated mast cell degranulation should be elucidated for clarification in future studies.

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## Funding

Not applicable.

## Availability of data and materials

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

## Authors' contributions

ZhiyongY, WL, and GL conceived the experimental design; ZhiyongY, ZhipanY, KH, YC and CX performed the experiments; YL and QL performed  $\text{Ca}^{2+}$  measurement and analysis; ZhipanY and SZ aided in data analysis; WL and GL reviewed and approved the final draft of the manuscript.

$\beta$ -hexosaminidase and histamine. Propofol treatment results in decrease Akt phosphorylation, suggesting that the PI3K/Akt signaling pathway serves a role in the suppressive effect of propofol on mast cell degranulation.

Generally, mast cell activation results in the degranulation of preformed mediators, including histamine, and the secretion of newly synthesized mediators, including leukotrienes and inflammatory cytokines (28). An influx of extracellular  $\text{Ca}^{2+}$  is essential for mast cell mediator release (29). It has been reported that  $\text{Ca}^{2+}$  mobilization is associated with the regulation of mast cell function (29). A previous study demonstrated that  $\text{Ca}^{2+}$  influx served a key role in modulating the spontaneous motility and directional migration of mast cells towards stimulating antigens (30). Furthermore, it was reported that miR-221 promoted the IgE-mediated activation of mast cell degranulation via the PI3K/Akt/PLC $\gamma$ / $\text{Ca}^{2+}$  signaling pathway in a non-NF- $\kappa\text{B}$  dependent manner (31). Consistent with the

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare no competing interests.

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