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Angelica polysaccharide alleviates oxidative response damage in HaCaT cells through up-regulation of miR-126

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ABSTRACT

Background: Pressure ulcers (PUs) prevalence has been considered as an index for patient safety and cure quality of hospital and community. Skin cellular oxidative response damage is existed in the development of PUs. *Angelica* polysaccharide (AP) has the anti-oxidation function. Therefore, our goal was to investigate the mechanism of AP in relieving cellular oxidative damage.

Methods: Transfected HaCaT cells with miR-126 inhibitor, pre-treated by AP, and then treated by H_2O_2. CCK-8 assay and flow cytometry detection were set to test viability and apoptosis of cells respectively. qRT-PCR and western blot tested levels of miR-126 and oxidative damage relative factors. ROS assay tested the production of ROS in cells.

Results: Cellular oxidative response damage was induced by H_2O_2 at concentration of 300 μM. We found that AP could attenuate cellular oxidative response damage caused by H_2O_2 that it elevated cell viability, attenuated cell apoptosis and production of ROS and promoted activation of PI3K/AKT and mTOR signal pathways. Further, miR-126 was up-regulated by AP. The up-regulation of miR-126 could activate the PI3K/AKT and mTOR signal pathways.

Conclusion: Our study demonstrated that AP attenuated cellular oxidative response damage in HaCaT cells by positively regulated miR-126.

1. Introduction

Pressure ulcers (PUs), called pressure sores and hemorrhoids, are caused by long-term compression of local organization, and persistent ischemia, hypoxia and malnutrition, resulting in tissue ulceration and necrosis (Taylor, 2017). Skin PUs are a common problem in rehabilitation and nursing. Although there is sufficient debridement and subsequent vascular tissue to successfully complete defect closure (Djedovic et al., 2017), the prevalence of PUs is still high nationally and internationally (Baath et al., 2014; James et al., 2010; Kottner et al., 2009; Moore et al., 2013). Recently, there is no evidence-based cure to prevent the development of skin ulcers in the early stages of PU. Considering PUs always accompany the skin oxidative damage reaction in the development process (Liu et al., 2018), searching for appropriate anti-oxidant damage drugs is of great significance for clinical treatment of PUs.

Angelica sinensis (Oliv.) Diels (Apiaceae), a perennial herb whose root is a Chinese herbal medicine, has been widely used for treating anemia, cardiovascular disease, rheumatism and liver fibrosis due to its nourishment, blood supplement and anti-inflammatory effects (Bunel et al., 2015). *Angelica* polysaccharide (AP), the most important biologically active ingredient in *Angelica sinensis*, has a variety of biological activities, including promoting hematopoietic function (Xiao et al., 2017), immune regulation (Qin et al., 2013), anti-tumor activity (Cao et al., 2010) and anti-oxidant activity (Zeng et al., 2015). Based on these functions, the application of AP may be an effective cure for oxidative damage. Previous studies have showed that AP reduces the oxidative damage of human bone marrow/stromal cells and protect them (Xiao et al., 2017). Besides, AP delays aging of hematopoietic stem cells by inhibiting oxidative damage (Zhang et al., 2013). However, whether AP has the effect of reducing oxidative damage in the clinical treatment of PUs is still unknown.

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MicroRNAs (miRNAs) are non-coding RNA molecules, regulating genes expression in post-transcriptional levels (Lee et al., 1993). MiR-126 was reported to work as the most common endothelial cell-specific miRNA (Zernecke et al., 2009). It was involved in protecting human umbilical vascular endothelial cells (HUVECs) against oxidative damage (Sui et al., 2014). However, whether miR-126 has a protective role in oxidative damage of PUs remains unknown. Besides, the PI3K/AKT and mTOR signal pathways are central regulator in multiple cellular roles, such as cell proliferation, apoptosis and autophagy (Porta et al., 2014). So it will be interesting to explore the biological function and mechanism of these two pathways in cellular oxidative damage of PUs.

Human skin, acted as a protective barrier between the environment and internal organs, is often exposed to potentially harmful compounds and radiation, accompanied by damage such as oxidative damage (Guo et al., 2010). HaCaT human immortalized keratinocytes cells are commonly used models for studying cell damage (Klaus et al., 2010; Zi et al., 2018). Therefore, our study investigated the protective effects of AP against oxidative damage and its regulation mechanism in HaCaT cells. This will be of important significance for clinical cure of PUs.

2. Materials and methods

2.1. Cell culture and treatment

HaCaT cells were bought from Chinese Academy of Sciences (Kunming, China) and cultured in Dulbecco's Modied Eagle Medium (DMEM)/F-12 medium (Gibco, Carlsbad, CA, USA) adding with 10% fetal bovine serum (FBS, Gibco) under environment of 5% CO_2 and 95% air at 37 °C. AP was bought from Ci Yuan Biotechnology Co., Ltd. Shanxi (Xi'an, China). Dilute AP to phosphate-buffered saline (PBS) at a concentration of 8 mg/ml. Prior to the treatment of H_2O_2, cells were pre-treated with AP for 8 h at a concentration of 50 μM. Cells were treated for 12 h with a range of concentrations of H_2O_2.

2.2. Cell viability

Cell viability was tested via a Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD, USA). Cells were seeded in 96-well plate with 5000 cells/well. Add CCK-8 solution to medium after stimulation, and keep cells in a humidified environment with 95% air and 5% CO_2 at 37 °C for 1 h. Absorbance was tested at 450 nm via a Microplate Reader (Bio-Rad, Hercules, CA, USA).

2.3. Apoptosis assay

We performed cell apoptosis analysis through propidium iodide (PI) and fluorescein isothiocynate (FITC)-conjugated Annexin V staining (BD Pharmingen, San Diego, CA, USA). Cells were washed in PBS for 3 times and stained in PI/FITC-Annexin V with 50 μg/ml RNase A (Sigma-Aldrich, St. Louis, MO, USA). Next keep cells at room temperature in the dark for 1 h. FACS can (Beckman Coulter, Fullerton, CA, USA) was used to differentiate apoptotic cells and necrotic cells. Our data was analyzed via FlowJo software (Tree Star Software, San Carlos, California, USA).

2.4. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Extraction of RNA was done through Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) following producer's directions. Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II with the TaqMan MicroRNA Assay of miR-126 and U6 (Applied Biosystems, Foster City, CA, USA) were used to test expression of miR-126, with U6 as internal control.

