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The anti-apoptosis effect of isovitexin on human keratinocytes by regulating miR-98-5p/Bcl-2/Bcl-xL and MAPKs/NF-κB signaling pathways

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ABSTRACT

Isovitexin is a highly bioactive food-derived flavonoid currently attracting attention for its signal transduction-based antioxidant effects. This study focused on the potential molecular mechanisms by which isovitexin protects HaCaT cells from oxidation-induced apoptosis. It was found that isovitexin significantly reversed oxidative stress-induced apoptosis by reducing ROS level. In addition, isovitexin inhibited apoptosis in a dose-dependent manner by upregulating the expression level of Bcl-xL and Bcl-2 and downregulating the expression level of Bax and caspase-3. It is important to note that the effect of isovitexin may be achieved by targeting miR-98-5p, an upstream target of Bcl-xL and Bcl-2, and overexpression of miR-98-5p counteracted isovitexin-mediated upregulation of antiapoptotic proteins. Isovitexin also inhibited apoptosis by reducing the level of IL-6, IL-1β and TNF-α, which was achieved by regulating MAPKs/NF-κB signaling pathway. Overall, this study confirmed the mechanism of multi-targeted inhibition of apoptosis by targeting miR-98-5p/Bcl-2/Bcl-xL and MAPKs/NF-κB signaling pathway by isovitexin.

1. Introduction

The skin is an essential organ that protects and regulates internal oxidative equilibrium. Environmental factors including ultraviolet radiation, smoking, and pollution frequently cause oxidative damage to skin cells, which results in DNA damage, abnormal calcium signal and mitochondrial alterations that lead to apoptosis (Wang et al., 2020). One of the main causes of cell death is apoptosis. The proteins B-cell lymphoma-xL (Bcl-xL) and B-cell lymphoma-2 (Bcl-2) are extremely critical for the regulation of cell apoptosis, which negatively regulate the discharge of pro-apoptotic proteins from mitochondria. According to studies, H_2O_2 can induce apoptosis in keratinocytes, and microRNAs (miRNAs) (e.g. miR-145, MiR-489, miR-181a, etc.) may be involved in this process (Zhang et al., 2017).

MiRNAs could block the translation of Bcl-2 and Bcl-xL to regulate cell apoptosis in disease prevention and treatment. For example, miR-181b-5p affects the cognitive function of schizophrenia patients by targeting Bcl-2 mRNA (Gou et al., 2021); let-7a can inhibit the tumorigenicity of JEG-3 cells by down-regulating Bcl-xL and YAP to enhance apoptosis (Zha et al., 2020). There is growing evidence that miR-98-5p participates in apoptosis and other biological processes (Wang et al., 2020). Moreover, the transcription of Bcl-2 can be inhibited by MAPK (ERK, p38 MAPK and JNK) and NF-κB, which are crucial in oxidative stress-induced cell death (Zhang et al., 2022). MAPK and NF-κB could also suppress apoptosis-related proteins caspase-9 and caspase-3 (Zhang et al., 2022), and promote cell apoptosis by promoting the release of inflammatory factors. Numerous studies have reported that IL-6, IL-1β and TNF-α could enhance the cleavage level of caspase-9, which increases the rate of apoptosis (Wang et al., 2020).

Flavonoids are phenolic compounds widely found in diet and plants, which show significant anti-apoptosis activity in animal studies and human experiments (Hu et al., 2019). Dietary flavonoids can mediate the apoptosis of many kinds of cells by regulating miRNA-mRNA axis as well as MAPKs/NF-κB signaling pathway. Isovitexin (also known as apigenin-6-C-glucoside) is a kind of dietary flavonoid with remarkable oral bioavailability. As one of the main active components in mung bean peel, isovitexin is gaining more attentions for its various biological properties, such as antioxidant, anti-inflammatory and anticancer activities. Isovitexin can protect $PM_{2.5}$-mediated oxidative stress and induce stemness in epidermal cells *via* scavenging ROS (Chowjarean et al. 2019). Isovitexin can also protect against oxidative damage by regulating apoptosis. It has been reported that isovitexin protected PC12

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cells from apoptotic *via* decreasing the activity of caspase-8 (Lin et al., 2009). Lv et al. found that the intravenous injection of isovitexin inhibited H_2O_2-induced cytotoxicity and apoptosis, showing significant anti-inflammatory and anti-apoptosis ability (Lv et al., 2016). Besides, isovitexin can affect apoptosis and inhibit the proliferation of tumor cells by increasing miR-34a and decreasing its target gene Bcl-2 (Liang et al., 2019).

In this study, HaCaT cells were used to investigate how isovitexin would exert anti-apoptosis functions in skin keratinocyte cells and its underlying mechanism. On top of broadening our view on the mechanisms of miRNA and Bcl-2-mediated apoptosis, this study also bridges the gap in our understanding of the involvement of miRNA and Bcl-2/Bcl-xL signaling in the protection of heterovascular letters.

2. Materials and methods

2.1. Cell culture and cell survival rate determination

Cultivate HaCaT cells (purchased from Shanghai Enzyme Research Biological Company) refers to previous studies (Wang et al., 2020). In conformity with quondam approach, MTT assay was used to measure cell survival rate (Rong et al., 2022). The cells were added with the prepared complete medium and cultured in an incubator at 37°C and 5% CO_2. After being treated with or without isovitexin and hydrogen peroxide, MTT was added for two hours. Then dissolved in DMSO and measure absorbance (Supplementary materials).

