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The Dietary Polyphenol Isorhapontigenin Activates Nrf2 via Targeting AKT to Exert Antioxidant and Anti-Ferroptotic Effects in SH-SY5Y Cells

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Abstract

Isorhapontigenin (ISO) is a natural polyphenol found in grapes and several common Chinese medicinal herbs. As an analog of resveratrol, it exhibits a wide spectrum of biological activities. Notably, ISO has been reported to possess neuroprotective effects, although the underlying mechanisms remain incompletely understood. Oxidative stress and ferroptosis are key pathological processes leading to neuronal damage in neurological disorders. In this study, we used an oxygen-glucose deprivation/reoxygenation (OGD/R)-induced SH-SY5Y cell model to investigate the neuroprotective effects of ISO. We examined its ability to alleviate oxidative stress and inhibit ferroptosis, and further explored the potential molecular mechanisms. This study found that ISO exerts antioxidant and anti-ferroptotic effects by targeting AKT to activate Nrf2. The results of this study aim to provide experimental evidence supporting the development of ISO as a functional food ingredient for neuroprotection.

Keywords

Isorhapontigenin, Neuroprotection, Dietary Polyphenol, AKT, Nrf2

1. Introduction

Due to the high safety profile and extensive biological activities of functional foods, they have attracted considerable attention in the field of disease prevention and clinical treatment in recent years. Isorhapontigenin (ISO), a naturally occurring dietary

polyphenol belonging to the stilbene class and a structural analog of resveratrol [1]. This bioactive compound is widely present in common food sources and food related by-products, including grapes (particularly concentrated in grape skins, seeds, and pomace, a major by-product of wine and grape juice processing) as well as edible parts or food-grade raw materials derived from a variety of traditional Chinese herbal medicines [2]. Similar to resveratrol, ISO possesses a stilbene backbone with specific hydroxyl and methoxyl substitutions, which not only ensures its good bioaccessibility in food matrices and during gastrointestinal digestion but also endows it with diverse food-relevant pharmacological activities, such as antioxidant, anti-inflammatory, and neuroprotective effects [3]. These properties make ISO a promising functional component for the development of functional foods, nutraceuticals, and food additives.

When the balance between oxidation and antioxidant reactions in the body is disrupted, oxidative stress occurs. Oxidative stress triggers a cascade of reactions that ultimately lead to neuronal death [4]. Excessive oxidative stress can trigger severe lipid peroxidation, which in turn initiates a distinct form of programmed cell death known as ferroptosis [5]. This iron-dependent process is characterized by the accumulation of reactive oxygen species (ROS) and the degradation of membrane lipids, ultimately leading to cellular demise. Given the critical roles of oxidative stress and ferroptosis in mediating neuronal injury across various neurological disorders, targeting these two interconnected pathological processes has emerged as a pivotal and promising therapeutic strategy for the treatment of such diseases.

Nrf2 is a key transcription factor that regulates oxidative stress [6]. Under normal physiological conditions, Nrf2 binds to Keap1 and is sequestered in the cytoplasm. When cellular oxidative stress increases, Nrf2 dissociates from Keap1, translocates into the nucleus, and binds to antioxidant response elements (AREs), thereby initiating downstream antioxidant signaling pathways and upregulating the expression of antioxidant factors [7]. Furthermore, studies have demonstrated that Nrf2 is also a critical regulator of ferroptosis, and activation of Nrf2 can effectively inhibit neuronal ferroptosis [8]. The PI3K/AKT pathway serves as an upstream signaling cascade governing Nrf2-mediated antioxidant responses [9]. In this study, we investigated whether ISO activates Nrf2 to exert antioxidant and anti-ferroptotic effects, and explored whether this action is mediated by targeting the AKT within the PI3K/AKT signaling pathway.

2. Experimental Section

2.1. Cell Culture and OGD/R Procedure

SH-SY5Y cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and maintained in a humidified incubator with 5% CO_2. For the OGD/R group, the culture medium was replaced with glucose-free DMEM, and cells were incubated in a hypoxic chamber

for 4 h, followed by restoration to normoxic culture conditions. ISO and the positive drug NBP (N-butylphthalide) were administered at the time of reperfusion.

2.2. Assessment of Oxidative Stress Levels

The levels of oxidative stress markers, including SOD (Beyotime, S0101S), MDA (Beyotime, S0131S), GSH (Beyotime, S0053), and H_2O_2 (Beyotime, S0038), were determined using commercial kits from Beyotime Institute of Biotechnology. The experimental procedures were performed strictly in accordance with the manufacturer's instructions.