2.5. Transfection

Synthetic miR-126 mimic, miR-126 inhibitor and their relative NC (Life Technologies Corporation) were transferred into cells through the Lipofectamine 3000 reagent (Life Technologies Corporation) following manufacturer's instructions. Culture medium containing 0.5 mg/ml G418 (Sigma-Aldrich, St Louis, Mo, USA) was used to choose calm transfected cell. G418-resistant clones were produced after about 4 weeks. 48 h post-transfection was regarded as the harvest time in following assays because the highest transfection efficiency was appeared at this moment.

2.6. Western blot

Total protein was extract through RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) adding with protease inhibitors (Roche, Basel, Switzerland) and quantified through BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Western blot system was established via a Bio-Rad Bis-Tris Gel system following producer's directions. Primary antibodies specific against p53 (ab1101, Abcam, MA, USA), p21 (ab109520), β-actin (ab8226), cleaved-PARP (ab32064), cleaved-caspase-3 (ab49822), cleaved-caspase-9 (ab2324), t-PI3K (ab140307), p-phosphatidylinositol 3 kinase (PI3K) (ab182651), t-protein kinase B (AKT) (ab179463), p-AKT (ab38499), t-mammalian target of rapamycin (mTOR) (ab2732) and p-mTOR (ab84400) were prepared in 5% blocking buffer. Incubate primary antibody with membrane at 4 °C overnight. Then wash it and incubate with secondary antibody, marking by horseradish peroxidase at room temperature for 1 h. Next, blots and antibodies were carried on the Polyvinylidene Difluoride (PVDF) membrane and then were transferred into the Bio-Rad ChemiDoc™ XRS system, following with 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, USA) to cover the membrane surface. Finally, signals were got and intensity of the strip was quantified via Image Lab™ Software (Bio-Rad).

2.7. ROS assay

Measurement of ROS was done by flow cytometry through 2, 7-dichlorofluorescein diacetate (DCFH-DA) (Nanjing Jiancheng, Nanjing, China). Log phase cells were seeded on 6-well cell culture plates. Treated cell culture medium was collected to the flow-specific tube and were washed with PBS twice and co-incubated with serum-free medium including 10 μM DCFH-DA (20 min, 37 °C, in dark). Next, cells were washed with PBS and collected by a trypsin digestion method. Centrifuge sample and discard the supernatant. Cells were resuspended in 500 μl PBS and fluorescent intensities were tested via a flow cytometer (488 nm excitation, 521 nm emission).

2.8. Statistics analysis

All experiments were repeated for 3 times. Results of multiplex assays are revealed as mean + SD. Statistical analyses were done via Graphpad Prism 6.0 software (GraphPad Software Inc., La Jolla, CA, USA). *P*-values were counted via a one-way analysis of variance (ANOVA). *P*-value of < 0.05 indicated statistical significant data.

3. Results

3.1. H_2O_2 caused oxidative damage in HaCaT cells

In order to obtain cells damaged by oxidative damage in vitro, we used H_2O_2 to induce HaCaT cells. HaCaT cells were handled with diverse concentrations (0, 50, 100, 200, 300, 400 and 500 μM) of H_2O_2. As shown in Fig. 1A, there was significantly discrepancy in inhibiting cell viability when the concentration of H_2O_2 was 200, 300, 400 and 500 μM ($P < .05$, $P < .01$, $P < .01$ and $P < .001$). We selected

