Effects of Resveratrol on Tight Junction Proteins and Notch1 Pathway in HT-29 Cells Inflammation Model Induced by Lipopolysaccharide

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Research Article

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Abstract

Ulcerative colitis (UC) is closely associated with disruption of the intestinal epithelial tight junction proteins. A variety of studies have confirmed that resveratrol (RSV), a natural polyphenolic compound, has a potential anti-inflammatory effect and can regulate the expression of tight junction proteins. However, the mechanism by which RSV regulates the expression of tight junction proteins in the intestinal epithelium remains unclear. Therefore, we investigated the RSV potential effect on tight junction proteins in the HT-29 cell inflammation model induced by lipopolysaccharide (LPS) and explored its mechanism of action. Firstly, the downregulated expression of tight junction proteins occludin, ZO-1, and claudin-1 in the HT-29 cells inflammation model induced by LPS were reversed by incubation with RSV, accompanied by the decrease in the expression of tumor necrosis factor α-converting enzyme (TACE). Also, the Notch1 pathway was inhibited and the expression of inflammation factors IL-6 and TNF-α was decreased by treatment with RSV. Secondly, after Jagged-1 was used in combination with RSV to reactivate the Notch1 pathway, the protective effects of RSV against LPS-induced reduction of tight junction proteins occludin, ZO-1, and claudin-1, and the decrease of inflammation factors IL-6 and TNF-α were abolished. These results suggest that RSV might regulate the expression of tight junction proteins by inhibiting the Notch1 pathway.

Introduction

Ulcerative colitis (UC) is an immune-mediated chronic nonspecific intestinal disease characterized by recurrent abdominal pain, diarrhea, mucous pus, and bloody stools. In recent years, the incidence of UC has gradually increased globally and is expected to affect 30 million people worldwide by 2025 [1]. However, the conclusion on the pathogenesis of UC is still not unclear. The current view is that the pathogenesis of UC is closely related to the abnormal structure and function of the intestinal epithelial barrier and the imbalance of the intestinal microbial population [2]. As the first barrier between the body and the outside world, the intestinal epithelial barrier prevents microorganisms in the gut from passing through the intestinal mucosa and entering the body to cause abnormal immune responses. The intestinal epithelial barrier is composed of a mechanical barrier, biological barrier, immune barrier, and chemical barrier. The mechanical barrier function of the intestinal epithelium is maintained by the intestinal mucus layer, intestinal epithelial cells, and tight junction proteins between intestinal epithelial cells. The tight junction proteins are located at the top of the contact between adjacent intestinal epithelial cells, which seal the intercellular space by assembling and connecting adjacent cells, thus maintaining the structural and functional stability of the intestinal epithelial barrier [3]. Previous studies have confirmed that abnormal expression of tight junction proteins such as occludin, ZO-1, and claudin-1 can lead to damage to the integrity of the intestinal epithelial barrier [4], causing diseases related to intestinal epithelial barrier damage (e.g. UC). Therefore, restoring the expression of tight junction proteins is one of the effective ways to treat UC.

The occurrence and development of UC are related to the abnormal activity of various signaling pathways in vivo, of which the Notch1 pathway is a key signaling pathway affecting UC [5, 6]. Notch1

pathway is a highly conserved pathway involved in a series of processes such as tissue development and homeostasis maintenance [7]. Previous studies have demonstrated that activation of the Notch1 pathway can increase the expression levels of inflammatory factors such as IL-6 and TNF-α, leading to the occurrence of inflammation [8]. In UC mice models induced by dextran sulfate sodium, after the Notch1 pathway activity was inhibited, the secretions of inflammatory factors such as IL-6 and TNF-α were reduced, the differentiation of intestinal epithelial cells populations restored balance, and intestinal mucus secretion was increased, thereby alleviating the damaged intestinal epithelial barrier and ameliorating colonic inflammation in mice [9–11].

