

Triptolide inhibits the Apoptosis and Reactive Oxygen Species Production in Hydrogen Peroxide-Treated Nasal Epithelial Cells by Regulating PTEN/PI3K/AKT Signaling Pathway

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Wang *et al.*: Triptolide inhibits the Nasal Epithelial Cells

Chronic rhinosinusitis is a chronic inflammatory condition that caused by many factors. However, the underlying mechanism of chronic rhinosinusitis remains unclear. The current study focused on the roles of triptolide on hydrogen peroxide treated nasal epithelial cells and the underlying mechanism. Hydrogen peroxide treated human nasal epithelial cells were cultured and treated by different concentrations of triptolide, cell growth and cell death were determined by cell counting kit-8 and flow cytometry methods. Moreover, effects of triptolide on expression of superoxide dismutase as well as malondialdehyde in nasal epithelial cells treated by hydrogen peroxide were evaluated by enzyme-linked immunosorbent assay analysis. Furthermore, effects of triptolide on expression of phosphatase and tensin homolog deleted on chromosome 10/phosphatidylinositol 3-kinase/protein kinase B molecules in hydrogen peroxide treated nasal epithelial cells have been examined by Western blot method. We found treatment of hydrogen peroxide markedly inhibited growth of the nasal epithelial cells in dose-dependent manner and triptolide could reverse the effect induced by hydrogen peroxide. Moreover, triptolide significantly decreased the apoptosis of the nasal epithelial cells induced by hydrogen peroxide in dose-dependent manner; moreover, triptolide reduced superoxide dismutase as well as malondialdehyde levels in dose dependent manner. Finally, triptolide downregulated phosphatase and tensin homolog deleted on chromosome 10 and upregulated the phosphatidylinositol 3-kinase related signalling in hydrogen peroxide treated cells *in vitro*. In conclusion, triptolide may exert anti-apoptotic and anti-oxidative behaviors in chronic rhinosinusitis *via* affecting phosphatase and tensin homolog deleted on chromosome 10/phosphatidylinositol 3-kinase/protein kinase B signaling. The results of current work study may provide new evidence for the research as well as management of chronic rhinosinusitis.

Key words: Chronic rhinosinusitis, chronic inflammatory, nasal epithelial cells, phosphatidylinositol 3-kinase/protein kinase B

Chronic Rhinosinusitis (CRS) is mainly due to the persistence of chronic inflammation of nasal cavity and paranasal sinus mucosa caused by many factors^[1,2]. Although the control of patients with nasal polyps has been greatly improved with the improvement of treatment measures such as drugs and surgery, there is still a high recurrence rate of CRS after treatment and this recurrence is more obvious in nasal polyps^[3,4]. The existence of these conditions may be related to the controversial and unclear etiology and pathogenesis of CRS. Many factors have been reported to be related to the pathogenesis of CRS, including bacterial and viral

infection; allergic factors; impairment of epithelial barrier function; obstruction of ostium nasal complex; immune dysfunction and so on^[5-9]. The role of epithelial cells in the pathogenesis of CRS has attracted more and more attention. As the first natural barrier to contact external stimuli and an important structure of innate immunity, epithelial cells on the surface of nasal mucosa play an important role in protecting the body from external harmful factors^[10-12]. Once the epithelial cell barrier function is damaged, it is mainly manifested in epithelial cell abscission and dysfunction in CRS, which will provide conditions for the invasion

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of external harmful substances, such as bacteria, virus invasion and allergen exposure, so as to cause and aggravate the chronic inflammation of nasal cavity and paranasal sinus mucus membrane and lead to the accumulation of inflammatory cells and inflammatory factors^[13,14]. There are many factors causing epithelial cell injury and abscission, such as inflammatory factor stimulation, but at present, Interferon (IFN) has been found-gamma (γ) it can cause epithelial cell apoptosis in CRS, but the specific mechanism is not clear.

Under normal conditions, the oxidation and antioxidant system in the human body maintain a relative balance. For example, some enzymatic and non-enzymatic antioxidants will prevent the invasion of Reactive Oxygen Species (ROS)^[15-17]. When this balance is broken by excessive ROS, it is called "oxidative stress". Oxidative stress can lead to diseases, including cancer, neurological diseases, hypertension, ischemia and reperfusion^[18-20], diabetes, chronic obstructive pulmonary disease and asthma^[21-23]. ROS plays an important role in the regulation of important cellular processes such as oxidative stress, apoptosis and gene expression. It is one of the most active frontier fields in life science and molecular medicine.