2.3. Measurement of reactive oxygen species (ROS) level

The method of measuring ROS level refers to previous studies (Hao et al., 2021a). After being treated with or without isovitexin and hydrogen peroxide, DCFH-DA (25 μM) was added, and multifunctional microplate reader (SpectraMax® M5, Shanghai, China)was used to detect fluorescence intensity after 30 min (Supplementary materials).

2.4. Apoptosis assay

Apoptosis assay in conformity with quondam approach (Liu et al., 2021). Annexin V-FITC/PI apoptosis detection kit (Wuhan Saiweier Biotechnology Co., Wuhan, China) was used to detect apoptosis rate. After being digested by trypsin without EDTA,collect cells and add V-FITC and PI. After incubation 10 min, cells were resuspended again by adding 400 μL binding buffer. The flow cytometry (Beckman Coulter, California, America) was used for detection and analysis (Supplementary materials).

2.5. Enzyme-linked immunosorbent assay (ELISA)

Extract protein and measure concentration. The enzyme activities of IL-6, IL-1β and TNF-α in protein were detected by ELISA kit (lengton, Shanghai, China), on the basis of the manufacturer's guidelines.

2.6. Quantitative real time polymerase chain reaction (qRT-PCR)

The qRT-PCR procedure was in conformity with the steps of previous assay (Liu et al., 2021), use the PrimeScript RT reagent kit or Mir-X miRNA First-Strand synthesis kit to synthesize cDNA and quantify mRNA or miRNA, respectively. Real-time fluorescence quantitative PCR was performed by PCR kit (Supplementary materials).

2.7. Western Blotting assay

The protein expression was detected by Hao's method (Hao et al., 2021b).[24] After the protein was transfected into PVDF membrane, the primary antibody and the anti-rabbit IgG (1:10000 dilution, abcam, Shanghai, China) were incubated for 10h and 2h respectively. The Image Lab TM version was used to examine bands (Supplementary materials).

2.8. MiRNA prediction

The bioinformatics software ENCORI (https://starbase.sysu.edu.cn) was used to predict miRNA.

2.9. Statistical analyses

The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. Statistical analysises were carried out by one-way analysis of variance (ANOVA) and Duncan's multiple range test using the SPSS 16.0 software. $P < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Isovitexin protected HaCaT cells from H_2O_2-induced damage by influencing cell apoptosis

Based on the IC_{50} values of H_2O_2, 900 μM H_2O_2 was used to treat cells for four hours to perform the subsequent studies (Fig. 1**A**). There was without significant disparation in cell viability with low concentrations of isovitexin (0-40 μM). Thereby, the isovitexin at concentrations of 5 μM, 10 μM and 20 μM in the non-toxic concentrations were selected for following experiment. Isovitexin was found to protect HaCaT cells from H_2O_2-induced oxidative damage through counteracting initiated decrease in cell viability. The treatments with isovitexin for 24 h significantly improved HaCaT cell survival rate, with the increase of 16%, 25% and 27%, respectively (Fig. 1**C**). And it is demonstrated the protection of isovitexin again by crystal violet staining experiment (Fig. 1**F**). And isovitexin notably suppressed H_2O_2-initiated increase in cellular ROS generation (Fig. 1**D, G**).

In addition, H_2O_2 is the activator of apoptosis, so the effect of isovitexin on cell apoptosis has been examined by flow cytometry experiments. Dealing with H_2O_2 induced the apoptosis in HaCaT cells with an increase of apoptosis rate by about 20% compared with the control group, while isovitexin dose-dependently dropped the percentage of apoptosis (Fig. 1**F, H**). When treatment of isovitexin at 20 μM, the apoptosis rate was reduced by16.59% compared with the H_2O_2 treatment group. The above experiments indicated that isovitexin protected HaCaT cells from H_2O_2-indiced oxidative damage *via* inhibiting cell apoptosis.

3.2. Isovitexin alleviated H_2O_2-induced apoptosis through Bcl-xL/Bcl-2/caspase-3 signaling pathway

The gene and proteins level of apoptosis was examined to demonstrate the effect of isovitexin in H_2O_2-induced HaCaT cells. Isovitexin of 10 μM observably reversed H_2O_2-induced down-regulating of Bcl-xL mRNA, and dissimilar concentrations of isovitexin also imposed Bcl-2 transcription (Fig. 2**A-B**). Isovitexin was found to increase expression of Bcl-xL and Bcl-2 and suppress the protein level of Bax (Fig. 2**F-H**). Likewise, high concentration of isovitexin (20 μM) inhibited H_2O_2-induced up-regulation of pro-apoptosis protein, Bax protein (Fig. 2**C**). As for the downstream apoptosis-related genes, isovitexin (10 μM and 20 μM) significantly inhibited caspase-3 mRNA level (Fig. 2**D**), and showed the similar changes in their protein level (Fig. 2**I**), suggesting the inhibitory action of isovitexin on the activity of caspase family. It is demonstrated that isovitexin inhibited apoptosis in HaCaT cells may through Bcl-xL/Bcl-2/caspase-3 axis.