Resveratrol (RSV) plays an important role in the regulation of Notch1 pathway activity [12, 13]. RSV is a natural polyphenol compound derived from metaplasia, grapes, and other plants, which has biological effects such as anti-tumor, antioxidant, and anti-inflammatory [14–16], so it has become a research hotspot in the development of new drugs for diseases such as cancer and UC. Previous studies have found that RSV, as a supplement therapy, can alleviate the symptoms of UC patients and improve the quality of life of UC patients [17, 18]. In addition, related experimental studies have confirmed that in UC mice induced by dextran sulfate-induced, RSV can inhibit the secretions of inflammatory factors through the PI3K/Akt/VEGFA pathway and NF-κB pathway, improving intestinal inflammation in mice [19, 20]. RSV also can up-regulate the expression of intestinal epithelial tight junction proteins in mice [21]. However, in UC-related studies, whether RSV can regulate Notch1 pathway activity and how RSV regulates the expression of tight junction proteins in intestinal epithelial cells is still unclear.

To elucidate the regulation mechanism of RSV on the expression of tight junction proteins in intestinal epithelial cells, this study intends to establish an intestinal epithelial cells inflammation model by inducing HT-29 with LPS in vitro experiments and explore the mechanism of RSV on the regulation of tight junction proteins in intestinal epithelial cells.

Materials And Method

Materials

RPMI-1640 mediums (cat.no. MA0215) and CCK-8 kit (cat.no. MA0218) were purchased from Meilun Biological Company (Suzhou, China). Fetal bovine serum (cat.no. C04001050) was purchased from Biological Industrie (VivaCell, Shanghai, China). Serum-free cell cryopreservation solution (cat.no. C40100) and 0.25% EDTA trypsin digestion solution (cat.no. C125C1) were purchased from New Saimei Biotechnology Company (Suzhou, China). Penicillin-streptomycin mixture (cat.no. BL505A) was purchased from the biosharp Company (Shanghai, China). Lipopolysaccharide (LPS, Escherichia coli serotype 055: B5, cat.no. L2880) was purchased from Sigma Company (USA). RSV (cat.no. A4182) was purchased from APE Company (USA). Jagged-1 (cat.no. P1846A) was purchased from MCE Company (USA). Antibodies against Notch1 (cat.no. ab52627), Hes1 (cat.no. ab108937), and claudin-1 (cat.no. ab211737) were purchased from Abcam Company (UK); Antibodies against occludin (cat.no. #91131), ZO-1 (cat.no. #8193), and GAPDH were purchased from cell signaling technology Company (USA);

PrimeScript™ RT reagent Kit with gDNA Eraser (cat.no.RR047A) was purchased from TARAKA Company(Beijing, China). PCR reagent (cat.no. A6002) was purchased from Promega Biotechnology Company (Beijing, China). Primer sequences were purchased from ShengGong Biotechnology Company (Shanghai, China). Other reagents were purchased from solarbio Company (Beijing, China) unless otherwise specified.

Cell Culture and Incubations

HT-29 cells were purchased from the Cell Resource Center, Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and were cultured in RPMI-1640 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C, 5% CO_2, and the cell culture medium was changed every 2 days.

When HT-29 cells reached about 70–80% fusion, they were starved overnight in a serum-free medium. HT-29 cells were preincubated for 4 h with Jagged-1 (10 μmol/L) to activate the Notch1 pathway. Then, cells were incubated for 4 h in the absence or presence of RSV (50–100 μmol/L) and subsequently added with LPS (100 μg/mL) for a further 24 h.

Cell Viability Assay

The effect of drugs on cell viability were detected by CCK-8 assay. HT-29 cells were seeded into a 96-well plate at a density of 5×10^3 /well, and HT-29 cells were incubated with different concentrations of LPS (0-100 μg/mL), RSV (0-100 μmol/L), Jagged-1 (0–10 μmol/L). After 24 h and 48 h incubation, the cells were washed twice with RPMI-1640 medium, and then a cell culture medium containing 10% CCK-8 solution was added to each well and further cultured for 1–2 h. After that, the 96-well plate was placed on a microplate reader at 450 nm to detect the absorbance of cells.

