Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.25622

Protective activity of sappanchalcone against oxygen glucose deprivation/re-oxygenation-induced renal tubular epithelial cell injury via TNFRSF1A/NF-kB pathway

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Abstract

Oxygen glucose deprivation/re-oxygenation (OGD/R)-mediated ischemia of kidney is frequently leads to enhanced apoptosis and amplified inflammatory response. Sappanchalcone (Sap) is a flavonoid compound that extracted from *Caesalpinia sappan L.* with a range of cell-protective activities. Herein, we primarily reconnoitered the influence of Sap in HK-2 cells under OGD/R treatment. In this research, the consequence revealed that Sap might linked with ischemia of kidney. Besides, Sap lessened OGD/R-mediated HK-2 cell injury by boosting cell viability, inhibiting apoptosis and inflammation. In addition, Sap hindered activation of the TNFRSF1A/NF-kB signaling. Moreover, upregulation of TNFRSF1A diminished the repressive influence of Sap on OGD/R-mediated apoptosis and inflammation. In conclusion, Sap declined the OGD/R-induced HK-2 cell injury by downregulation of TNFRSF1A/NF-kB signaling, thereby offered a theoretical basis for the handling of ischemia of kidney.

Keywords: sappanchalcone; oxygen glucose deprivation/re-oxygenation; ischemia of kidney; TNFRSF1A/NF-kB pathway.

Practical Application: Sappanchalcone alleviates oxygen glucose deprivation/re-oxygenation-induced renal tubular epithelial cell injury.

1 Introduction

Acute kidney injury (AKI) is a clinical syndrome of abrupt chaos of kidney function that causes symptoms such as renal ischemia reperfusion (IR) and sepsis (Tang et al., 2019). Ischemia of kidney can seriously affect the function of renal tubules and lead to acute renal failure in most clinical cases (Jung et al., 2009; Ray et al., 2019). Ischemia of kidney can also lead to necrosis, apoptosis, detachment or differentiation of renal tubular cells (Jung et al., 2009). In addition, reperfusion injury caused by the restoration of blood supply can also lead to local secondary injury, including inflammation, oxidative stress, and apoptosis (Wang & Jin, 2021). With the further study of renal IR, it has been found that IR-mediated AKI is associated with various inflammatory cytokines (Xu et al., 2021). But so far, the clinical treatment effect of ischemia of kidney is still not ideal. Therefore, there is a pressing need for us to discover more efficient treatments.

In recent years, with the deepening of new drug research, many natural compounds with biological activity gradually come into people's view. Among them, the special role of flavonoids compounds can not be ignored. For example, eriocitrin had the effects of anti-diabetes, anti-cancer, anti-oxidation and alleviated oxidative damage of rat liver tissue (Guo et al., 2019; Minato et al., 2003). Coincidentally, sappanchalcone (Sap) is also a flavonoid compound that separated from *Caesalpinia sappan L.* (Seo et al., 2020). Jung *et al.* confirmed that Sap was utilized as an anti-inflammatory agent in the cure of rheumatoid arthritis (Jung et al., 2015). In addition, Sap took part in regulating cell growth and apoptosis in oral cancer (Lee et al., 2011). Therefore, we speculate that Sap may also have a certain influence on ischemia of kidney-mediated cell apoptotic and inflammation, which will be the focus of this paper.

TNF receptor superfamily member 1A (TNFRSF1A) serves as a key role in the TNF signaling pathway. Additionally, TNFRSF1A can activate the TNF signaling pathway *in vitro* (Hu et al., 2019). Besides, TNFRSF1A is also an upstream regulatory factor of NF-kappa B signaling pathway (Egusquiaguirre et al., 2018). What's more, Ren et al. proved that fisetin could alleviate LPS-induced AKI mice kidney inflammation and apoptosis through impeding NF-kappa B signaling pathway (Ren et al., 2020). In this article, we will examine whether Sap plays its protective role through above signal pathway.

Herein, we built oxygen glucose deprivation/reoxygenation (OGD/R) model in HK-2 cell to mimic ischemia of kidney *in vitro* (Xu et al., 2021). We found that Sap could weaken the OGD/R-induced HK-2 cell injury by modulating TNFRSF1A/NF-kB signaling pathway, which will deliver a possible new option for the cure of ischemia of kidney.