3.3. miR-98-5p mediated the protective effect of isovitexin in H_2O_2-induced HaCaT cells

3.3.1. The role of miR-98-5p in the protection of isovitexin against H_2O_2-induced cell damage

Although it has been proved that miRNAs have the ability to regulate apoptosis, it is not clear whether miRNAs are involved in the

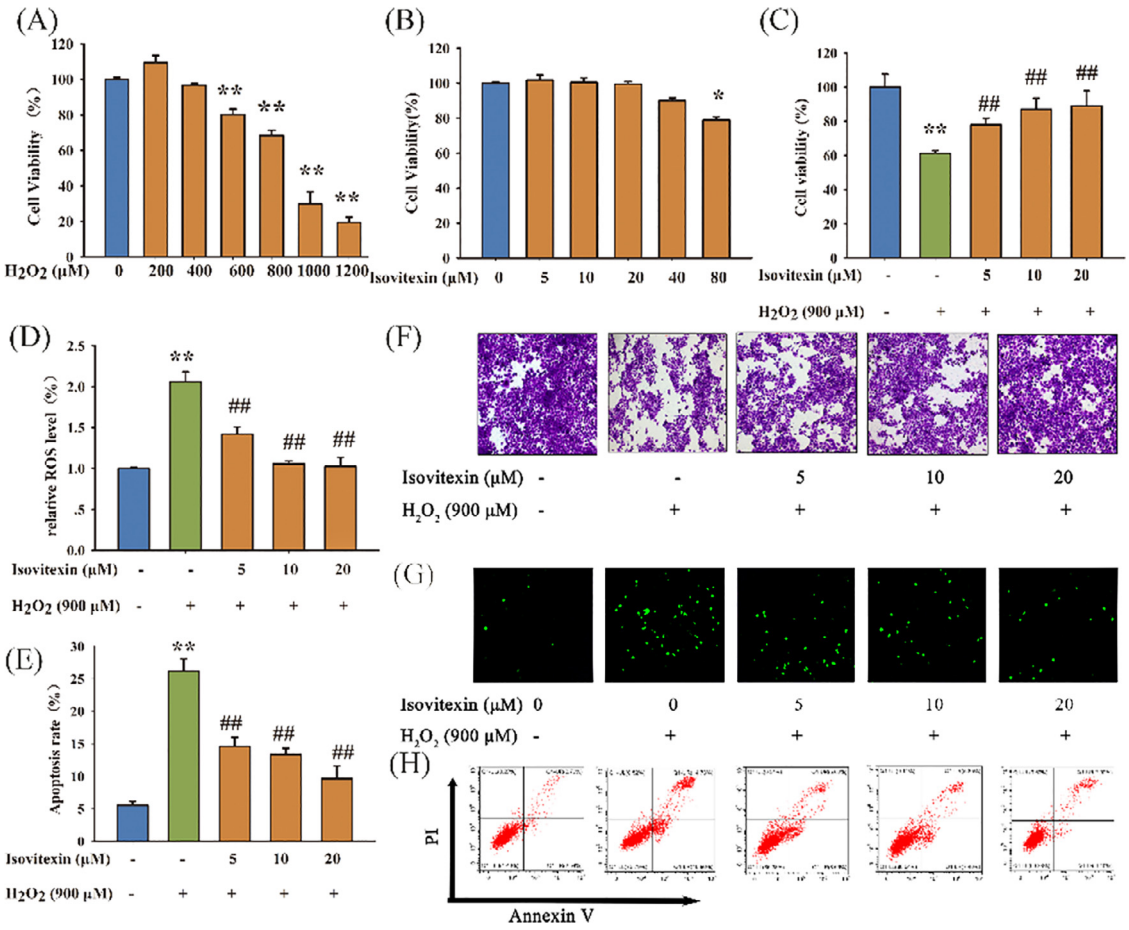

Fig. 1. The protection of isovitexin in H_2O_2-indiced HaCaT cells. (A-C) Influence of H_2O_2, isovitexin and both on HaCaT Cell survival rate; (D) ROS level; (E) The percentage of apoptosis; (F) Crystal violet staining assay; (G) DCF fluorescent probe staining; (H) Cell apoptosis. The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ vs. the control group; $\#(P < 0.05)$, $\#\#(P < 0.01)$ vs. the H_2O_2 treatment group.