Western Blot

After the experiments, HT-29 cells were washed with PBS buffer, then transferred to a sterile centrifuge tube, and we prepared cell lysates according to the ratio of "RIPA high-efficiency lysis buffer: protease inhibitor = 100:1". According to the number of cells in the centrifuge tube, suitable cell lysates were added to the cells and then placed the cells on ice for 30 minutes to fully lyse. After the cells were fully lysed, the cells were centrifuged at 12000 r/min for 15 min in a low-temperature centrifuge at 4°C and collected the supernatant. The protein concentration was detected by a BCA protein detection kit. After the detection of protein concentration, the appropriate amount of protein loading buffer was added to the proteins, and then proteins were boiled in boiling water for 7 mins to denature. An equal number of proteins from each group was separated by SDS-PAGE gel, and then proteins were transferred to the PVDF membrane. The PVDF membranes were blocked with TBST solution containing 5% nonfat milk powder at room

temperature for 1 h, and then the corresponding PVDF membranes were respectively mixed with antibodies against occludin, claudin-1, ZO-1, Notch1, Hes1, and GAPDH were incubated overnight at 4°C. After the specific antibody incubation, the PVDF membranes were washed three times with TBST buffer for 10 mins each time. After washing, the PVDF membranes were incubated with antibodies against IgG with the rabbit at room temperature for 1 h. After incubation, the PVDF membranes were washed with TBST buffer in the dark 3 times and for 10 mins each time. After washing, Odyssey was used to scan the image bands, and Image J software was used to analyze the protein expression level of the bands.

qRT-PCR

After the experiments, the cells were washed with PBS buffer. According to the instructions, RNAiso plus solution, isopropanol, and chloroform were used to extract and purify the total RNA of HT-29 cells, and then we reverse-transcribed the RNA by PrimeScrip™ RT reagent Kit. In HT-29 cells, mRNA expressions of GAPDH, Notch1, Hes1, occludin, ZO-1, claudin1, TNF-α, TACE, and IL-6 were amplified by GoTaq qPCR Master Mix and SteponePlus™ instrument with the following number of cycles: the initial denaturation step was carried out at 95°C for 10 mins, followed by denaturation at 95°C for 15 seconds, 40 cycles, and finally annealed at 60°C for 1 min. The relative mRNA expressions of the target gene were calculated by the $2^{-\triangle\triangle CT}$ method. Forward and reverse primers are shown below: TACE (F) 5'-AATCTCTGTCTCTGTTTCACCC-3', (R) 5'-AAAGGGTTTGATAATGCGAACC-3'; TNF-α (F) 5'-AGTGCCACTTTGGCATTATGAGA-3', (R) 5'-CTTGTGGCAGCAATTGGAAAC-3'; IL-6 (F) 5'-CACTGGTCTTTTGGAGTTTGAG-3', (R) 5'-GGACTTTTGTACTCATCTGCAC-3'; occludin (F) 5'-AGTGCCACTTTGGCATTATGAGA-3',(R) 5'-CTTGTGGCAGCAATTGGAAAC-3'; claudin-1 (F) 5'-GGGCAGATCCAGTGCAAAG-3', (R) 5'-GGATGCCAACCACCATCAAG-3'; ZO-1 (F) 5'-GACCAATAGCTGATGTTGCCAGAG-3', (R) 5'-TGCAGGCGAATAATGCCAGA-3'; Notch1 (F) 5'-TCCACCAGTTTGAATGGTCAAT-3', (R) 5'-CGCAGAGGGTTGTATTGGTTC-3'; Hes1 (F) 5'-AACACTGATTTTGGATGCTCTG-3', (R) 5'-CACTGTCATTTCCAGAATGTCC-3'; GAPDH (F) 5'-GCACCGTCAAGGCTGAGAAC-3', (R) 5'-TGGTGAAGACGCCAGTGGA-3'.