2.3. Western Blot

Intracellular proteins were extracted using a protein extraction kit. Target proteins were separated by SDS-PAGE and electrotransferred onto PVDF membranes. The membranes were incubated overnight with primary antibodies against Nrf2 (Proteintech, 16396-1-AP), AKT (CST, 9272), p-AKT (CST, 9271), GPX4 (Abmart, T56959S), and COX2 (Abcam, ab179800), respectively. On the following day, the membranes were incubated with the corresponding secondary antibodies. Protein expression was subsequently detected using chemiluminescence, and the results were recorded.

2.4. Molecular Docking

AutoDock was used to simulate the interaction between ISO and AKT (PDB: 3O96). During the protein preparation process, all H_2O were removed.

2.5. CETSA

The cells were collected and repeatedly frozen and thawed using liquid nitrogen. The processed cell supernatant was incubated with ISO and DMSO for 60 min and then denatured at different temperatures, followed by WB detection.

2.6. DARTS

The cell lysates were mixed with ISO and DMSO, respectively and incubated at room temperature for 1 h. Then, protease inhibitor was added for 30 min. The addition of protease inhibitor was to terminate the reaction. After the experiment, WB detection was performed.

2.7. Data Statistics

Statistical analyses were conducted using SPSS 22.0 software. For comparisons among multiple groups, data were subjected to one-way analysis of variance (ANOVA), followed by either the least significant difference (LSD) test or Dunnett's T3 test. Results are presented as the mean ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Cell Viability

When cells were treated with different concentrations of ISO, MTT assay results showed that cell viability was significantly reduced at 30 μM ISO, indicating obvious cellular injury (**Figure 1(A)**). Therefore, ISO concentrations of 5, 10, and 20 μM were used in subsequent experiments. As shown in **Figure 1(B)**, ISO treatment attenuated the OGD/R-induced decrease in SH-SY5Y cell viability in a concentration-dependent manner.

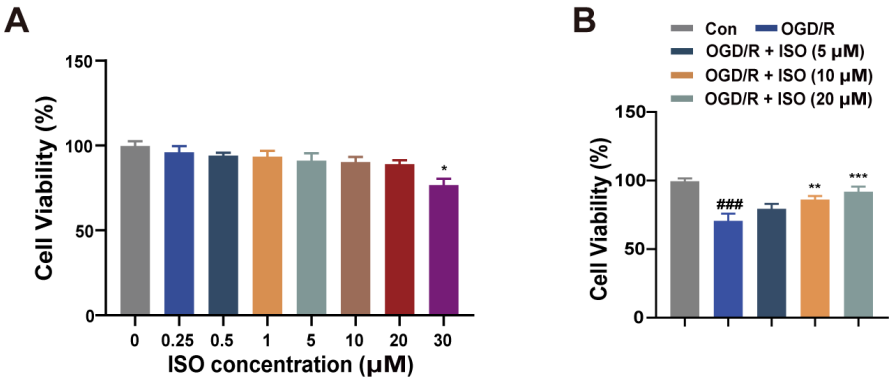

Figure 1. MTT assay. (A) Effects of different concentrations of ISO on the viability of normally cultured SH-SY5Y cells. (B) Effects of ISO on the viability of SH-SY5Y cells subjected to OGD/R. Data are presented as the mean ± SD (n = 5). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{###}p < 0.001$.

3.2. The Effect of ISO on ROS and Lipid Peroxidation Products

The results of experiments evaluating the ROS scavenging and lipid peroxidation inhibitory effects of ISO demonstrated that ISO significantly reduced the levels of H_2O_2 and MDA (**Figure 2**).

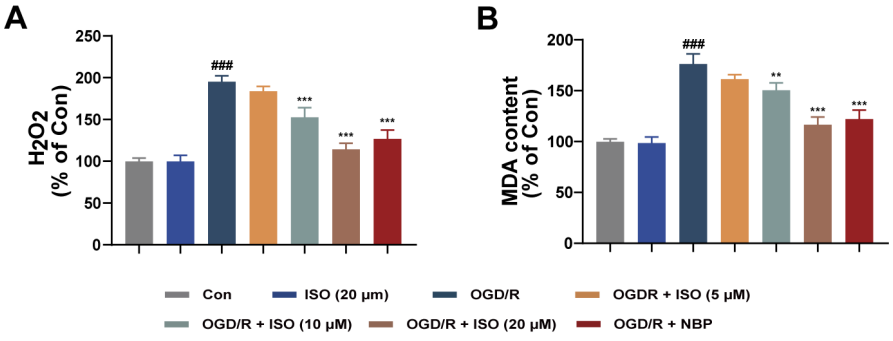

Figure 2. (A) H_2O_2 level. (B)MDA content. Data are presented as the mean ± SD (n = 3). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{###}p < 0.001$.