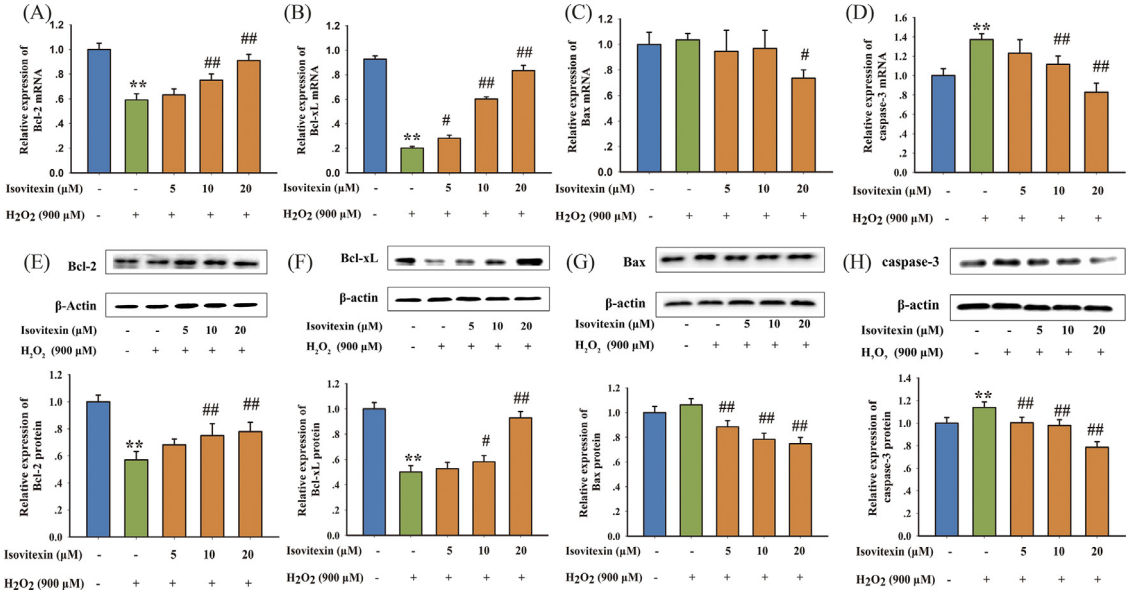

Fig. 2. Effects of isovitexin on Bcl-2 (A, E), Bcl-xL (B, F), Bax (C, G) and caspase-3 (D, H). The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ vs. the control group; $\#(P < 0.05)$, $\#\#(P < 0.01)$ vs. the H_2O_2 treatment group.

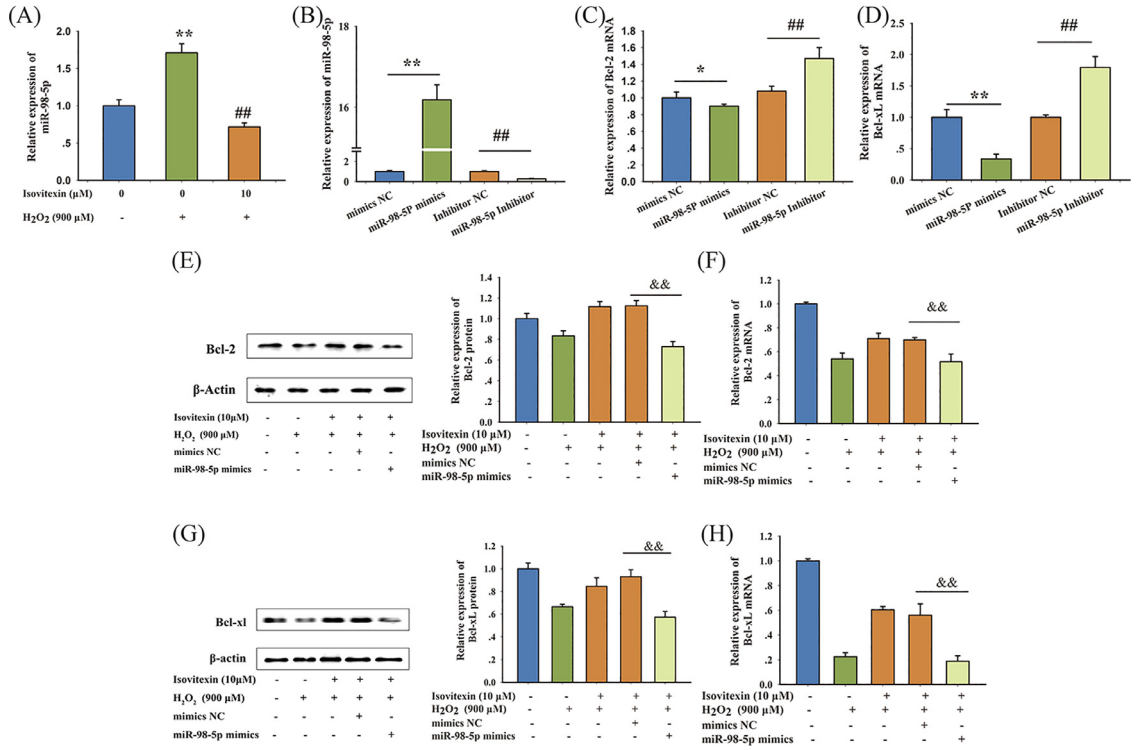

Fig. 3. Influences on Bcl-xL and Bcl-2 after overexpression or inhibition of miR-98-5p in HaCaT cells. (A) The inhibition of isovitexin on miR-98-5p expression in H_2O_2-induced HaCaT cells by RT-qPCR method; (B) The miRNA level of miR-98-5p detected by RT-qPCR in order to verify the efficiency of transfection; (C, D) The Bcl-2 (C) or Bcl-xL (D) mRNA level after overexpression or inhibition of miR-98-5p by RT-qPCR.; (E) The protein level of Bcl-2; (F) The mRNA level of Bcl-2; (G) The protein level of Bcl-xL (H) The mRNA level of Bcl-xL. The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ vs. the mimics NC; #($P < 0.05$, ##)$P < 0.01$ vs. the inhibitor NC; &(P < 0.05), &&(P < 0.01) vs. the isovitexin+H_2O_2+mimics NC group.

anti-apoptosis effect of isovitexin in HaCaT cells. Therefore, miRNAs which can directly target Bcl-2 or Bcl-xL gene were predicted by ENCORI. Interesting, miR-98-5p was predicted to directly target both Bcl-xL and Bcl-2 gene. It can be hypothesized that isovitexin might target to miR-98-5p, simultaneously regulating Bcl-xL and Bcl-2 gene to exert enhanced anti-apoptosis effect. In this study, isovitexin markedly inhibited H_2O_2-initiated up-regulation of miR-98-5p by qPCR (Fig. 3**A**), and the targeting relationships between the anti-apoptosis gene (Bcl-2 and Bcl-xL) and miR-98-5p in HaCaT cells were examined by qPCR after overexpression or inhibition of miR-98-5p. And the overexpression and inhibition of miR-98-5p cells were successfully constructed (Fig. 3**B**). It is shown that overexpressing miR-98-5p down-regulated both Bcl-xL and Bcl-2 at the level of transcription, while the inhibition of miR-98-5p was contrary to the overexpression (Fig. 3**C, D**). Furthermore, overexpressing miR-98-5p significantly lessen impact of isovitexin on the Bcl-2 **(**Fig. 3**E, F)** and Bcl-xL **(**Fig. 3**G, H)** mRNA and protein level. Our results are also consistent with previous ones, which have been confirmed that miR-98 directly targeted 3′-UTR of Bcl-2 and Bcl-xL through luciferase reporter gene system (Wang et al., 2016; Xia et al., 2014). The above results showed that isovitexin protected cells from H_2O_2-induced oxidative damage might through inhibiting miR-98-5p, negatively regulating both Bcl-2 and Bcl-xL as well as downstream apoptosis related signaling pathway.