2 Materials and methods

2.1 Bioinformatics analysis

HERB (http://herb.ac.cn/) was implemented to predict the diseases that Sap might be involved in (Fang et al., 2021). Then, the similarity ensemble approach (SEA; https://sea.bkslab.org/)

Received 05 Mar., 2022
Accepted 01 May, 2022
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was applied to analyse the targets of Sap. GeneCards (https://www.genecards.org/) was enforced to predict the the targets of ischemia of kidney. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was exploited to carry out the gene product metabolic pathway forecast.

2.2 Cells culture and treatment

In this paper, the human renal tubular epithelial cell line HK-2 was acquired from Procell Life Science&Technology Co.,Ltd. (Procell, Wuhan, Chian) and cultivated in Dulbecco's modified eagle medium (DMEM; Solarbio, Beijing, China) with 10% heat inactivated fetal bovine serum (Solarbio). Next, HK-2 cells were exposed to 5% CO_2 and 95% air at 37 °C.

Cell sectionalization and treatment: Control group: Not to deal with. OGD/R group: treated on the base of the OGD/R model founding process. Sap groups: HK-2 cells were exposed to Sap (5, 10, 20, 40 μM) for 36 h at the same time as OGD/R treatment, and Sap was unceasingly administered at the equal amount throughout the OGD and reoxygenation. Besides, TNFRSF1A overexpression plasmid (TNFRSF1A) and control plasmid (Vector) were acquired from Ribobio (Guangzhou, China). Lipofectamine 2000 (Solarbio) was engaged in the transfection process in keeping with its directions.

2.3 Founding of OGD/R cell model

At the end of the 6 days cultivation, HK-2 cells were rinsed 3 times with glucose-free DMEM (Solarbio) that had been balanced in the incubator befor, added with 1% O_2, 5% CO_2, and 94% N_2 at 37 °C. Afterwards, the previous medium was substituted, and the HK-2 cells were moved to an incubator comprising 1% O_2, 5% CO_2, and 94% N_2, and sustained at 37 °C for 12 h. Later, the medium was altered back to the previous medium, and the HK-2 cells were transferred to a standard incubator for 6, 12, or 24 h. Likewise, the control cells were cultivated in complete medium (pH = 7.2; Solarbio) with 5% CO_2 for equal time. Lastly, after 6, 12, or 24 h, the HK-2 cells viability were evaluated.

2.4 Cell Counting Kit-8 (CCK-8) assay

HK-2 cells viability were measured via a CCK-8 kit (Solarbio). Initially, HK-2 cells (5×10^3/mL) were sowed in 96-well plates. After adherence, the HK-2 cells were coped with CCK-8 solution (10 μL; Solarbio) and hatched for 1 h. In conclusion, the absorbance was examined at 450 nm.

TUNEL assay A TUNEL In Situ Cell Death Detection Kit (Solarbio) was implemented to carry out the TUNEL assay. In short, the fixed HK-2 cells were exposed to TUNEL (5 μM; Solarbio) and the DNA was coped with DAPI for image analysis. Finally, a fluorescent microscope (1 × 400 magnification; Leica, Shanghai, China) was enforced to observe these images.

2.5 Lactate dehydrogenase (LDH) measurement

After various treatments, the supernatant of HK-2 cells were collected and the activity of LDH was detected by an LDH Cytotoxicity Assay Kit (C0016; Beyotime, Shanghai, China) in line with the guidelines.

2.6 Enzyme linked immunosorbent (ELISA) assay

After several treatments, the liquids supernatant of HK-2 cells were collected for indicator detection. These ELISA kits (Abcam, Shanghai, China) were applied to confirm the HK-2 cells culture supernatant factors caspases-3 (ab285337; Abcam), TNF-α (ab181421; Abcam), IL-1β (SEKH-0002; Solarbio), IL-6 (ab178013; Abcam), and IL-18 (ab215539; Abcam) in line with the directions.

2.7 Western blot

The protein contents of TNFRSF1A, p-p65, and p65 were appraised by western blot according to previous steps (Song et al., 2019). The antibodies were listed as follows: anti-TNFRSF1A (K108969P; 1:1,000; Solarbio), anti-p-p65 (sc-136548; 1:1000; Santa Cruz, Shanghai, China), anti-p65 (ab32536; 1:1000; Abcam), and anti-β-actin (ab8226; 1:1000; Abcam). Finally, the protein bands images were observed through an Enhanced chemiluminescence reagent (ECL; Solarbio).