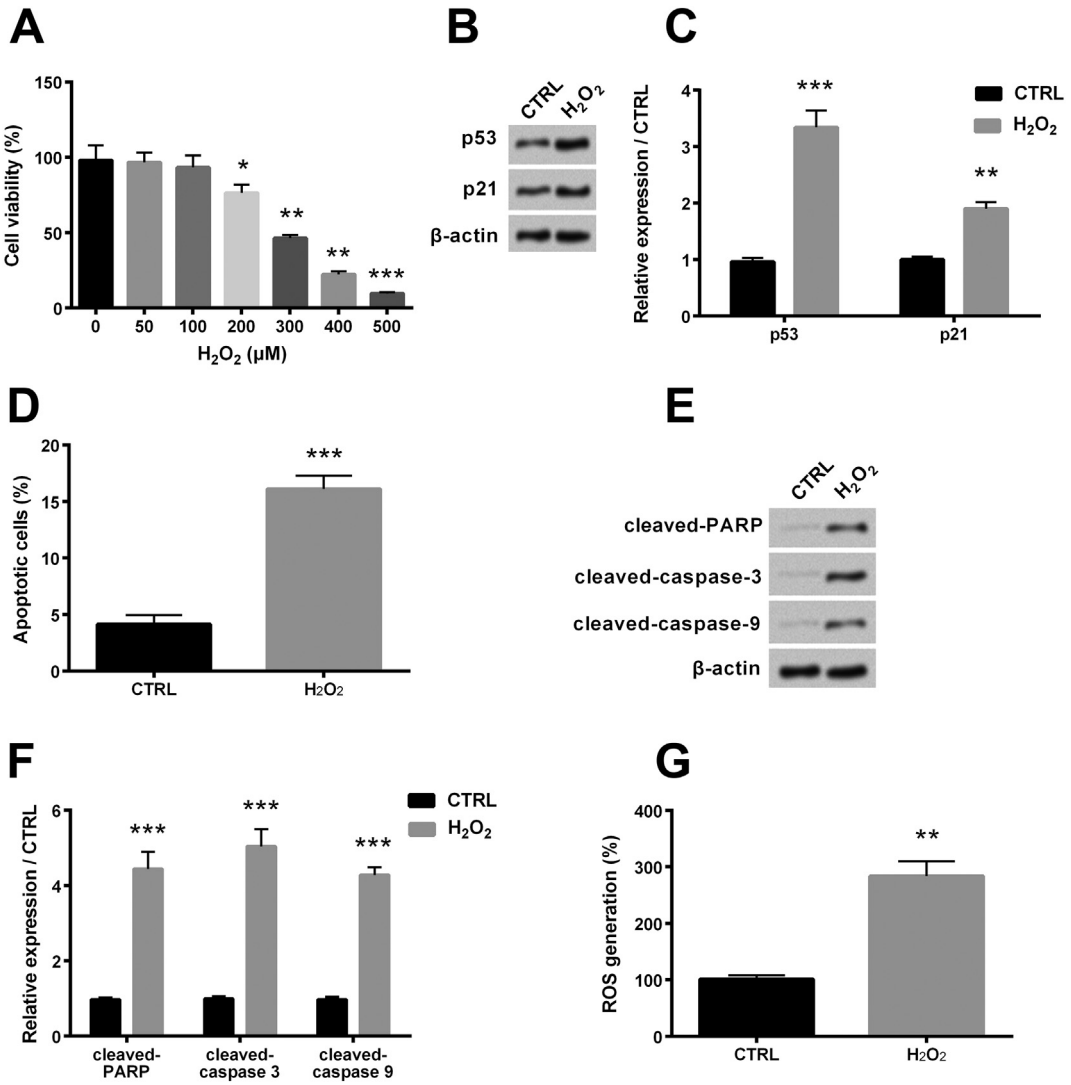

Fig. 1. The effects of H_2O_2 on inducing cellular oxidative response damage. (A) Cell viability was tested under diverse concentrations of H_2O_2 (0, 50, 100, 200, 300, 400 and 500 μM). 300 μM was selected in subsequent experiments. (B) Expression of p53 and p21 was tested via western blot. (C) Expression of p53 and p21 was tested via western blot quantitative. (D) Cell apoptosis treated by H_2O_2 was tested via flow cytometry. (E) Related proteins levels of apoptosis were tested via western blot. (F) Expression of related factors of apoptosis was detected via western blot quantitative. (G) Generation of ROS was detected via ROS assay. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with control group.

300 μM as the working concentration in following assays because 300 μM was cell viability semi-lethal concentration.

To further test the effects of H_2O_2, we test the expression of cell growth inhibition factors (p53 and p21). Western blot test showed the same trends that levels of p53 and p21 were obviously enhanced in H_2O_2-treated cells (Fig. 1B). Levels of p53 and p21 in H_2O_2-treated cells were notably increased than those in control group ($P < .001$ and $P < .01$, Fig. 1C). These results indicated that H_2O_2 could reduce cell viability. For cell apoptosis, we found that apoptosis was notably enhanced by H_2O_2 ($P < .001$, Fig. 1D) and western blot test showed that cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were apparently overexpressed after H_2O_2 treatment (Fig. 1E). Besides, levels of cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were notably increased via western blot quantitative (all $P < .001$, Fig. 1F). These findings suggested that H_2O_2 had a positive effect on cell apoptosis. In addition, ROS assay revealed that ROS generation was significantly increased in H_2O_2-treated cells ($P < .01$, Fig. 1G). This result further demonstrated that H_2O_2 could induce HaCaT cells oxidative damage. Above all, H_2O_2 could successfully induce cellular oxidative damage.

3.2. AP attenuated H_2O_2-induced cellular oxidative damage

According to our experiment, Fig. 2A showed that AP had no influence in cell viability. H_2O_2 could significantly reduce cell viability ($P < .05$), while AP could relieve this reduction ($P < .05$, Fig. 2B). For p53 and p21, H_2O_2 notably promoted expression of p53 and p21 (both $P < .01$), while AP could relieve this promotion (both $P < .05$, Fig. 2C-D). These findings suggested that AP could attenuate H_2O_2-induced cell viability loss. Moreover, apoptosis was significantly increased by H_2O_2 ($P < .001$), while AP could significantly reduce apoptosis in comparison with H_2O_2 group ($P < .05$, Fig. 2E). Similar to Fig. 2F, western blot test showed that cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were apparently down-regulated after AP treatment compared with H_2O_2 group. Levels of cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were notably enhanced through H_2O_2 ($P < .01$, $P < .001$ and $P < .01$), while AP could reduce their levels ($P < .01$, $P < .05$ and $P < .05$, Fig. 2G). These findings suggested that AP could attenuate apoptosis. In addition, ROS assay revealed that ROS generation notably was reduced in H_2O_2 + AP group in comparison with H_2O_2 group ($P < .05$, Fig. 2H). This result indicated that AP