3.3.2. The biological functions of miR-98-5p in the protection of isovitexin against H_2O_2-induced cell damage

To verify if miR-98-5p affected the anti-apoptosis effect of isovitexin on HaCaT cells, another isovitexin +H_2O_2 group was set to overexpress miR-98-5p. The group of transfecting miR-98-5p mimics significantly suppressed the effect of isovitexin on cell viability (Fig. 4**A**). The overexpression of miR-98-5p also notably counteracted the inhibition of isovitexin on both ROS level and cell apoptosis (Fig. 4**B-D**). In brief, miR-98-5p negatively regulates the effects of isovitexin on cell survival rate, ROS and apoptosis rate of HaCaT cells.

3.4. Isovitexin alleviated the inflammatory response induced by H_2O_2 through MAPKs/NF-κB signaling pathway

As we known, oxidative stress and inflammation seem to be "evil twins of aging" (Hindmarch, 2002). ROS plays essential role in redox signaling and active multiple pathways, including NF-κB and MAPK, in response to stress. Therefore, PCR and ELISA were used to test inflammatory factors level (Fig. 5). The treatment of H_2O_2 increased IL-6, IL-1β and TNF-α mRNA level, and isovitexin counteracted the increased expression. The IL-6, IL-1β and TNF-α protein level by isovitexin also have the similar changes while high concentration of isovitellin restored it to normal level (Fig. 5**D-F**).

To further elaborate on the mechanism of how isovitexin inhibited the expression of inflammatory factors to protect against oxidative damage, the upstream signaling pathways, such as NF-κB and MAPK were examined through Western Blot. The exposure to 900 μM H_2O_2 significantly increased the relative protein expression of p-p65 (Fig. 5**I**), and isovitexin inhibited the up-regulation of p-p65 protein. Isovitexin also significantly suppressed H_2O_2-induced phosphorylation of JNK and p38 (Fig. 5 **G, H**). These data proved that isovitexin protected cells from oxidative damage might also through down-regulating MAPKs/NF-κB signaling pathway.

4. Discussions

As the largest human organ, skin can be easily exposed to ultraviolet radiation, air particles or external trauma and vulnerable to oxidative stress. Oxidative stress, due to the accumulation of excessive ROS including hydrogen peroxide, superoxide anion and peroxyl radicals, can