2.8 Statistical analysis

In this paper, all tests were repeated three times as a minimum. All statistical investigation was implemented by utilizing GraphPad 7.0 software. The figures were communicated as mean ± standard deviation, and the contrast between groups was enforced by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison. $P < 0.05$ was considered as significant.

3 Results

3.1 Sap mitigated OGD/R-mediated cell damage

Primarily, we predicted that Sap was related to ischemia of kidney via HERB (Figure 1A). To discover the effect of Sap in ischemia of kidney, we established an OGD/R model of HK-2 cells and exposed to diverse doses of Sap. The outcomes presented that HK-2 cell viability was damaged by OGD/R treatment. Besides, the longer OGD/R treatment time, the stronger the inhibition effect of cell viability (Figure 1B). Therefore, in subsequent tests HK-2 cells were coped with OGD/R treatment for 24 h. Next, we treated HK-2 cells with diverse levels of Sap, and the outcomes displayed that 5, 10, and 20 μM Sap did not affect cell viability, while 40 μM Sap significantly reduced cell viability (Figure 1C). Therefore, in the follow-up test, 5, 10, and 20 μM Sap were selected in order to eliminate the cytotoxicity caused by Sap treatment. In addition, treatment with Sap subdued the OGD/R-mediated diminution in cell viability in a dose-dependent mode (Figure 1D). Moreover, OGD/R treatment tempted a more serious apoptosis (Figure 2A), augmented LDH release (Figure 2B) and caspase-3 activity (Figure 2C). Nevertheless, treatment with Sap repressed the OGD/R-mediated cell apoptotic in a dose-dependent mode (Figures 2A-2C). These outcomes submitted that Sap defended HK-2 cells from the OGD/R-tempted damage.