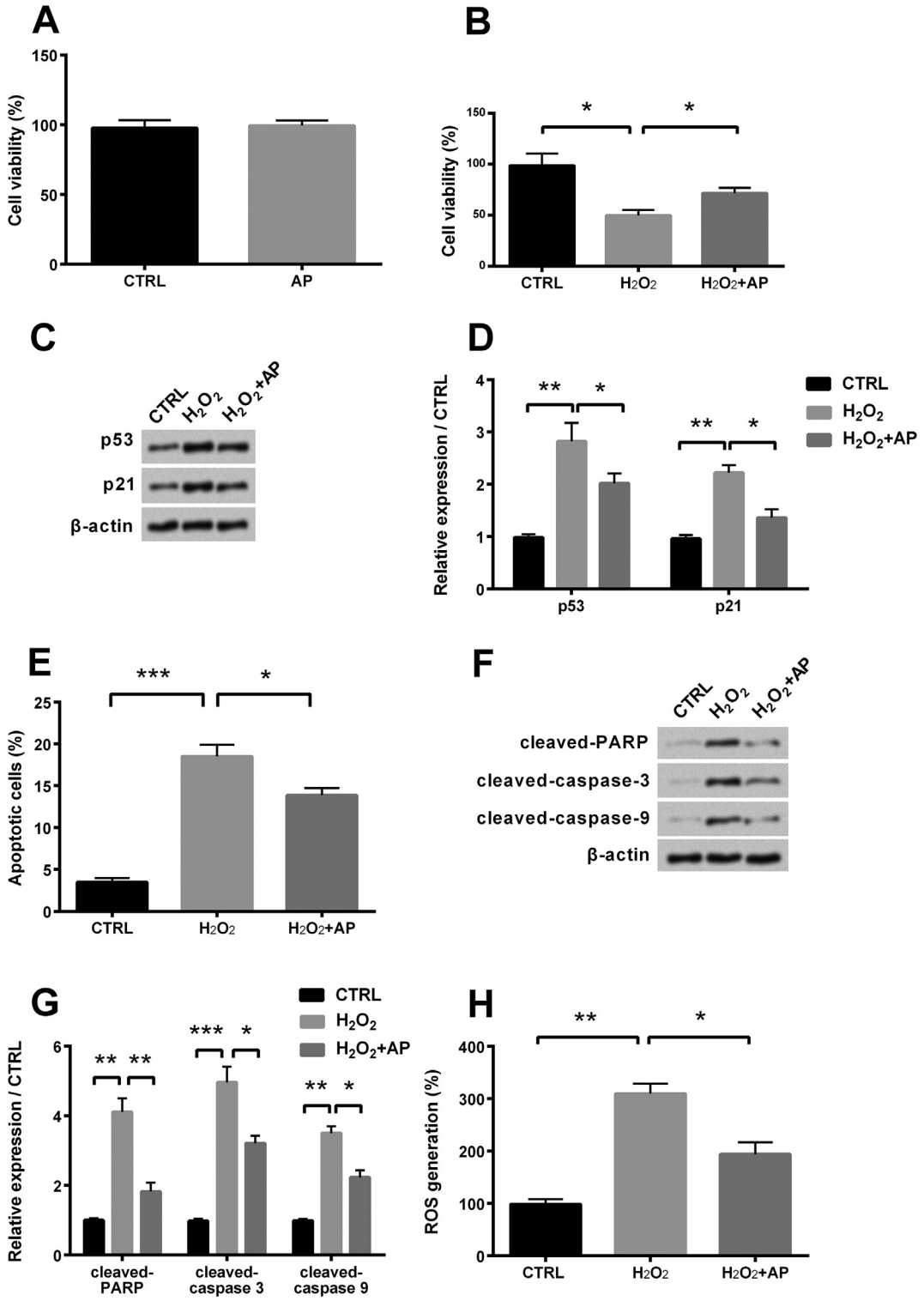

Fig. 2. The defensive effects of AP on cellular oxidative response damage. (A) Cell viability was tested via CCK-8 after AP treatment in HaCaT cells. (B) AP attenuated H_2O_2-induced inhibition of cell viability in HaCaT cells. (C) Expression of p53 and p21 was tested via western blot. (D) Expression of p53 and p21 was tested via western blot quantitative. (E) AP attenuated apoptosis caused by H_2O_2 in HaCaT cells. (F) Related protein levels of apoptosis were tested via western blot. (G) Expression of related factors of apoptosis was detected via western blot quantitative. (H) AP attenuated ROS generation caused by H_2O_2 in HaCaT cells. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with indicated group.

could relieve ROS production to attenuate cellular oxidative damage. Above all, we concluded that AP could attenuate H_2O_2-induced cellular oxidative damage.

3.3. AP promoted activation of PI3K/AKT and mTOR signal pathways

We detected the PI3K/AKT and mTOR signal pathways to study regulation mechanisms of AP. Western blot test showed that levels of p-PI3K, p-AKT and p-mTOR were notably reduced after treatment with

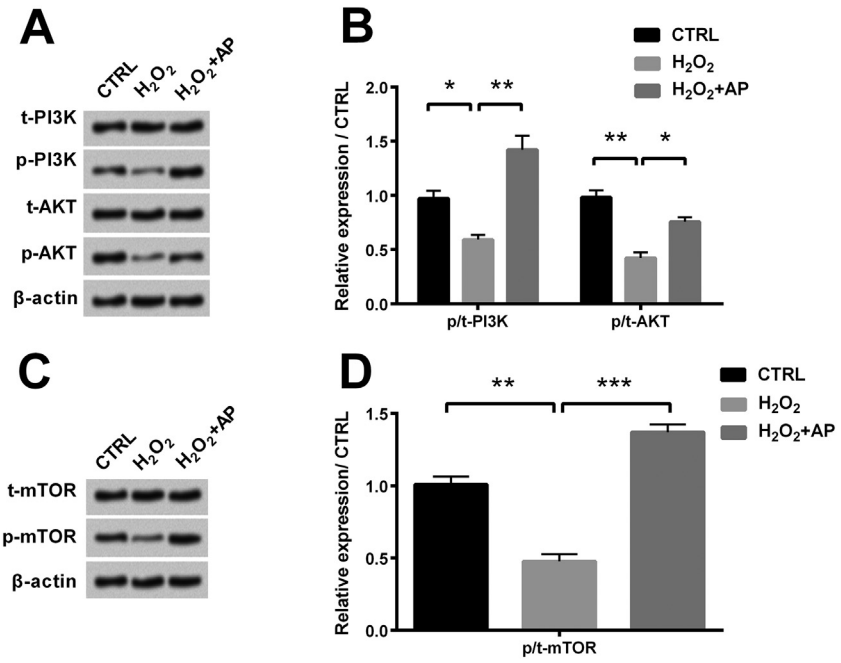

Fig. 3. Effects of AP on PI3K/AKT and mTOR signal pathways in HaCaT cells. (A) Levels of PI3K/AKT signal pathways related proteins were tested via western blot. (B) Expression of PI3K and AKT were measured via western blot quantitative. (C) Levels of mTOR was tested via western blot. (D) Level of mTOR was tested via western blot quantitative. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with indicated group.

H_2O_2 ($P < .05$, $P < .01$ and $P < .01$, Fig. 3A–D). However, the reduction effects on p-PI3K, p-AKT and p-mTOR were notably eliminated after treatment with AP, their levels in H_2O_2 + AP group were notably enhanced in comparison with H_2O_2 group ($P < .01$, $P < .05$ and $P < .001$, Fig. 3A–D). These findings indicated that AP could elevate the activation of PI3K/AKT and mTOR signal pathways.

3.4. AP up-regulated expression of miR-126

From Fig. 4A, we found that miR-126 was notably up-regulated by AP ($P < .01$). Besides, level of miR-126 was reduced when cells were treated with H_2O_2 in comparison with control ($P < .01$), while was specifically increased after AP treatment ($P < .01$). These findings suggested that AP notably up-regulated miR-126.