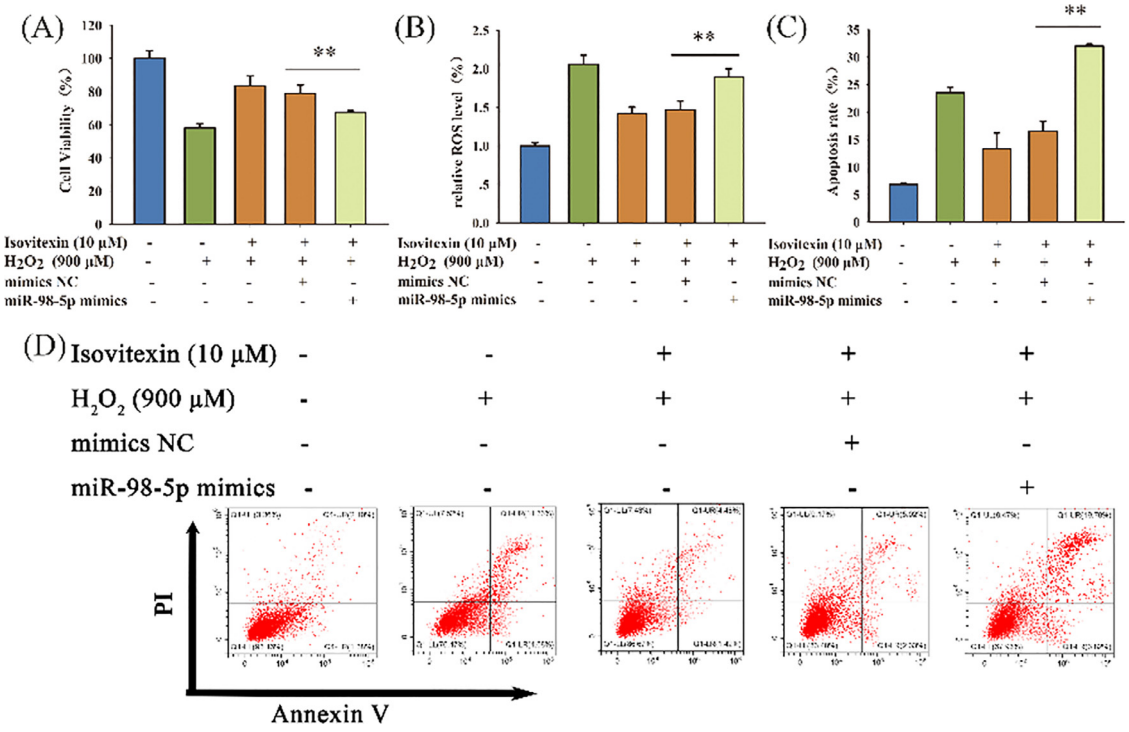

Fig. 4. Influence of miR-98-5p on the protection of isovitexin in H_2O_2-induced HaCaT cells. (A) Survival rate of HaCaT cells overexpressing miR-98-5p; (B) ROS level of HaCaT cells overexpressing miR-98-5p; (C-D) apoptosis rate of HaCaT cells overexpressing miR-98-5p. The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. $^{*}(P < 0.05)$, $^{**}(P < 0.01)$ vs. the isovitexin+H_2O_2+mimics NC group.

damage DNA, protein and other biomolecular, leading to aging, inflammation and cancer. H_2O_2 is the most common active oxygen, and various diseases are accompanied by over-expression of H_2O_2 (Mauler et al., 2019). H_2O_2 can easily cross the cell membrane and react with intracellular iron ions through Fenton reaction to generate high electrophilic free radicals, which then trigger a chain of cell-damaging events. Keratinocytes located in the outermost layer of human skin, thus acting as a barrier to protect the skin from external stimuli. And keratinocytes could continuously produce lipids, ceramides, free fatty acids and cholesterol during epidermal differentiation, which is vital to the body's defense system.

Therefore, phytochemicals derived from food, such as flavonoids, which are crucial for maintaining skin health by preventing oxidative stress, have become the focus of our attention. Flavonoids, which are abundant dietary polyphenols in plants, can inhibit oxidation and apoptosis of skin cells, prevent endogenous pigmentation, and relieve skin diseases such as psoriasis, acne and atopic dermatitis (Kang et al., 2014). It has been observed that apigenin affected the expression of hyaluronic acid synthase, hyaluronic acid and antibacterial peptide of HaCaT, thus improving physical and chemical skin barrier (Park et al., 2020). Gu, Y et al found that 4,4′-Dimethoxychalcone induced autophagy activation through phosphorylation of ULK1, alleviating HaCaT cell aging and UVB-induced photoaging in mice (Gu et al., 2022). Isovitexin is a monosaccharide flavonoid, which has been found in many natural plants and fruits. Isovitexin has the advantages of easy extraction, stable properties, high bioavailability, and weak or even no toxicity (Choo et al., 2012),which plays a crucial role in reducing oxidative stress, inflammation and apoptosis (Liu et al., 2020).

In this study, we found that isovitexin could protect human keratinocyte HaCaT by attenuating H_2O_2-induced apoptosis. Many studies have proved the protective effect of isovitexin through cell experiments, which is an effective method to study the mechanism of action. Our team recently discovered that isovitexin could protected HaCaT cells from oxidative damage *via* regulating Nrf2/Keap1/HO-1 axis (Hao et al., 2022). Moreover, isovitexin reduced the release of lactate dehydrogenase and the production of NO in PC12 cells, and protected PC12 cells from the toxicity induced by amyloid-β25-35 peptide (Guimarães et al., 2015). In this work, we found that isovitexin protected HaCaT cells from oxidative damage by significantly improving cell survival, reducing ROS and inhibiting apoptosis in a non-toxic dose range (Fig. 1). Therefore, in the following experiment, we investigated the mechanism of the isovitexin regulating apoptosis.

Apoptosis is a programmed cell death that is regulated and controlled by genes. Anti-apoptosis proteins Bcl-xL and Bcl-2 can sequester BH3 domain protein in stable mitochondrial complexes. It hinders the capacity of apoptotic stimulation to cause the opening of permeability transition pores, which prevents the activation of Bax. Moreover, they inhibit caspase-9 activating, subsequently suppressing of downstream caspase-3 processing and activation, and eventually preventing cell apoptosis. Previous research has pointed out that when cells stimulated by inflammation or oxidative stress, mitochondrial signals involving Bcl-xL, Bcl-2, Bax and caspase-3 in disparate cell lines can be activated to induce apoptosis, and flavonoids can reverse this phenomenon (Chen et al., 2018). This study revealed that isovitexin significantly reversed the H_2O_2-induced down-regulation of Bcl-xL and Bcl-2 and the up-regulation of Bax and caspase-3 at the transcription and translation level (Fig. 2). These findings suggested the potential anti-apoptosis mechanism of isovitexin, which is consistent with the results of other researcher's research that isovitexin protects mice from acute lung injury through anti-apoptosis mechanism (Lv et al., 2016).

According to an ever-increasing research reports, miRNAs are abnormally expressed in cancer, cardiovascular, neurological diseases, diabetes, toxicology and many diseases (Rong et al., 2022). However, flavonoids can prevent various diseases by regulating the expression of miRNAs (Hao et al., 2022; Liu et al., 2020). MiRNAs also play a key role in regulating apoptosis, but their underlying mechanism is still poorly elucidated. It was indicated that Bcl-2 and Bcl-xL were the key proteins of isovitexin in protecting HaCaT cells from oxidative damage. Accord-