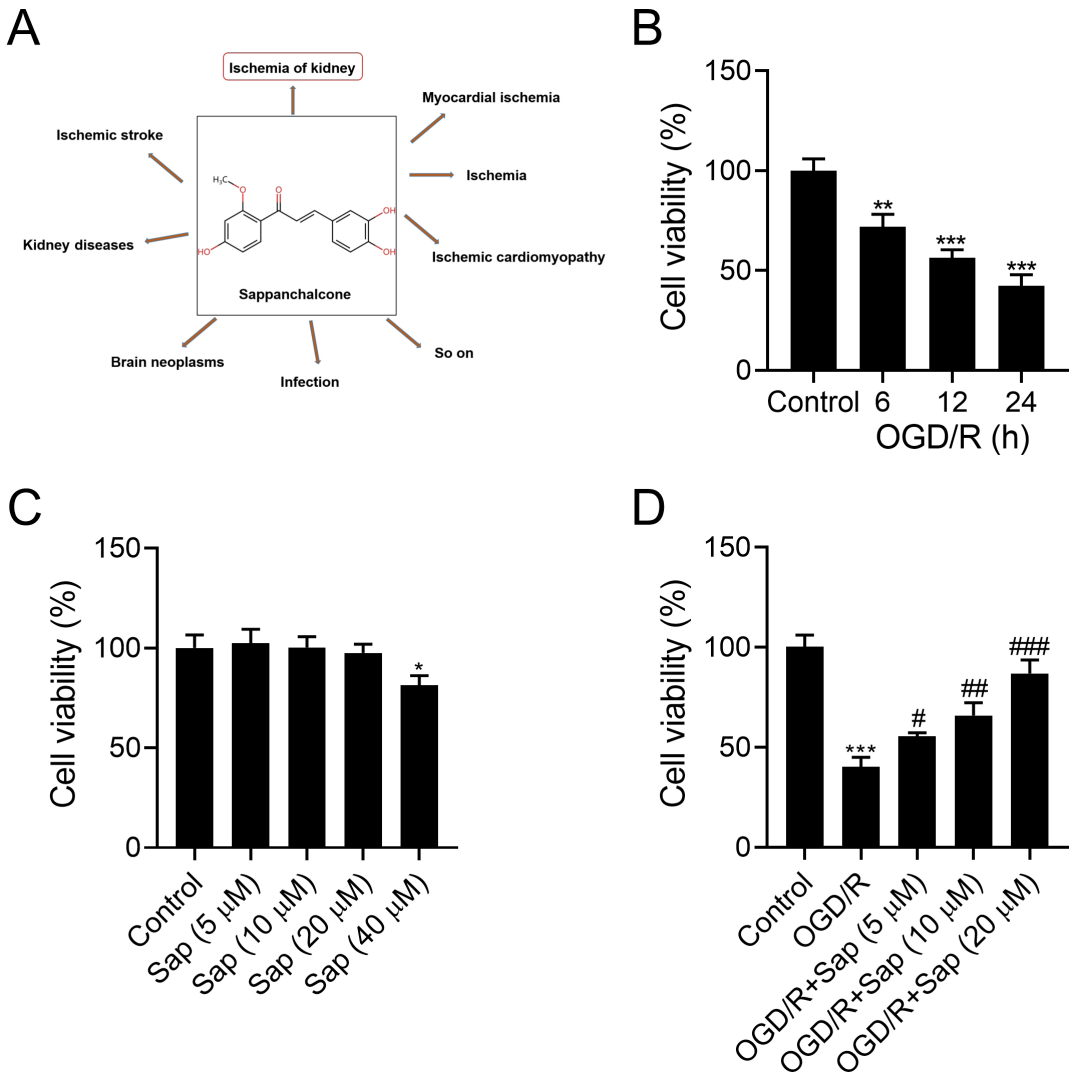

Figure 1. Sap diminished OGD/R-induced cell injury. (A) HERB was used to predict the correlation between Sap and ischemia of kidney. (B-D) The cell viability was appraised by CCK-8 assay. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ for the comparison with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ for the comparison with the OGD/R group.

3.2 Sap curbed OGD/R-mediated inflammation

In order to reconnoiter the influence of Sap on OGD/R-mediated inflammation in HK-2 cells, we carried out ELISA assay to validate the abundances of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-18. We uncovered that these above pro-inflammatory factors abundances in HK-2 cells were noticeably amplified by OGD/R treatment, while were substantially subdued by Sap in a dose-dependent way (Figures 3A-3D). These consequences demonstrated that Sap markedly lessened OGD/R-tempted inflammation in HK-2 cells.

3.3 Sap hindered activation of the TNFRSF1A/NF-kB signaling

Then, we discovered the mechanism of Sap on HK-2 cells. Firstly, we reconnoitered the link between Sap and ischemia of kidney. Herein, 81 targets of Sap were predicted through SEA. Meanwhile, 643 targets of ischemia of kidney were predicted via GeneCards (Figure 4A). Among these targets, 24 were overlapped, and KEGG analysis was implemented on these targets. Interestingly, these targets were linked with TNF signaling pathway and NF-kappa B signaling pathway (Figure 4B). TNFRSF1A is a vital factor in the TNF signaling pathway and acts as an upstream gene of NF-kappa B signaling pathway (Egusquiaguirre et al., 2018; Hu et al., 2019). We confirmed that OGD/R treatment obviously enhanced TNFRSF1A and p-p65 levels (Figures 4C-4E). More, OGD/R treatment boosted the level of p-p65/p65 to activate the NF-kappa B signaling pathway (Figure 4E). Besides, Sap curbed the influence of OGD/R treatment on the TNFRSF1A content (Figures 4C-4D) and level of p-p65/p65 (Figures 4C and 4E). Furthermore, the TNFRSF1A content and level of p-p65/p65 were enhanced by TNFRSF1A overexpression in HK-2 cells with OGD/R and Sap treatment (Figures 4C-4E). These outcomes proved that Sap might curb TNFRSF1A expression and repress the activation of the NF-kB signaling.