In recent years, more and more attention has been paid to the role of oxidative stress in the pathogenesis of airway diseases. The airway is the only organ exposed to high oxides and antioxidants. Under normal conditions, oxides and antioxidants maintain a relative balance, which is very necessary to protect the normal function of the airway. When the airway is exposed to environmental factors such as animal hair, dust, mites, fungi, pollen, cigarette smoke, volatile chemicals, occupational dust, automobile exhaust and sulfur dioxide^[18,19,23,24], these irritants stimulate epithelial cells to produce too many harmful oxides, break the balance between oxides and antioxidants, and finally lead to oxidative stress and airway inflammation.

Triptolide, also known as triptolide and triptolide alcohol, is the most deeply studied traditional Chinese medicine immunosuppressant^[25,26]. Triptolide is a diterpene compound isolated from the roots of *Tripterygium wilfordii* in Celastraceae. Triptolide is a light yellow crystal with stable physical and chemical properties^[27,28]. It is insoluble in water, slightly soluble in alcohol and easily soluble in organic solvents. It has the dual effects of anti-inflammatory and immunosuppression^[25,29,30]. Through clinical application and experimental research, triptolide has anti-inflammatory and immunosuppressive effects.

In the present work, we will focus on the function of triptolide for the treatment of CRS using nasal epithelial cells as cell models. Hydrogen peroxide (H_2O_2) has been used to induce ROS and modulate the oxidative stress. We hypothesized that triptolide may inhibit the apoptosis and ROS production in H_2O_2 .

MATERIALS AND METHODS

Cells and cell culture:

The Human Nasal Epithelial Cells (HNEpC) have been placed in Dulbecco's modified eagle's medium (Thermo Fisher Scientific, Waltham, MA, USA) combining with 10 % fetal bovine serum (Gibco), 1 % penicillin as well as streptomycin. Cells have been cultured in an incubator with 5 % Carbon dioxide (CO_2) at 37°.

Cell growth analysis:

Cell Counting Kit-8 (CCK-8) method was used to detect cell growth in each group. First, 4×10^3 cells were seeded into 96-well plates. Then, a total of 4×10^3 cells were inoculated with 96-well plates per hole. CCK-8 reagent (Tokyo Dojindo, Japan) was added at 0, 24, 48, 72 h respectively. The plate was then incubated at 37° for 60 min using an automatic microboard reader (BioTek, USA). The measurement of Optical Density (OD) was performed with a microplate at the wavelength of 450 nm.

Cell apoptosis analysis:

After 48 h of culture, cells were washed twice with Phosphate Buffered Saline (PBS), followed by digestion and resuspension, Then cells were double-stained with FITC Annexin V/Propidium Iodide (PI) at 4°. Cell apoptosis was calculated with FACSCalibur™ flow cytometry (BD Biosciences, USA) using FlowJo software (version 7.6.5; Tree Star, USA).

Enzyme-Linked Immunosorbent Assay (ELISA) assay:

Treated HNEpC cells were cultured at 37°, 5 % CO_2 environment for 12 h and treated with Radio Immunoprecipitation Assay (RIPA) lysis buffer. Then, the supernatant was obtained by centrifugation at 4°, 10 000 r/min for 5 min. Finally, the Superoxide Dismutase (SOD) and Malondialdehyde (MDA) contents of each group were measured using corresponding commercial ELISA kits.

Western blot:

Cells were harvested at 48 h and lysed by RIPA (Sigma-Aldrich, Germany). Protein concentration was calculated by a bicinchoninic acid kit (Pierce, USA).

Then the protein was separated with 12 % Sodium Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride membranes, which were blocked with 5 % non-fat milk. Next, the membranes were incubated with primary antibodies, purchased from Boster Biological Technology, China, overnight at 4°C; 2nd d, the membranes were incubated with Horseradish Peroxidase (HRP)-goat-anti-rabbit antibody (Beyotime, China) and treated with BeyoECL Plus (Beyotime, China). The bands were visualized with a microscope (Bio-Rad, USA).

Statistical analysis:

All the data were analyzed by Statistical Package for the Social Sciences (SPSS) 19.0 software and expressed as mean±standard error. When the sample size is two groups, the T-test was used to compare the mean value and analysis of variance for multi-group. $p < 0.05$ was considered a significant difference.