3.3. The Effect of ISO on Cellular Antioxidant Capacity

Assessment of the intracellular antioxidant capacity of ISO revealed that ISO enhanced SOD activity and increased GSH content in OGD/R-injured SH-SY5Y

cells (**Figure 3**).

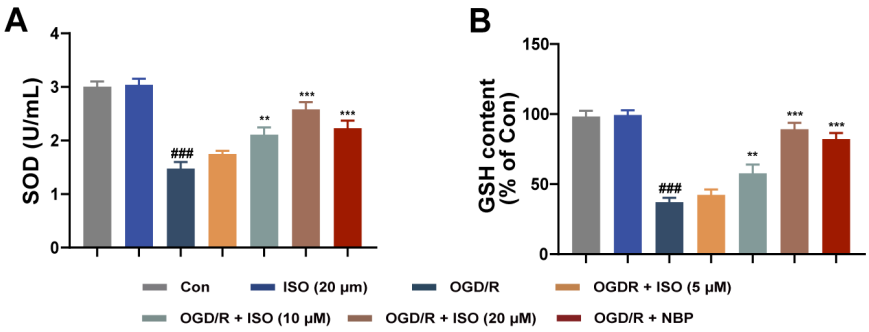

Figure 3. (A) SOD activity. (B) GSH content. Data are presented as the mean ± SD (n = 3). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{###}p < 0.001$.

3.4. Activation of Nrf2 and Inhibition of Ferroptosis by ISO

Western blot results showed that ISO inhibited the expression of Nrf2 in the cytoplasm and increased its expression in the nucleus in a dose-dependent manner, indicating that ISO could promote the nuclear translocation of Nrf2, thereby activating the Nrf2 signaling pathway (**Figure 4(A)**).

In addition, Western blot analysis was performed to evaluate the regulatory effect of ISO on the expression of ferroptosis-related proteins. The results showed that OGD/R treatment downregulated GPX4 expression and upregulated COX2 expression, while ISO treatment reversed this phenomenon, indicating that ISO could inhibit OGD/R-induced neuronal ferroptosis (**Figure 4(B)**).

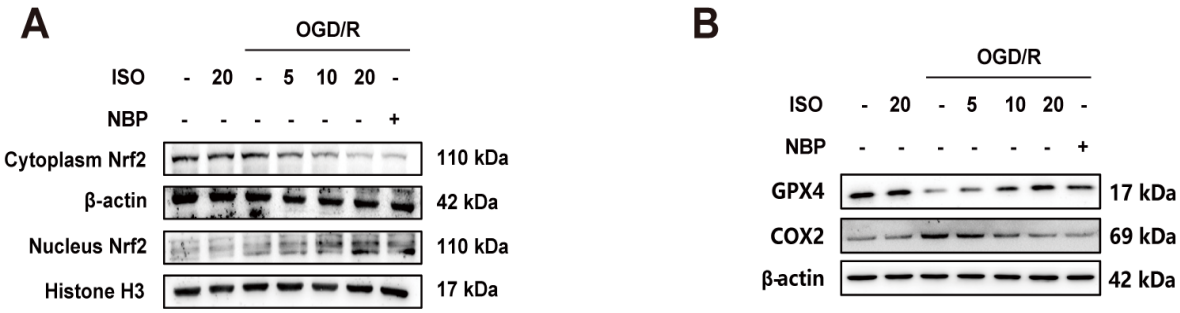

Figure 4. Western blot analysis was performed to evaluate the activation of Nrf2 and the regulation of ferroptosis-related proteins by ISO. (A) The expression of Nrf2 in the cytoplasm and nucleus was detected by Western blot analysis. (B) The protein expression levels of ferroptosis-related proteins GPX4 and COX2.

3.5. ISO Targeted AKT

Western blot results indicated that ISO could promote the phosphorylation of AKT, suggesting that ISO is capable of activating the PI3K/AKT signaling pathway (**Figure 5(A)**). Meanwhile, molecular docking assay results showed that ISO could bind to AKT with a binding energy of −7.713 kcal/mol (**Figure 5(B)**). The DARTS experimental results indicated that ISO can inhibit the degradation of AKT protein caused by protease, and this effect was concentration dependent (**Figure 5(C)** & **Figure 5(D)**). The CETSA results indicated that ISO can improve the thermal

stability of AKT (**Figure 5(E)** & **Figure 5(F)**).