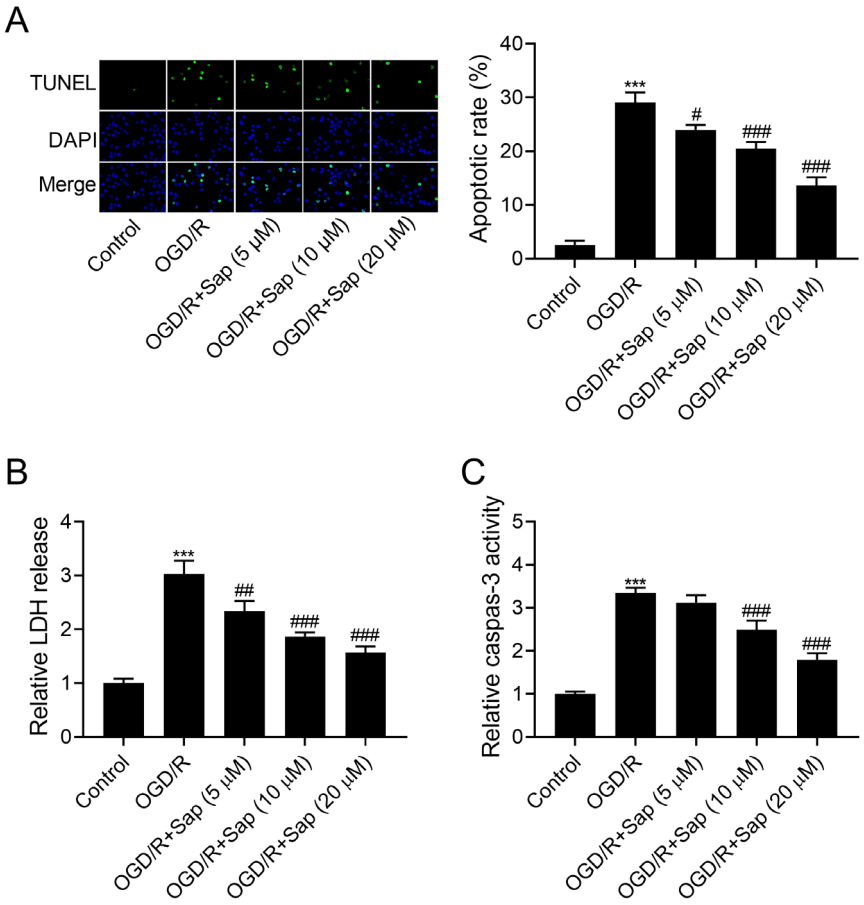

Figure 2. Sap curbed OGD/R-induced cell death. (A) The apoptotic was confirmed by TUNEL assay. (B) The level of LDH release was measured by commercial kit. (C) The caspas-3 activity was detected by ELISA kit. $^{***}P < 0.001$ for the comparison with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ for the comparison with the OGD/R group.

3.4 TNFRSF1A overexpression mitigated the repressive effects of Sap on OGD/R-mediated cell apoptotic and inflammation

To search whether the TNFRSF1A overexpression overturned the suppressive effects of Sap in HK-2 cells, we implemented rescue experiments. The consequences displayed that TNFRSF1A overexpression inverted the effects of Sap by increasing apoptosis rate (Figure 5A), enhancing LDH release (Figure 5B), and boosting caspase-3 activity (Figure 5C) in HK-2 cells with OGD/R and Sap treatment. Meanwhile, the outcomes also verified that TNFRSF1A overexpression obviously augmented the abundances of the pro-inflammatory factors TNF-α, IL-1β, IL-6, and IL-18 versus the OGD/R + Sap group in HK-2 cells (Figures 6A-6D). These upshots validated that Sap lessened the damage of HK-2 cells in the OGD/R model dose-dependently by downregulation of TNFRSF1A.

4 Discussion

Renal IR is the most common reason of AKI. With the development of research, it has been found that inflammatory response and apoptosis are the main reasons of IR-induced AKI, and cell protection and anti-inflammatory therapy are the central treatment approaches (Lorenzen, 2015). Renal tubular cell dysfunction that induced by ischemia of kidney serves as a vital role in the progress of renal failure (Jung et al., 2009). Several traditional Chinese medicines have been shown to have immunosuppressive property and antioxidant activity. It is of great significance to develop the active components of traditional Chinese medicines for the treatment of kidney disease (Liu et al., 2021). Meanwhile, Sap played an anti-inflammatory role in a variety of human sicknesses, like rheumatoid arthritis (Jung et al., 2015). Herein, we firstly revealed that Sap curbed TNFRSF1A expression and repressed the activation of the NF-kB signaling pathway, thereby diminishing apoptosis and inflammation in HK-2 cells with OGD/R treatment.