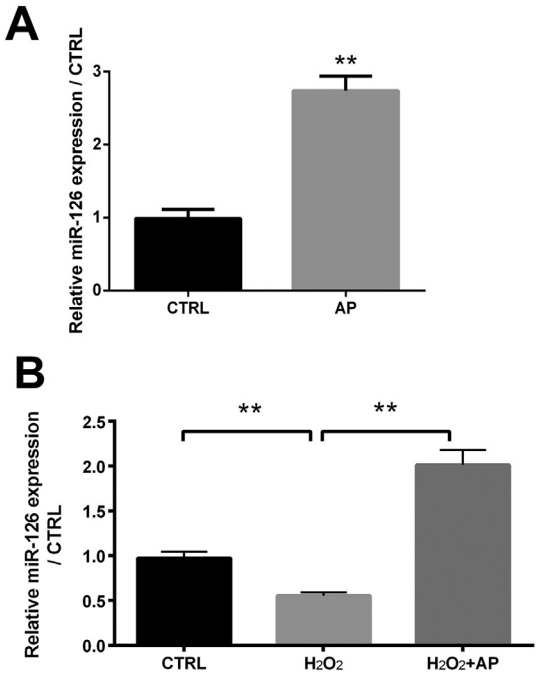

Fig. 4. MiR-126 was up-regulated by AP. mRNA level of miR-126 was measured via qRT-PCR. ** $P < .01$ in comparison with indicated group.

3.5. AP attenuated cellular oxidative damage by up-regulating miR-126

First, we found that level of miR-126 was notably increased ($P < .001$, Fig. 5A) after miR-126 mimic transfection. Besides, H_2O_2-induced cell viability attenuation was notably eliminated ($P < .05$, Fig. 5B) and levels of p53 and p21 were notably decreased (both $P < .05$, Fig. 5C-D) by miR-126 mimic. Moreover, H_2O_2-induced apoptosis was alleviated by miR-126 mimic ($P < .05$, Fig. 5E). Similarly, levels of cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were also attenuated (all $P < .05$, Fig. 5F-G) after transfection with miR-126 mimic. In addition, H_2O_2-induced ROS generation was notably attenuated by miR-126 mimic ($P < .05$, Fig. 5H). These findings indicated that miR-126 mimic could block H_2O_2-induced damage on HaCaT cells.

Then, expression of miR-126 was obviously inhibited after miR-126 inhibitor transfection ($P < .01$, Fig. 7A). Cell viability was significantly increased after treatment with AP ($P < .05$, Fig. 7B), however, inhibition of miR-126 could significantly decrease cell viability in comparison with its NC group ($P < .01$, Fig. 7B). Similarly, from Fig. 7C-D, levels of p53 and p21 were significantly decreased after treatment with AP (both $P < .05$), while were apparently increased by miR-126 inhibitor (both $P < .05$). For cell apoptosis, it was significantly decreased after treatment with AP ($P < .05$), while was notably increased after miR-126 inhibitor transfection ($P < .05$, Fig. 7E). Similarly, levels of cleaved-PARP, cleaved-caspase-3 and cleaved-caspase-9 were notably up-regulated after transfection with miR-126 inhibitor ($P < .05$, $P < .05$ and $P < .01$, Fig. 7F-G). In addition, ROS generation was significantly increased after H_2O_2 treatment, while was notably decreased by the adding of AP ($P < .01$, Fig. 7H). However, it was notably increased after transfection with miR-126 inhibitor ($P < .05$, Fig. 7H), indicating that miR-126 inhibitor could inhibit the attenuation role of AP in H_2O_2-induced ROS generation. Above all, our results suggested that AP could reduce cellular oxidative damage possibly by up-regulating miR-126.