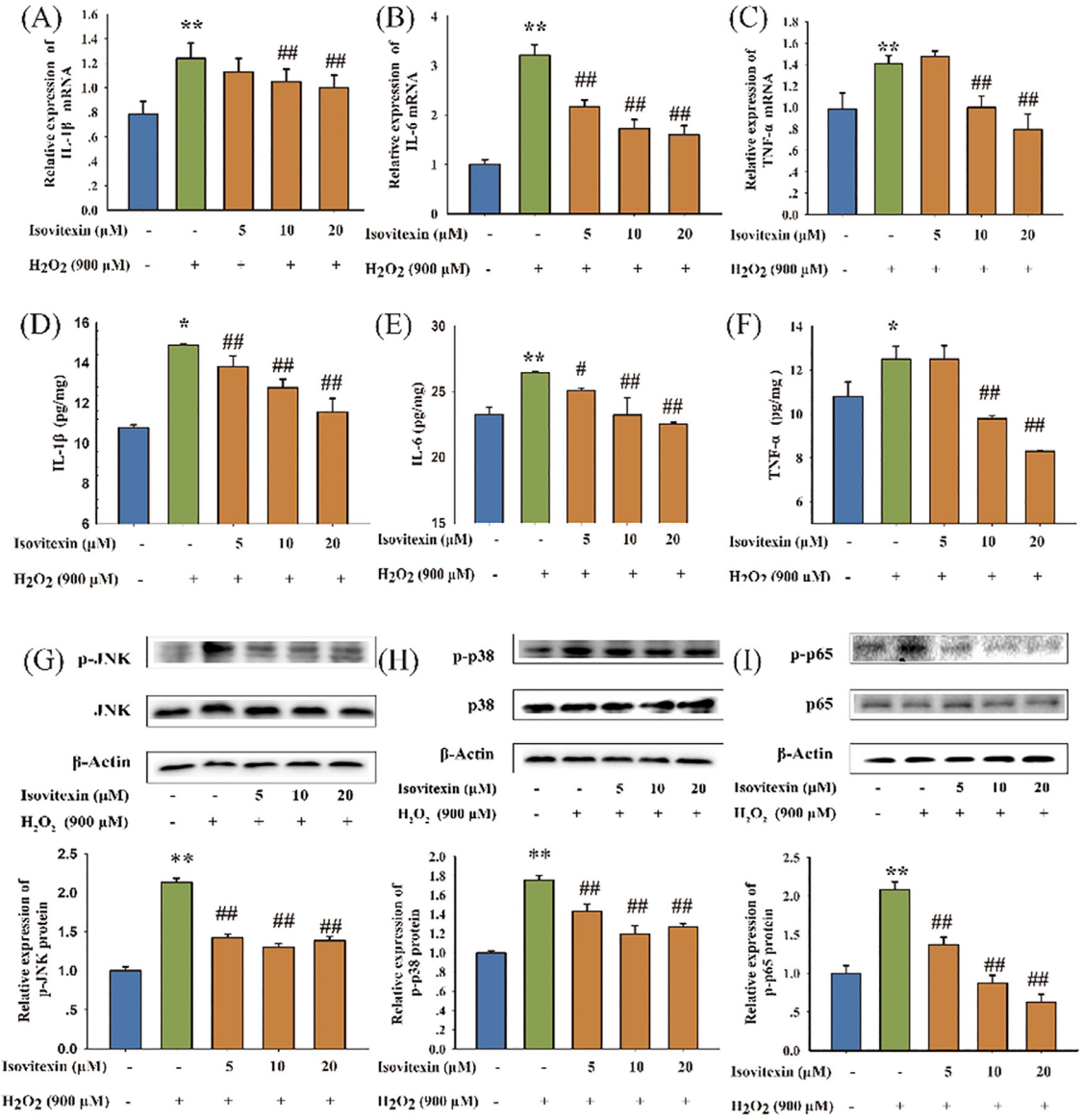

Fig. 5. Effects of isovitexin on inflammation in H_2O_2-induced HaCaT cells. (A) The mRNA level of IL-1β; (B) The mRNA level of IL-6; (C) The mRNA level of TNF-α; (D) The level of IL-1β; (E) The level of IL-6; (F) The level of TNF-α; (G) The protein level of p-JNK; (H) The protein level of p-p38; (I) The protein level of p-p65. The data were expressed as "mean ± standard deviation" and obtained from triplicate dependent experiments. *(P<0.05), **(P<0.01) vs. the control group.

ing to bioinformatics predictions and extensive literatures, miR-98-5p can target Bcl-2 and Bcl-xL (Wang et al., 2016; Xia et al., 2014), and the repeated PCR experiments also showed the same expression trend. We found that isovitexin could significantly reduce the miR-98-5p level in H_2O_2-induced HaCaT cells, which may be the key to the cell protection by isovitexin (Fig. 3**A**). Previous studies have been proved the vital function of miR-98 in regulating cell apoptosis. Wenya Ma et al. indicated that H_2O_2 accounted for the increase of miR-98 level in cardiac progenitor cells, while miR-98 inhibitors suppressed H_2O_2-induced cell proliferation and apoptosis (Ma et al., 2018). Besides, the transfection of miR-98 mimics resulted in the increase of apoptosis and the decrease of proliferation in HaCaT cells (Khan et al., 2020). MiR-98-5p plays a significant role in the cell apoptosis and oxidation induced by hydrogen peroxide. It seems reasonable that miR-98-5p may play a significant role in the cell apoptosis and oxidative damage in HaCaT cells. Consistent with the previous opposite trend of miR-98-5p and Bcl-2, Bcl-xL (Wang et al., 2016; Xia et al., 2014), this study found that miR-98, as the pivotal non-coding RNA, participated in inhibitory effect of isovitexin on HaCaT apoptosis. And miR-98-5p overexpression reduced the increase of Bcl-xL and Bcl-2 triggered by isovitexin (Fig. 3). Furthermore, we demonstrated that miR-98-5p contributed in the protection of isovitexin against HaCaT cell injury using MTT, DCF fluorescent probe staining and flow cytometry experiments (Fig. 4). Therefore, this study showed that isovitexin can promote Bcl-xL and Bcl-2 level by down-regulating miR-98-5p, and protect cells from H_2O_2-induced damage and apoptosis.