At the present time, there are various researchs about the influences of naturally extracted compounds on renal IR-tempted AKI. Such as, Propolis combined with boric acid inhibited apoptosis and inflammatory response in rats, thereby alleviating AKI that induced by renal IR (Geyikoglu et al., 2019). In addition, preventive could distinctly lessen IR-induced inflammatory response in mice kidney to act as a cellular protector (Topdağı et al., 2020). Moreover, mallow extract participated in protecting kidney against IR-induced liver damage (Najafi et al., 2017). Besides, garlic combined with telmisartan also took part in

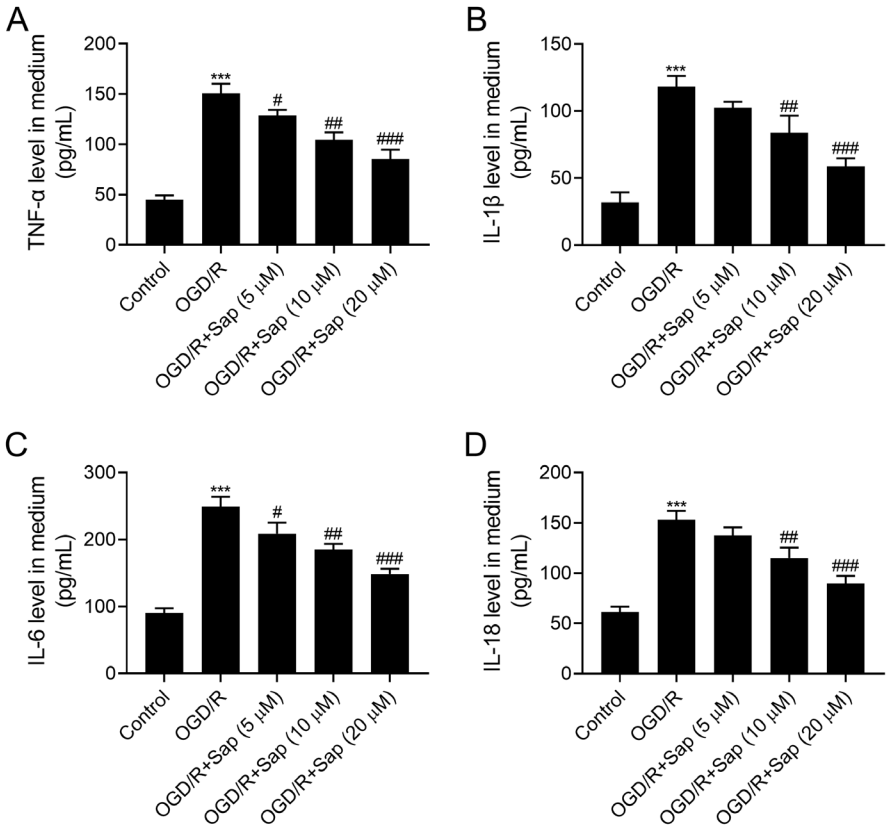

Figure 3. Sap weakened OGD/R-induced inflammation. (A-D) The abundances of TNF-α, IL-1β, IL-6, and IL-18 were examined by ELISA kits. $^{***}P < 0.001$ for the comparison with the control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ for the comparison with the OGD/R group.