RESULTS AND DISCUSSION

First, H_2O_2 treated nasal epithelial cells have been randomly divided to five groups. Cells have been cultured using triptolide of different concentrations. The growth of the cells has been examined by CCK-8 assay. We found that H_2O_2 inhibited nasal epithelial cell growth and treatment of triptolide markedly increased growth of the nasal epithelial cells in dose-dependent manner

as shown in fig. 1. Nasal epithelial cells were stimulated with H_2O_2 and treated with different concentrations of triptolide. CCK-8 analysis was applied for determining cell viability. $**p < 0.01$, H_2O_2 vs. control group.

Furthermore, H_2O_2 treated nasal epithelial cells have been randomly divided to five groups and the cell death have been determined by flow cytometry assay. We found that after exposing to H_2O_2 , cell apoptosis was novelty increased; however, treatment of triptolide markedly inhibited the apoptosis of the nasal epithelial cells in a dose-dependent manner as shown in fig. 2. Nasal epithelial cells were triggered with H_2O_2 and treated with different concentrations of triptolide. Flow cytometry analysis was conducted to evaluate cell apoptosis. $***p < 0.001$, H_2O_2 vs. control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ vs. H_2O_2 .

Furthermore, effects of triptolide on activity of SOD as well as MDA in nasal epithelial cells treated by H_2O_2 were evaluated by ELISA analysis. In fig. 3, H_2O_2 markedly increased the levels of SOD and MDA. And 25, 50 and 100 $\mu\text{mol/l}$ of triptolide decreased SOD as well as MDA levels induced by H_2O_2 in dose dependent manner as shown in fig. 3. Nasal epithelial cells were triggered with H_2O_2 and treated with different concentrations of triptolide. $***p < 0.001$, H_2O_2 vs. control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. H_2O_2 .

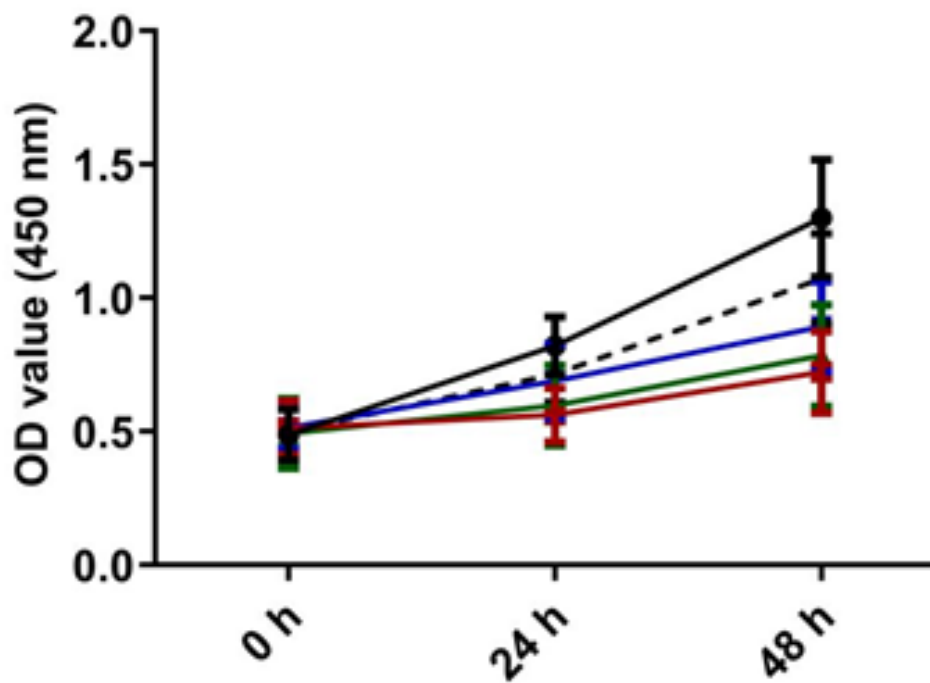


Fig. 1: Triptolide inhibited the growth ability of nasal epithelial cells, (◆): H_2O_2 +100 $\mu\text{mol/l}$ triptolide; (▼): H_2O_2 +50 $\mu\text{mol/l}$ triptolide; (▲): H_2O_2 +25 $\mu\text{mol/l}$ triptolide; (■): H_2O_2 and (●): Control