The MAPK family plays a vital role in many physiological processes such as apoptosis and inflammation, while JNK and p38 MAPK belong to the classic MAPK family and are widely expressed in mammalian cells. By acting on IKK or p65 subunit, MAPK can directly or indirectly regulate the activity of NF-κB, which is the key transcription factor to promote the expression of inflammatory cytokines (Cuadrado & Nebreda, 2010). Thereby, MAPK can affect cell apoptosis by regulating the expression of inflammatory cytokines. Recently, it has been reported that H_2O_2 induced the increase of inflammatory factors in HaCaT cells, while the production of inflammatory factors was inhibited by the active plant components (Ashida et al., 2003). And isovitexin is an effective NF-κB inhibitor, which can inhibit the production of IL-6, COX-2, IL-1β

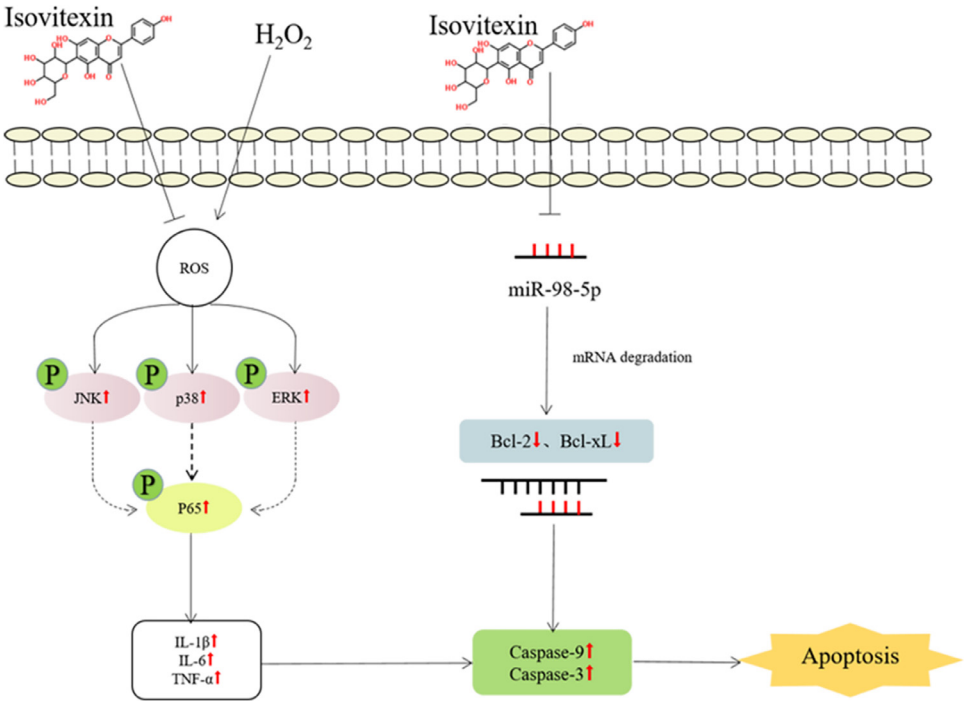

Fig. 6. The possible regulatory mechanisms underlying protection of isovitexin in H_2O_2-induced HaCaT cells

and the rest (Attiq et al., 2021). Therefore, we examined the level of IL-6, IL-1β and TNF-α, the results showed that isovitexin inhibited the expression and secretion of IL-6, IL-1β and TNF-α in HaCaT cells (Fig. 5). The results showed that the H_2O_2 treatment decreased the phosphorylation level of p65 protein, however, isovitexin reversed this H_2O_2 impact (Fig. 5**I**). Also, isovitexin significantly inhibited JNK and p38 phosphorylation induced by hydrogen peroxide (Fig. 5**G, H**), which was coincident with the published studies that isovitexin did its work to MAPK signaling pathway (Zhang et al., 2021). Little is known about the impact of isovitexin on MAPKs/NF-κB axis at oxidative environment, while this study suggests that isovitexin may accommodate inflammatory factors expression by mediating MAPKs/NF-κB signaling pathway to inhibit cell apoptosis.

To sum up, isovitexin plays a vital role in improving apoptosis *via* Bcl-xL/Bcl-2/caspase-3 axis. At the same time, miR-98-5p, which can directly target the 3'-UTR of Bcl-xL and Bcl-2, may be the key molecular target of isovitexin. In addition, isovitexin reduces cytokine level through MAPKs/NF-κB signaling pathway, thus inhibiting cell damage. This research shows that isovitexin has a very prominent role in apoptosis through miR-98-5p/Bcl-2/caspase-3 and MAPKs/NF-κB signaling pathways, and protects HaCaT cells from H_2O_2 damage. Although cell experiments can effectively verify the mechanism of action of isovitexin, it cannot directly verify its protective effect like animal experiments. In the future, the further study of isovitexin by animal experiments or human models will be examined to systematically clarify its protective effect on skin.

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Declaration of Competing Interest

No potential conflict of interest was reported by the author(s).

CRediT authorship contribution statement

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Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.focha.2023.100238.

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