Collectively, these results suggest that ISO can target AKT to activate the PI3K/AKT signaling pathway, which serves as the upstream regulatory pathway of Nrf2-mediated antioxidant response.

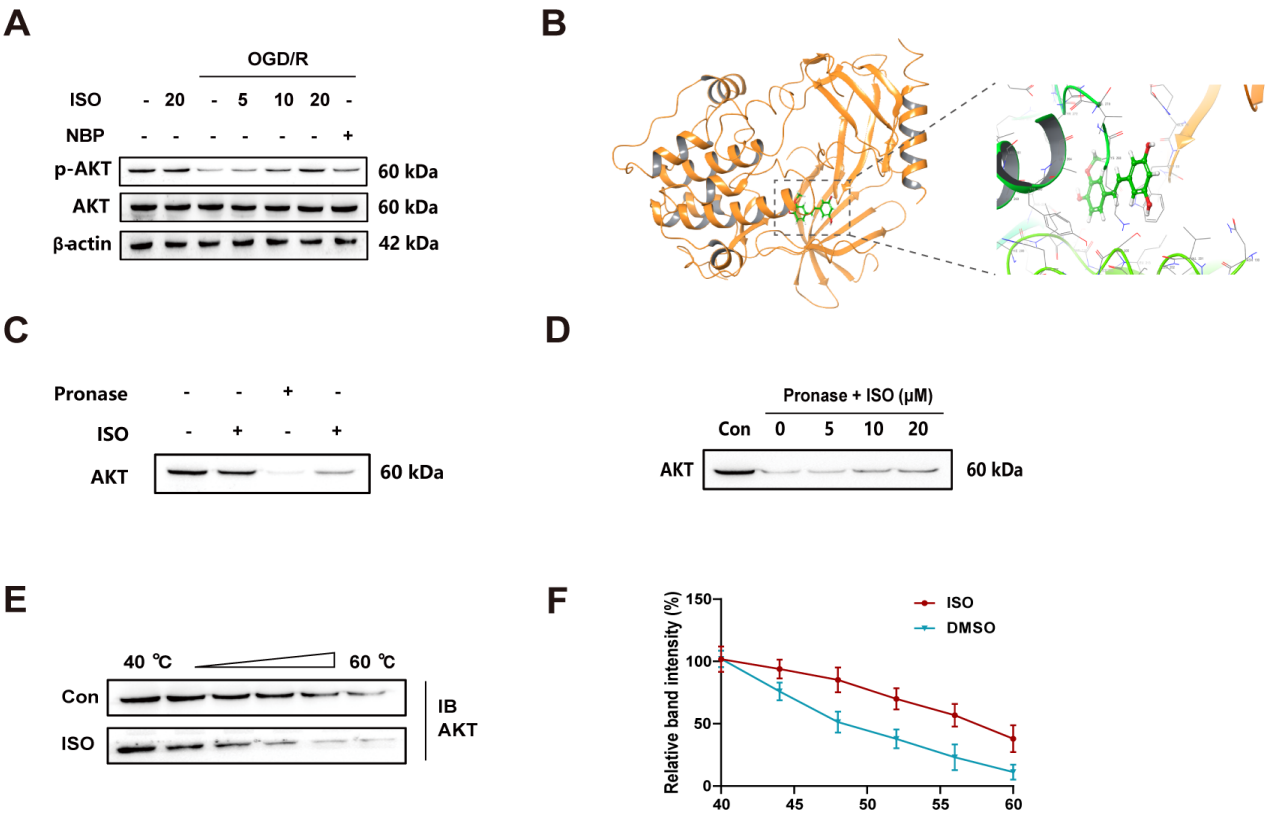

Figure 5. ISO activates the PI3K/AKT pathway by targeting AKT. (A) ISO can promote AKT phosphorylation. (B) Molecular docking between ISO and AKT. (C-D) DARTS detected the stability of protease binding to ISO and AKT. (E-F) CETSA detected that ISO improved the thermal stability of AKT.

4. Conclusion

This study found that ISO dose-dependently improved the viability of SH-SY5Y cells subjected to OGD/R, indicating that ISO exerts a clear neuroprotective effect. Combined with the results of molecular biological experiments, ISO not only inhibited oxidative stress but also enhanced cellular antioxidant capacity. This effect may be achieved by activating the Nrf2 pathway. Meanwhile, ISO regulated the abnormal expression of ferroptosis-related proteins GPX4 and COX2. Further investigation revealed that ISO targeted the PI3K/AKT pathway, an upstream regulator of Nrf2, thereby modulating intracellular oxidative stress and ferroptosis. These findings provide additional scientific evidence for the application of ISO as a functional food.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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