Transmission Electron Microscope

After the experiments, the cells were washed with PBS solution. Cells were added with 2.5% glutaraldehyde fixative and then fixed at room temperature in the dark for 2 mins. After fixation, we collected cells in a sterile centrifuge tube and centrifuge at 1000 r/min for 2 mins. After centrifugation, we discarded the fixative and re-add an appropriate amount of electron microscope fixative. Continued to fix for 30 mins at room temperature in the dark. After fixation, 1% agarose was heated into a liquid and poured into a centrifuge tube containing cells to make a cell-agarose block. Subsequently, 0.1% osmic acid was added to the cell-agarose block to fix the cells again, and the cells were fixed at room temperature in the dark for 2 h. After fixation, put the cell-agarose block into 30%-50%-70%-80%-95%-100%-100% alcohol in sequence, and dehydrate at room temperature for 20 mins

each time. After dehydration, put the cell-agarose block into 90% acetone solution three times for 10 mins each time. Finally, the cell-agarose block was embedded and sliced, observed, and filmed by transmission electron microscope.

Statistical Analysis

All experimental results were repeated at least three times, and the data were expressed as mean ± SD. SPSS 22.0 was used for statistical analysis of experimental data, and GraphPad Prism 9.0 was used to draw statistical graphs. One-way ANOVA and Tukey's test was used to compare differences among each group. When $P < 0.05$, it means the difference is statistically significant.

Results

The Effect of Drugs on Cell Viability

The results of the effect of drugs on the viability of HT-29 cells were shown in Fig. 1a-c. HT-29 cells were incubated with Different concentrations of LPS, RSV, and Jagged-1 for 24 h and 48 h, and the effect of the drugs on the viability of HT-29 cells were detected. The results of CCK-8 showed that HT-29 cells were incubated with drugs for 24 h, and the cell viability was not affected at the highest concentration (the highest concentrations of LPS, RSV, and Jagged-1 were 100 μg/mL, 100 μmol/L, and 10 μmol/L, respectively); However, after HT-29 cells were incubated with drugs for 48 h, cell viability could be affected to varying degrees. To ensure that the subsequent experimental results were not caused by drug-induced cell proliferation or apoptosis, the selected drug intervention concentration and intervention time did not affect the viability of HT-29 cells.

LPS Inhibits the Expression of Tight Junction Proteins in HT-29 Cell Inflammation Model

LPS can reduce the expression of tight junction proteins, so we detected the expression levels of tight junction proteins occludin, ZO-1, and claudin-1 after HT-29 cells were exposed to different concentrations of LPS (0-100 μg/mL) for 24h by Western Blot. The results were shown in Fig. 2a-d: when the concentrations of LPS were lower than 10 μg/mL, it did not affect the expression levels of occludin, ZO-1, and claudin-1. When the concentration of LPS was 100 μg/mL, it down-regulated the expression levels of occludin, ZO-1, and claudin-1. Therefore, the concentration of LPS in subsequent experiments was 100 μg/mL.

RSV Inhibits the Expression of TACE in HT-29 Cell Inflammation Model

The activation of IL-6 and TNF-α are regulated by TACE. Therefore, we detected the inhibition of RSV on LPS-stimulated TACE in the HT-29 cell inflammation model by qRT-PCR, the results were shown in Fig. 3: Compared to control cells, higher levels of TACE were observed after 24 h incubation with LPS. RSV prevented this increase in a dose-dependent manner in the HT-29 cell inflammation model, suggesting RSV can inhibit the expression of TACE.