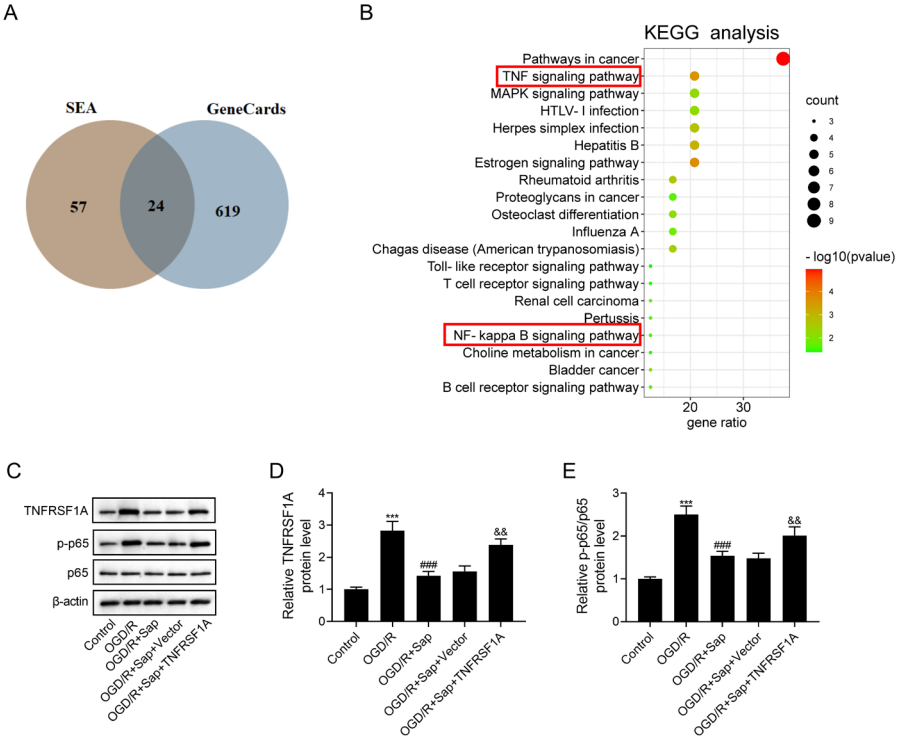

Figure 4. Sap inhibited activation of the TNFRSF1A/NF-kB signaling. (A) Venn diagram of overlapped targets in Sap and ischemia of kidney. (B) KEGG analysis of 24 targets and bubble plot for top 20 pathways. (C-E) The contents of TNFRSF1A, p-p65, and p-65 were inspected by western blot. $^{***}P < 0.001$ for the comparison with the control group. $^{\#\#\#}P < 0.001$ for the comparison with the OGD/R group. $^{\&\&}P < 0.01$ for the comparison with the OGD/R + Sap + Vector group.

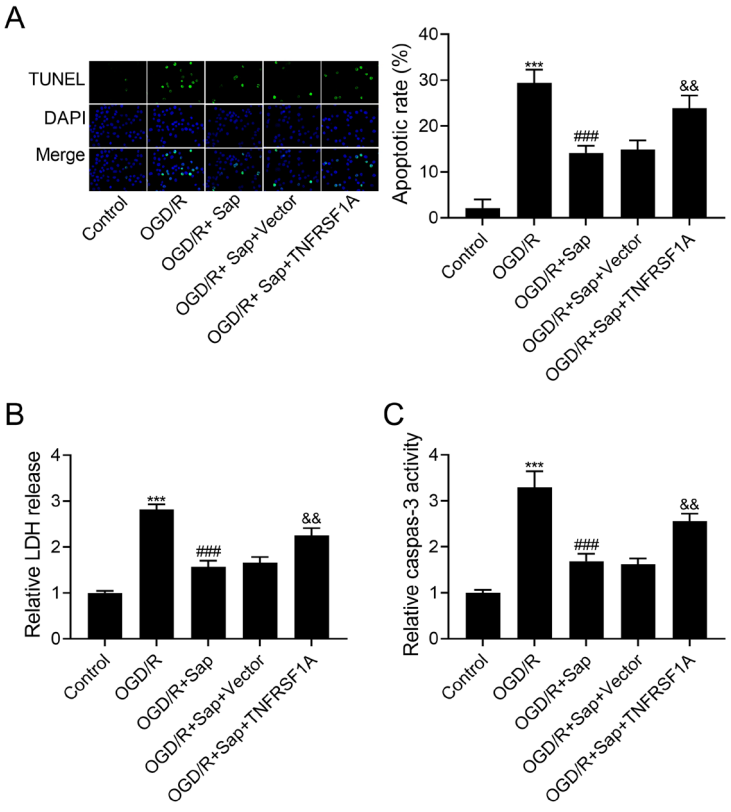

Figure 5. TNFRSF1A overexpression mitigated the suppressive influence of Sap on OGD/R-mediated cell death. (A) The apoptotic was evaluated by TUNEL assay. (B) The LDH release was examined by commercial kit. (C) The caspas-3 activity was distinguished by ELISA kit. $^{***}P < 0.001$ for the comparison with the control group. $^{###}P < 0.001$ for the comparison with the OGD/R group. $^{\&\&}P < 0.01$ for the comparison with the OGD/R + Sap + Vector group.