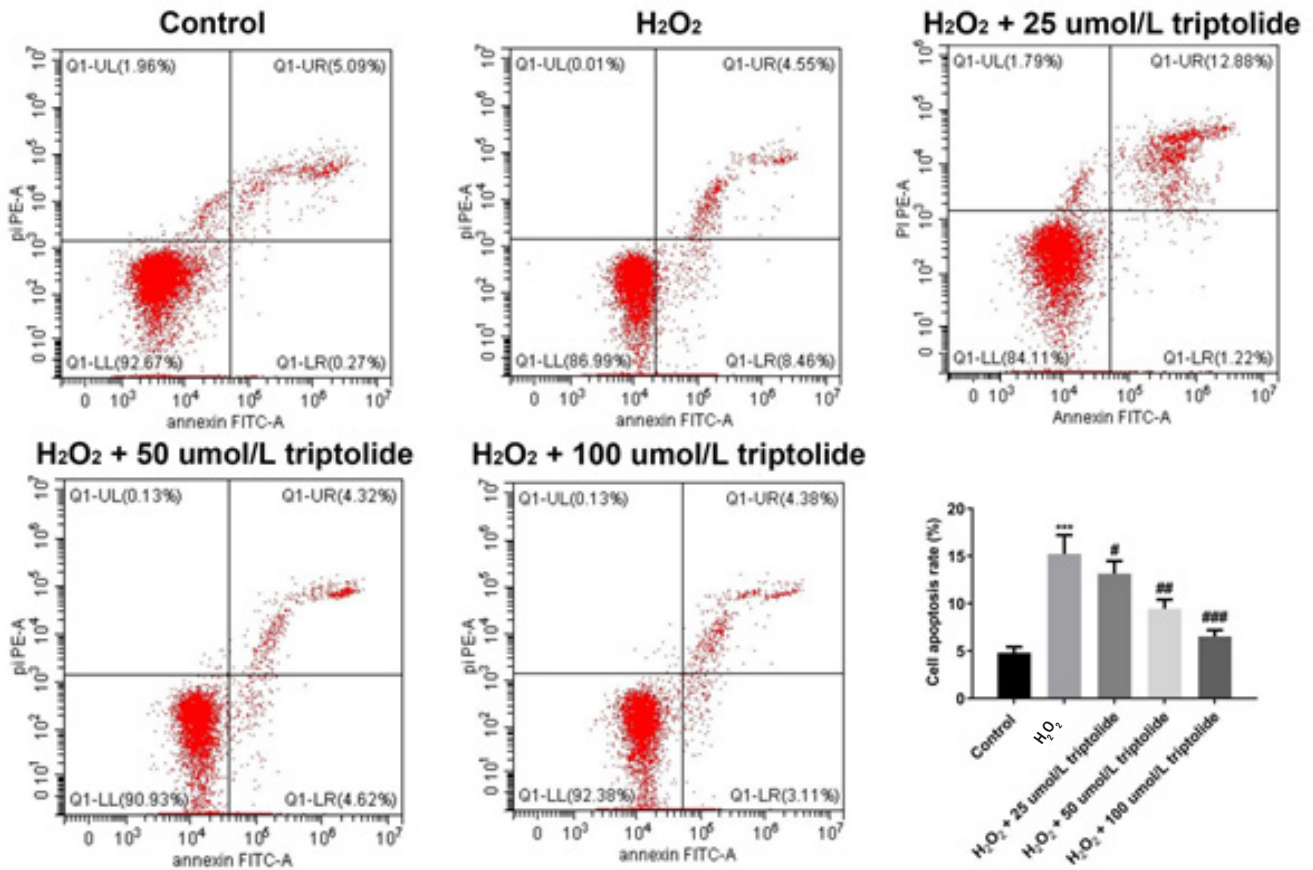


Fig. 2: Triptolide inhibited nasal epithelial cell death *in vitro*

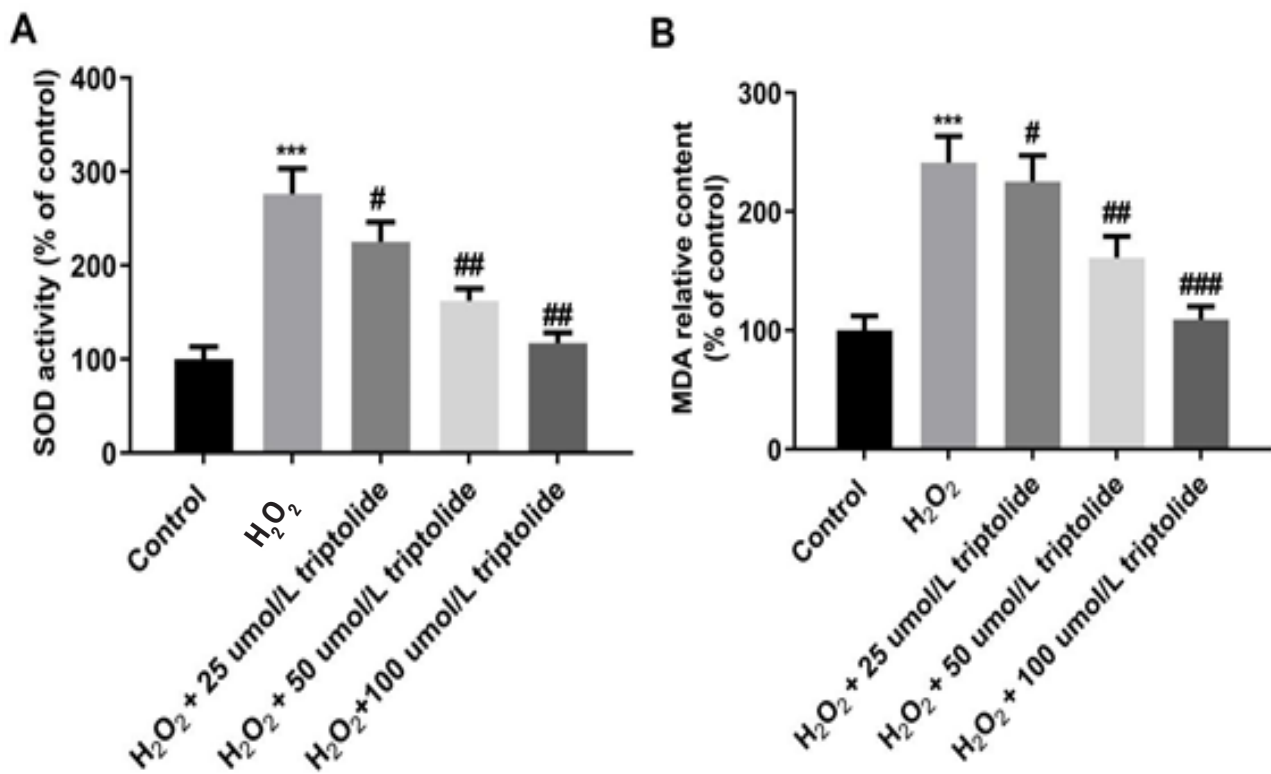


Fig. 3: Triptolide can affect the level of SOD and MDA nasal epithelial cells treated by H₂O₂

Finally, effects of triptolide on expression of Phosphatase and Tensin homolog deleted on chromosome 10/ Phosphatidylinositol 3-Kinase/Protein Kinase B (PTEN/PI3K/AKT) molecules in H_2O_2 treated nasal epithelial cells have been examined. We found that H_2O_2 significantly promoted PTEN expression and inhibited phosphorylated-PI3K (p-PI3K) as well as p-AKT expression, while on the other hand, triptolide inhibited PTEN and the PI3K related signaling in H_2O_2 treated cells *in vitro* as shown in fig. 4. Nasal epithelial cells were triggered with H_2O_2 and treated with different concentrations of triptolide. Western blot assay was utilized to determine PTEN/PI3K/AKT activation under H_2O_2 conditions. *** $p < 0.001$, H_2O_2 vs. control; # $p < 0.05$ and ## $p < 0.01$ vs. H_2O_2 .

In current work, we investigated the effects of triptolide on growth, apoptosis and ROS production of nasal epithelial cells *in vitro*. The results suggested that triptolide may regulate growth as well as apoptosis and the ROS production of nasal epithelial cells through PTEN/PI3K/AKT signaling. Our results found that triptolide may function as alternative medicine for CRS management.