RSV Inhibits the Expression of IL-6 and TNF-α in HT-29 Cell Inflammation Model

Inflammatory factors IL-6 and TNF-α are important indicators for judging the degree of cellular inflammation. Therefore, we detected the inhibition of RSV on LPS-stimulated IL-6, and TNF-α in the HT-29 cell inflammation model by qRT-PCR. The results were shown in Fig. 4a-b: Compared to control cells, LPS increased the expression levels of inflammatory factors IL-6 and TNF-α in HT-29 cells. After HT-29 cells were incubated with RSV, the expression levels of inflammatory factors IL-6 and TNF-α in the HT-29 cell inflammation model were significantly decreased. These results indicated that RSV has a good anti-inflammation effect.

RSV Up-regulates the Expression of Tight Junction Proteins in HT-29 Cell Inflammation Model

Occludin, ZO-1, and claudin-1 are important components of tight junction proteins, which are involved in the maintenance of the structure and function of the intestinal epithelial barrier. Therefore, we detected the expression of occludin, ZO-1, and claudin-1 by Western Blot and qRT-PCR. The results were shown in Fig. 5a-g: Compared to control cells, LPS down-regulated the expression levels of tight junction proteins occludin, ZO-1, and claudin-1. On the contrary, the expression of occludin, ZO-1, and claudin-1 in the HT-29 cell inflammation model were up-regulated after being incubated with RSV.

We further observed the tight junction structure by transmission electron microscope. The results were shown in Fig. 6: the normal tight junction structure between HT-29 cells was narrow and continuous strips under the transmission electron microscope. After HT-29 cells were incubated with LPS, the tight junction structure was loose, the intercellular space was widened, and the continuity of bands of the structure was interrupted. When the HT-29 cell inflammation model was incubated with RSV, the tight junction structure became tighter, the gap between cells was narrowed, and the disruption of tight junctions was improved.

Altogether, these results suggested that RSV can up-regulate tight junction proteins to protect the integrity of tight junction structures.

RSV Inhibits the Activity of Notch1 Pathway in HT-29 Cell Inflammation Model

The Notch1 pathway is involved in the signal transduction of cell differentiation, apoptosis, and survival, and plays an important role in the maintenance of the structure and function of the intestinal epithelial barrier. Therefore, we detected the expression of Notch1 pathway-related indicators by Western Blot and qRT-PCR. The results were shown in Fig. 7a-e: LPS led to the activation of Notch1 pathway that was prevented by RSV in a dose-dependent manner.

RSV Up-regulates the Expression of Tight Junction Proteins in HT-29 Cell Inflammation Model by Inhibiting Notch1 Pathway Activity

To verify the mechanism of RSV regulating the expression of tight junction proteins in the HT-29 cell inflammation model, we verified its mechanism by the Notch1 pathway activator Jagged-1. The results were shown in Fig. 8a-e: Jagged-1 abolished the inhibition effect of RSV against LPS-induced the activation of Notch1 pathway.

After Jagged-1 abolished the inhibition effect of RSV against LPS-induced the activation of Notch1 pathway, we detected the expression of tight junction proteins occludin, ZO-1, and claudin-1 in the HT-29 cell inflammation model incubated by RSV via Western Blot and qRT-PCR. The results were shown in Fig. 9a-g: Compared to cells incubated by RSV, the expression levels of tight junction proteins occludin, ZO-1, and claudin-1 in the HT-29 cell inflammation model incubated with RSV and Jagged-1 were decreased.

We further observed the tight junction structures by transmission electron microscope, and the results were shown in Fig. 10: Compared to cells incubated by RSV, the tight junction structure of cells incubated with RSV and Jagged-1 was loose, and the gap between cells became wider.

All these results supported that RSV can up-regulate the expression of tight junction proteins by inhibiting the activity of the Notch1 pathway.

Activation of Notch1 Pathway Promotes the Expression of TACE in RSV-Incubated HT-29 Cells Inflammation Model

The expression levels of TACE in the RSV-incubated HT-29 cell inflammation model were detected by qRT-PCR after the Notch1 pathway was activated by Jagged-1. The results were shown in Fig. 11: Compared to cells incubated by RSV, the expression levels of TACE in the HT-29 cell inflammation model incubated with RSV and Jagged-1 were increased.