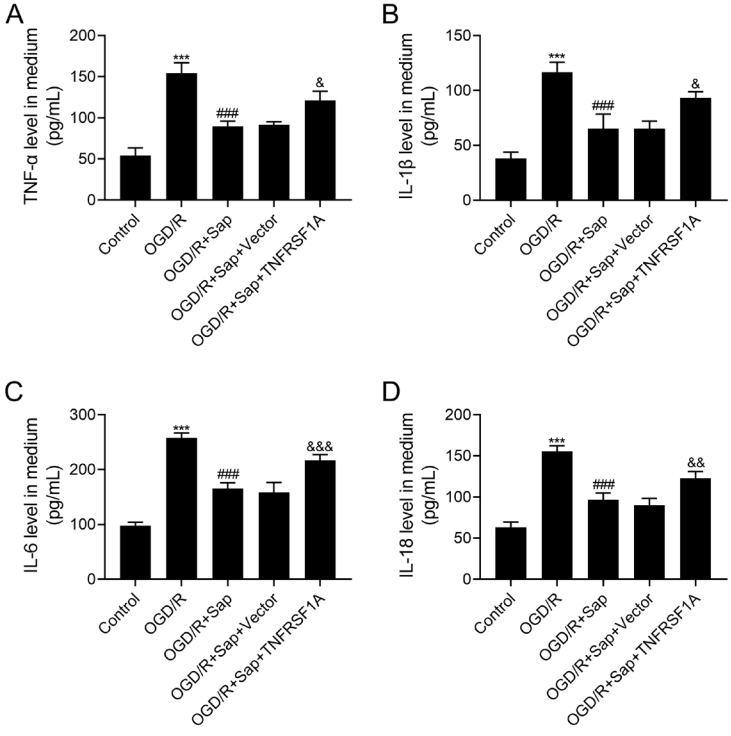

Figure 6. TNFRSF1A upregulation lessened the suppressive effect of Sap on OGD/R-mediated inflammation. (A-D) The levels of TNF-α, IL-1β, IL-6, and IL-18 were assessed by ELISA kits. $^{***}P < 0.001$ for the comparison with the control group. $^{###}P < 0.001$ for the comparison with the OGD/R group. $^{\&}P < 0.05$, $^{\&\&}P < 0.01$, and $^{\&\&\&}P < 0.001$ for the comparison with the OGD/R + Sap + Vector group.

protecting kidney from IR-induced mice renal injury (Ali et al., 2016). Therefore, we could confirm that many natural extracts were efficient in lessening IR-induced inflammatory response and apoptosis in kidney.

In current years, the role of Sap has also been gradually studied. For example, Jeong et al. indicated that Sap could protect human dental pulp cells against H_2O_2-induced cell injury. Besides, Sap also exhibited anti-inflammation influence in LPS-mediated human periodontal ligament cells (Jeong et al., 2010). Moreover, Seo et al. verified that Sap took part in regulating cell apoptotic in human colon cancer cells (Seo et al., 2020). In this paper, we manifested that Sap decreased OGD/R-induced HK-2 cell injury via boosting cell viability, impeding apoptosis and inflammation. These outcomes were alike with the description of Jeong et al. (2010) and Seo et al. (2020).

Nuclear factor-kappa B (NK-κB) plays a vital role in the process of inflammatory response (Shin et al., 2021). Furthermore, some reports have verified that hindered NF-kB signaling pathway reduced renal injury in IR-mediated AKI (Zeng et al., 2020). In addition, Jing et al. proved that downregulation of TNFRSF1A and inactivated NF-kB signaling could protect human bronchial epithelial cells against cigarette smoke extract-mediated cell damage (Jing et al., 2021). Herein, Sap curbed activation of the TNFRSF1A/NF-kB signaling. Additionally, upregulated TNFRSF1A weakened the suppressive influence of Sap on OGD/R-induced apoptosis and inflammation, which was similar with the results of Zeng et al. (2020).

5 Conclusion

In a word, our paper illustrated that Sap restrained TNFRSF1A expression to block the activation of the NF-kB signaling in HK-2 cells with OGD/R treatment, thereby inhibiting apoptosis and inflammatory response to protect the kidney. In short, our consequences revealed that the potential application prospect of Sap in ischemia of kidney was good.

Conflict of interest

The authors declare that there is no conflict of interests.

Availability of data and material

The data underlying this article are available in the article. If needed, please contact the corresponding author. The email address is hongc31367@126.com.

Funding

This study was funded by the 2021 Medical Research Project of Wuhan Municipal Health Commission.

Author contributions

Quan Wang and Li Cheng designed the project, Huanlan Wang and Hongbo Li collected data, Taotao Hu and Shuai Fu analyzed the data, drafted the manuscript and did all the experiments, Quan Wang and Li Cheng involved in data collection and analysis, as well as revised and corrected the manuscript.

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