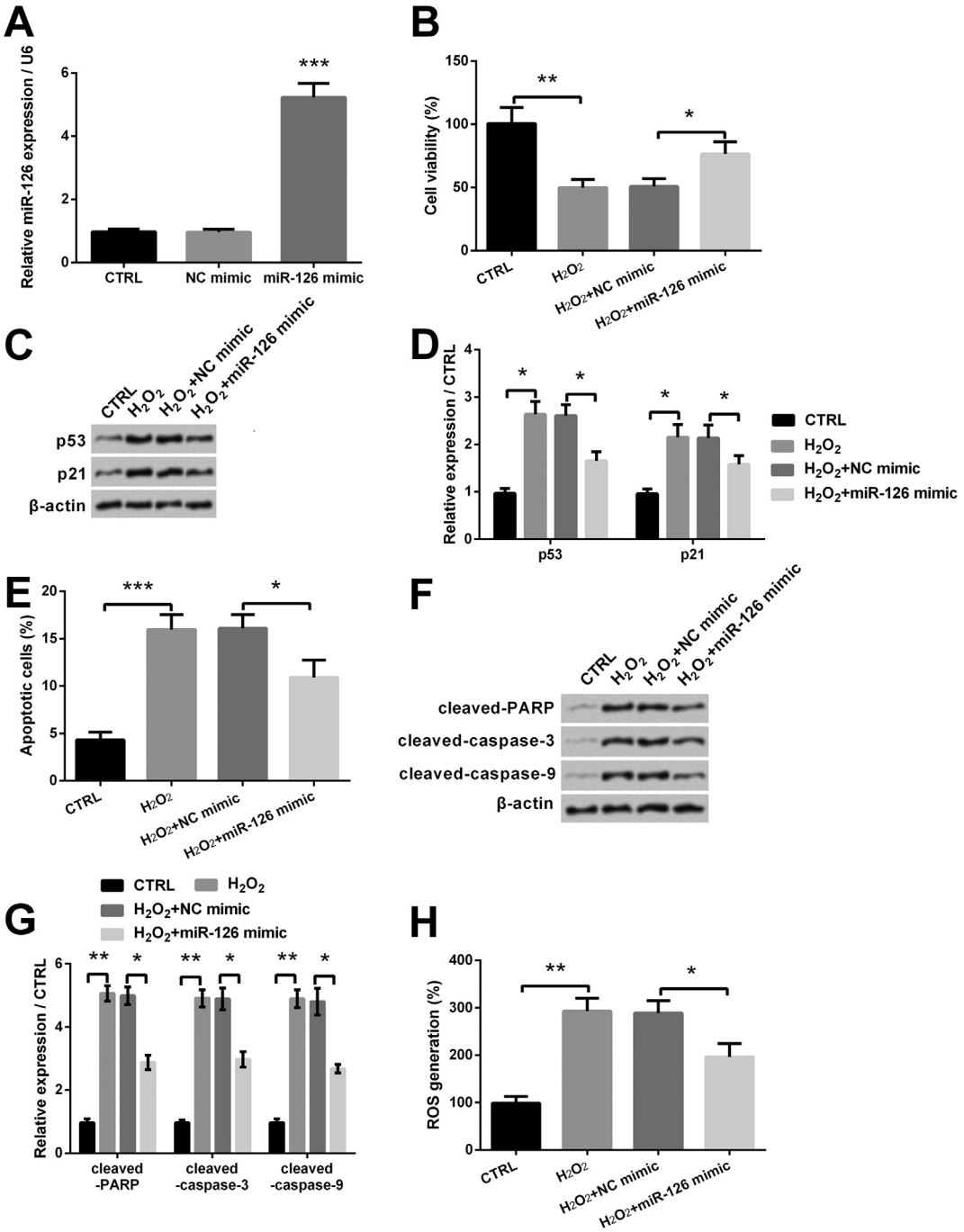

Fig. 5. Effects of miR-126 mimic on H_2O_2-induced HaCaT cells damage were tested after transfection with miR-126 mimic. (A) Level of miR-126 was tested via qRT-PCR after transfection with miR-126 mimic. (B) Cell viability was tested through CCK-8 assay (C) Expression of p53 and p21 was tested via western blot. (D) Levels of p53 and p21 were tested via western blot quantitative. (E) Cell apoptosis was tested through flow cytometry. (F) Expression of apoptosis relative proteins was tested via western blot. (G) Level of apoptosis relative proteins was detected via western blot quantitative. (H) Generation of ROS was tested through ROS assay. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with indicated group.

3.6. AP promoted the activation of PI3K/AKT and mTOR signal pathways by up-regulating miR-126

To further research the mechanism of AP, we detected the PI3K/AKT and mTOR signal pathways. From Fig. 6, we found that levels of p-PI3K, p-AKT and p-mTOR were notably reduced by H_2O_2 (all $P < .01$), while were significantly increased after transfection with miR-126 mimic (all $P < .01$). This finding indicated that miR-126 mimic could eliminate H_2O_2-induced the inhibition of PI3K/AKT and mTOR pathways. From Fig. 8, we found that levels of p-PI3K, p-AKT and p-mTOR were enhanced after treatment with AP ($P < .01$, $P < .05$ and $P < .01$), however, they were decreased when miR-126 were inhibited compared with its NC group ($P < .01$, $P < .05$ and $P < .01$). Our findings indicated that AP could promote activation of these two signal pathways, while AP plus miR-126 inhibitor could suppress these signal pathways. We concluded that AP promoted the activation of PI3K/AKT and mTOR signal pathways possibly by up-regulating miR-126.

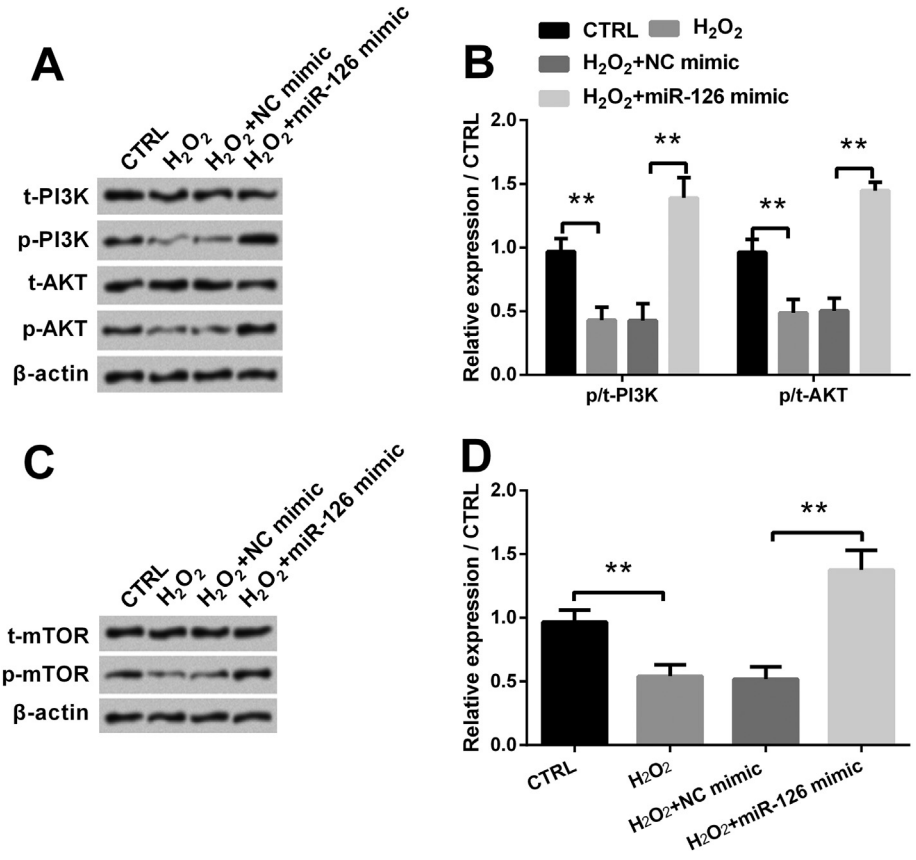

Fig. 6. Effects of miR-126 mimic on H_2O_2-induced inhibition of PI3K/AKT and mTOR signal pathways were tested after transfection with miR-126 mimic. (A) Expression of PI3K/AKT pathway relative factors was tested through western blot. (B) Levels of PI3K/AKT pathway relative factors were tested through western blot quantitative. (C) Expression of mTOR was tested through western blot. (D) Level of mTOR was tested through western blot quantitative. ** $P < .01$ in comparison with indicated group.

4. Discussion

PUs are common complications in elderly and nerve-damaged patients, resulting in changes in the morphology and function of the skin and internal organs. PUs are caused by unresolved pressure, shear or friction, accompanying by skin oxidative damage, resulting in damage to local areas of the skins and underlying tissue (Crenshaw and Vistnes, 1989). Therefore, relieving cellular oxidative damage can be an effective method to cure PU. AP, known as the effective component of *Angelica sinensis*, has been proved to exert protective effect on liver damage and anti-oxidation induced by carbon tetrachloride (CCl4) (Zhang et al., 2010). This finding suggested that AP has function in relieving oxidative damage. H_2O_2 had been considered as a common form of ROS (Zhuang et al., 2016), which is able to induce cell apoptosis (Nguyen et al., 2013). In this study we used H_2O_2 to induce oxidative damage in HaCaT cells. Our study firstly research the relieve mechanism of AP in H_2O_2-caused oxidative injury in HaCaT cells. We got that AP was to be effective in protecting HaCaT cells from H_2O_2-caused oxidative damage. AP could inhibit apoptotic process and reduce the production of ROS to attenuate cellular oxidative damage through the regulation of miR-126. These results indicated that AP played an important role in attenuating H_2O_2-caused cellular oxidative damage.