Activation of Notch1 Pathway Promotes the Expression of IL-6 and TNF-α in RSV-Incubated HT-29 Cells Inflammation Model

The expression of inflammatory factors IL-6 and TNF-α in the RSV-incubated HT-29 cell inflammation model was detected by qRT-PCR after the Notch1 pathway was activated by Jagged-1. The results were shown in Fig. 12a-b: Compared to cells incubated by RSV, the expression levels of IL-6 and TNF-α in the HT-29 cell inflammation model incubated with RSV and Jagged-1 were increased.

Discussion

In the intestine, downregulation of tight junction proteins can lead to an impaired defense of the intestinal barrier, which in turn promotes the occurrence and development of UC [22–24]. Therefore, restoring the expression of tight junction proteins can effectively relieve the symptoms of UC. RSV plays an important role in modulating intestinal inflammation of UC, given that RSV contributes to preserving tight junction proteins [21, 25], but the mechanism by which RSV regulates the expression of tight junction proteins remains unclear. So, this work aimed to explore the potential mechanism of RSV action at the expression of tight junction proteins.

In this study, we found that LPS triggered an increase in TACE, IL-6, and TNF-α in HT-29 cells. Also, exposure to LPS led to the activation of Notch1 pathway and led to the decrease of tight junction proteins in HT-29 cells. Accordingly, LPS caused the disruption of tight junction structure, manifested in widening of intercellular spaces, and disruption of the continuity of tight junction structure, suggesting that the structure of the intestinal epithelial barrier was damaged. These results were consistent with the results that the expression levels of inflammation factors, TACE, and the activity of Notch1 pathway are increased, the expression levels of tight junction proteins are decreased in UC patients [5, 26, 27], indicating that the LPS-induced intestinal epithelial cell inflammation model of HT-29 cells was successfully contracted. And all results induced by LPS could be inhibited by RSV, suggesting that RSV can inhibit the activation of the Notch1 pathway and alleviate cellular inflammation, and upregulate the expression of tight junction proteins in the intestinal epithelium. The results that RSV could up-regulate the expression levels of tight junction proteins and alleviate inflammation were consistent with those of previous studies in UC models [21, 25]. In subsequent experiments, after the Notch1 pathway activator Jagged-1 was used in combination with RSV, the Notch1 pathway was reactivated in the inflammation model of HT-29 cells cultured with RSV, and the expression levels of tight junction proteins occludin, ZO-1, and claudin-1 were decreased. The comprehensive experimental results showed that: In the HT-29 cell inflammation model, RSV up-regulated the expression levels of tight junction proteins occludin, ZO-1, and claudin-1, the mechanism was related to the inhibition of Notch1 pathway activity by RSV.

In this report, we demonstrated that the mechanism of RSV up-regulating the expression levels of intestinal epithelial tight junction proteins was related to the inhibition of Notch1 pathway activation,

which was inconsistent with the previous conclusion that " the activation of Notch1 pathway could stabilize the expression of tight junction proteins" [28], and this may be related to the decreased expression of inflammation factors IL-6 and TNF-α after the Notch1 pathway was inhibited. The Notch1 pathway, as a highly conserved signaling pathway in the body, is mainly composed of Notch1 receptors, Notch1 ligands, and DNA-binding proteins. When ligands and receptors of two adjacent cells are combined, the Notch1 pathway is activated mediated by TACE [29]. After the Notch1 pathway is activated, its ligands can promote the activation of the NF-κB pathway through TRAF6, thereby promoting the secretion of inflammatory factors IL-6 and TNF-α downstream of the NF-κB pathway [30]. When Notch1 pathway activity is inhibited, the expression levels of inflammation cytokines IL-6 and TNF-α would be decreased, which in turn alleviates inflammation. In this study, we found that RSV reduced the expression levels of TACE and inhibited the activity of the Notch1 pathway in the HT-29 cell inflammation model, which in turn reduced the expression of inflammatory factors IL-6 and TNF-α, and ultimately alleviated tight junction proteins damage in intestinal epithelial cells by inflammatory factors IL-6 and TNF-α. So, when Notch1 pathway activity is properly inhibited, the expressions of tight junction proteins are up-regulated in the HT-29 cell inflammation model.