With the in-depth research on the roles of traditional medicine in different diseases, the roles of traditional medicine in many types of diseases have been elucidated. For example, it has been reported that ginsenoside-Rg5 may decrease the proliferation and promote the cell death of tumor cells through decreasing BCL2 expression^[31]; on the other hand, wogonin could decrease the formation of nasal polyp *via* increasing the apoptosis of eosinophil *via* regulating the expression of Hypoxia-Inducible Factor (HIF)-1 alpha as well as surviving^[32]. The function of triptolide for the treatment of CRS remains unclear. In current work, nasal epithelial cells have been treated by triptolide, the growth of the cell, cell death and ROS production has been determined. Our data suggested that triptolide may regulate the proliferation as well as death of nasal epithelial cells. Furthermore, triptolide decreased ROS production in nasal epithelial cells. The present data suggested that triptolide may decrease the apoptosis as well as ROS production of nasal epithelial cells. Our results proved that triptolide may exert anti-apoptotic and anti-oxidative effects in CRS.

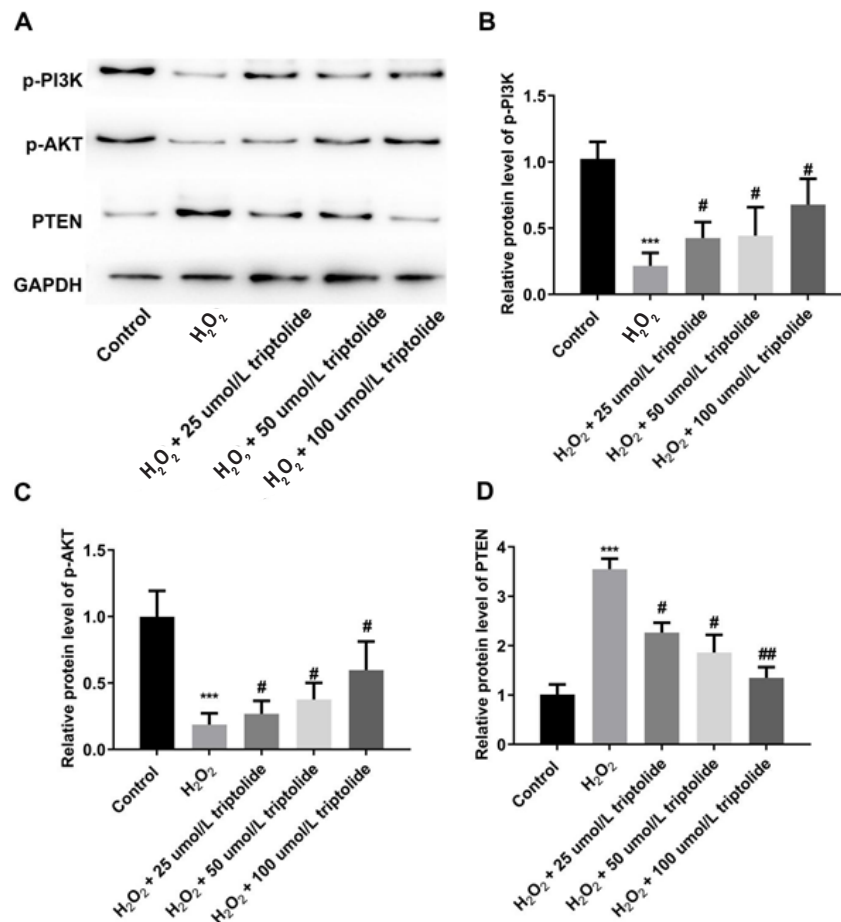


Fig. 4: Triptolide can inhibit the apoptosis in H_2O_2 -treated nasal epithelial cells *via* inhibiting PTEN/PI3K/AKT expression

The PI3K/AKT signaling was known as the key cellular signaling pathway that involved in many different biological activities, including the carcinogenesis process^[33-35]. The roles of PI3K/AKT signaling in CRS have also been discussed previously^[36,37]. Therefore, we further examined whether the management of triptolide may regulate the expression of PI3K/AKT signaling. As expected, PTEN was markedly decreased, while p-PI3K and p-AKT were significantly increased in triptolide treated nasal epithelial cells. Our results proved that triptolide may regulate nasal epithelial cells proliferation as well as ROS production *via* PI3K/AKT signaling pathway.

In conclusion, our data suggested triptolide may exert anti-apoptotic and anti-oxidative behaviors in CRS *via* affecting PTEN/PI3K/AKT signaling. The results of current work study may provide new evidence for the research as well as management of CRS.

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Conflict of interests:

The authors declared no conflict of interests.

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