Previous studied showed that AP had various pharmacological activities and relatively low toxicity (Jin et al., 2012). For example, AP played a liver protective role through regulating amino, energy and lipid metabolism (Ji et al., 2014). And it protected the nerve through controlling cerebral ischemia, increasing the number of microvessels and ameliorating blood flow after cerebral ischemia (Ai et al., 2013; Lei et al., 2014). These findings also revealed that AP had no side-effects on the organism. Additionally, there had been many studies testing the anti-oxidant activity of AP. For example, AP had been proved to exert anti-oxidant, anti-apoptotic and anti-inflammatory roles to protect chondrocytes from H_2O_2-caused damage (Zhuang et al., 2016). Moreover, AP attenuated H_2O_2-induced PC12 neuronal apoptosis and ROS levels (Lei et al., 2014). These reports suggested that AP exerted a key function in relieving oxidative damage. However, the influence of AP in oxidative damage in PUs still remains unknown. HaCaT cells are human immortalized epidermal cells derived from human skin. H_2O_2 treatment model of HaCaT cells has been established to investigate skin cellular oxidative damage (Nguyen et al., 2013). Our study was consistent with the above findings that apoptotic cells and generation of ROS were reduced after treatment with AP, suggesting that AP could attenuate cellular oxidative damage induced by H_2O_2.

To further investigate the regulation mechanism of AP, we turn our attention to miRNA. MiRNAs, which imperfectly pair with targeted mRNAs of protein coding genes and relative post-transcriptional regulation, play important roles in biological processes (Sun et al., 2010). MiR-126, reported to have anti-apoptosis and anti-inflammatory roles (Hao and Fan, 2017), is one of the most abundant miRNAs in human endothelial cells (Sui et al., 2014). It has been proved to exert a crucial function in angiogenesis signaling and cell survival (Santoro and Nicoli, 2013). Noratto et al. found that miR-126 was participated in the defensive influence of polyphenols on inflammation of HUVECs (Noratto et al., 2011). In addition, Sui et al. found that miR-126 may be participant in avoiding endothelial oxidative stress (Sui et al., 2014). These findings revealed the anti-inflammatory and anti-oxidation roles of miR-126. In the above, we mentioned that AP exerted anti-oxidative function. But whether there is a certain regulatory relationship between AP and miR-126 is still remain unclear. Our study firstly demonstrated the regulation mechanism between AP and miR-126 that AP could apparently up-regulate expression of miR-126 to play its role of attenuating cellular oxidative damage. Our study offers a target for studying the function of AP in cellular oxidative damage.

Furthermore, the development of disease is inseparable from the regulation of signal pathways. It is well known that PI3K/AKT and mTOR signal pathways play a role in variety of cellular functions,

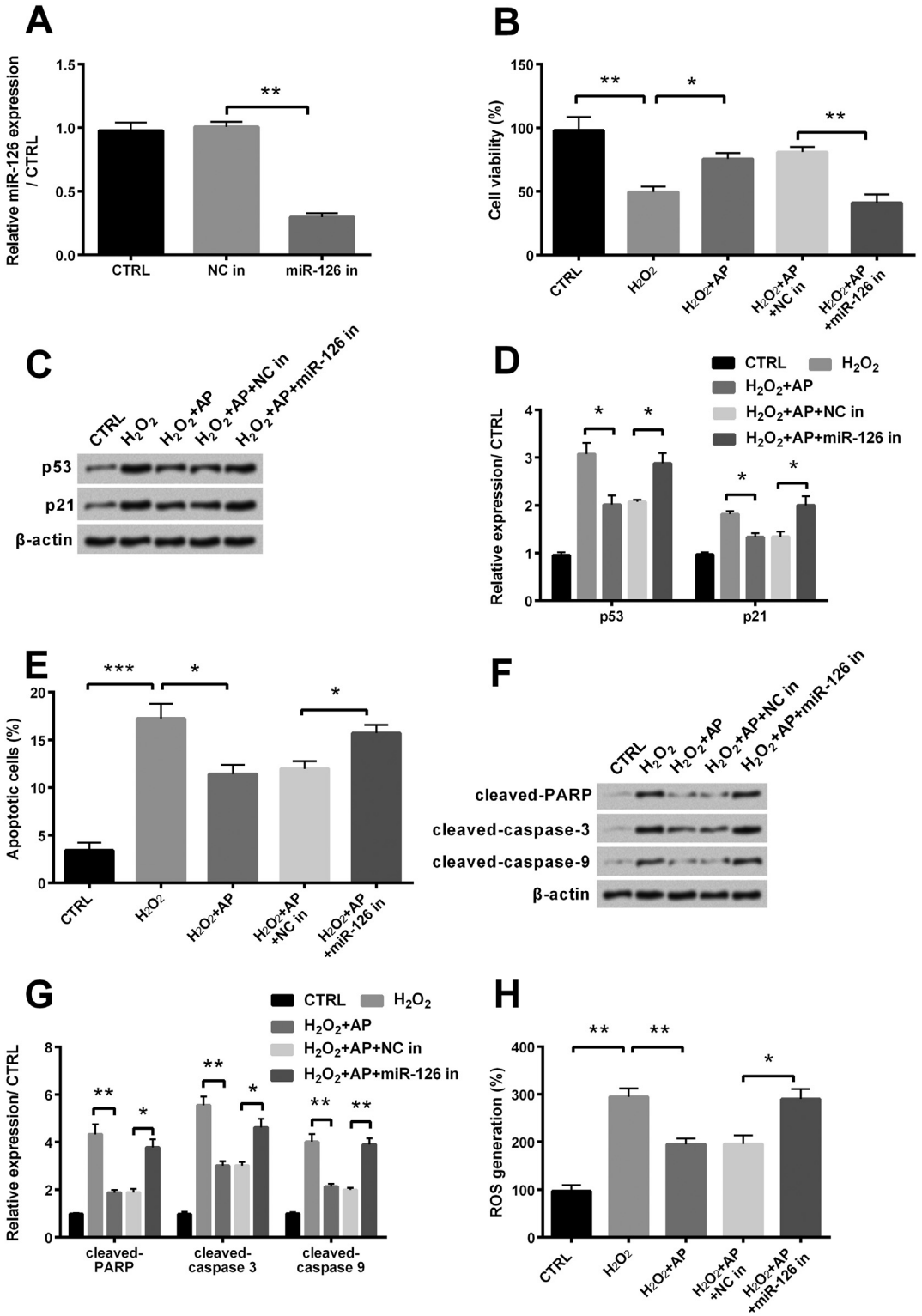

Fig. 7. Protective mechanism of AP was tested on HaCaT cells after transfection with miR-126 inhibitor. (A) Level of miR-126 was tested via qRT-PCR. (B) Cell viability was tested through CCK-8 assay. (C) Expression of p53 and p21 was tested via western blot. (D) Levels of p53 and p21 were tested via western blot quantitative. (E) Cell apoptosis was tested through flow cytometry. (F) Expression of related proteins of apoptosis was tested via western blot. (G) Levels of related factors of apoptosis were detected via western blot quantitative. (H) Generation of ROS was tested through ROS assay. * $P < .05$, ** $P < .01$ and *** $P < .001$ in comparison with indicated group.

including cell survival, cell proliferation, *etc* (Porta et al., 2014). PI3K activation leads to AKT phosphorylation, and then phosphorylation AKT leads to mTOR phosphorylation and activation (Tang and Yang, 2018). The activation of PI3K/AKT and mTOR signal pathways result in reducing cell apoptosis and promoting cell survival (Zhang et al., 2015). Consistently, we found that AP could promote the activation of these pathways to play a mitigation role in H_2O_2-induced oxidative damage. Moreover, Tang et al. found that miR-126 inhibited PI3K/AKT and

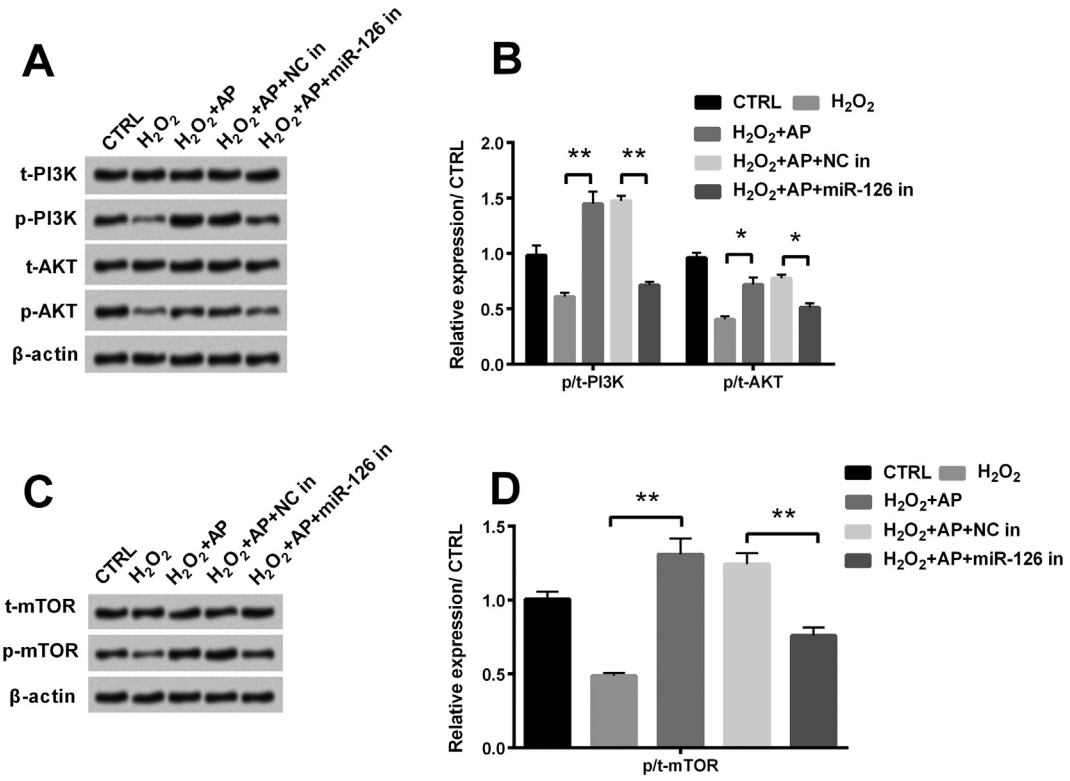

Fig. 8. Activation mechanism of AP on PI3K/AKT and mTOR signal pathways was tested after transfection with miR-126 inhibitor. (A) Expression of PI3K/AKT signal pathways related proteins was tested via western blot. (B) Levels of PI3K and AKT were measured via western blot quantitative. (C) Expression of mTOR was measured via western blot. (D) Level of mTOR was tested via western blot quantitative. * $P < .05$ and ** $P < .01$ in comparison with indicated group.

mTOR signal pathways to restore autophagic flux, attenuating ox-LDL-induced HUVECs damage (Tang and Yang, 2018). Kim et al. reported that there was a negative relationship between miR-126 and PI3K/AKT and mTOR pathways when mango polyphenolics reduced inflammation in intestinal colitis (Kim et al., 2017). Differently, we found that miR-126 mimic could increase levels of PI3K, AKT and mTOR, while the inhibition of miR-126 was accompanied by the decreasing of PI3K, AKT and mTOR. The regulatory relationship between miR-126 and these pathways may depend on the specific cell situation. Further work is needed to explore the regulatory mechanism between miR-126 and PI3K/AKT and mTOR pathways. Our findings firstly build up the regulatory relationship among AP, miR-126 and these pathways that PI3K/AKT and mTOR pathways were activated by miR-126, which was up-regulated by AP in relieving cellular oxidative damage, This study provide a theoretical basis for reducing clinical cure of cellular oxidative damage and PUs.

In conclusion, the results of our study indicated the underlying relieve mechanism of AP in cellular oxidative damage. AP could up-regulated miR-126, promote cell viability and the activation of PI3K/AKT and mTOR signal pathways, and inhibit apoptosis and the generation of ROS. Our findings suggested that AP could be an effective biomacromolecule of important significance for clinical cure of PUs.

Data availability statement

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

Declaration of Competing Interest

Authors declare that there is no conflict of interests.

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None.

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