Another possible reason for the up regulation of tight junction proteins expression after inhibition of the Notch1 pathway is that inhibition of the Notch1 pathway maintains the balance of intestinal epithelial cell spectrum without affecting the proliferation and renewal of intestinal epithelial cells. The Notch1 pathway plays a dual role in maintaining the stability of intestinal epithelial barrier structure and function. On the one hand, the over-activation of the Notch1 pathway leads to the overexpression of the Hes1 gene downstream of the Notch1 pathway, which in turn inhibits the Math1 gene, resulting in the increase in intestinal absorptive cells and the decrease in secretory cells. Finally, the defense function of the intestinal epithelial barrier is impaired and the intestinal inflammatory response is aggravated [31]; On the other hand, completing knockout of the Notch1 gene leads to the inhibition of intestinal epithelial cell proliferation and renewal in mice, leading to the decrease in expression of tight junction proteins, thereby aggravating intestinal inflammation [28]. Therefore, proper maintenance of the Notch1 pathway activity can promote the balance of intestinal epithelial cell spectrum, it can also ensure the proliferation and renewal of intestinal epithelial cells and maintain the intestinal epithelial barrier function. In this study, the activity of the Notch1 pathway was inhibited to a certain extent under the action of RSV, and the proliferation and renewal capacity of HT-29 cells were not affected (the results of CCK-8 indicated that RSV had no cytotoxicity to HT-29 cells after 24 h at concentrations ≤ 100 μmol/L), the expression levels of tight junction proteins in the intestinal epithelium were up-regulated, the gap of tight junction structure was narrow, and the band continuity was good. As the Notch1 pathway activator, Jagged-1 can alleviate LPS-induced intestinal epithelial cell inflammation by promoting cell proliferation[32]. In this study, CCK-8 and previous experiments found that when the concentration of Jagged-1 was 10 μmol/L, it did not affect the viability of HT-29 cells and could activate the Notch1 pathway activity. When RSV was combined with Jagged-1, the effect of RSV up-regulating intestinal epithelial tight junction proteins was reversed. Combined with this study and previous studies [28], it was concluded that: under the premise of not affecting the proliferation and renewal of intestinal epithelial cells, the inhibition of Notch1 pathway

activity can up-regulate the expression of intestinal epithelial tight junction proteins and alleviate LPS-induced inflammation in HT-29 cells.

In conclusion, this study demonstrated that RSV significantly reduced LPS-induced inflammatory response and ameliorated intestinal epithelial tight junction proteins injury by inhibiting the activation of TACE and Notch1 pathways, suggesting that RSV may be a potentially effective drug for the treatment for UC.

Declarations

Ethical Approval

Not applicable.

Content for Publication

All authors have read and approved the submission.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author.

Competing Interests

The authors declare that they have no conflict of interest

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Authors' Contributions

Xue Huang contributed to the conception of the paper and review the final manuscript. Yihua Luo performed most of the experiments shown in the manuscript and wrote the paper. Xueyan Yu performed data analysis. Peizhuang Zhao and Jun Huang contributed to constructive discussions.

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Figures

Figure 1

The effect of drugs on HT-29 cell viability. a-c Cells were incubated with different concentrations of LPS, RSV, and Jagged-1 for 24 h and 48 h, the effect of drugs on the viability of the HT-29 cells was detected by CCK-8. The values shown were mean ± SD, *P<0.05 *vs*. compared to the control group.

Figure 2

The effect of LPS on tight junction proteins in HT-29 cell inflammation model. a-d The protein levels of occludin, ZO-1, and claudin-1 were detected by Western Blot. The values shown were mean ± SD, *P<0.05.

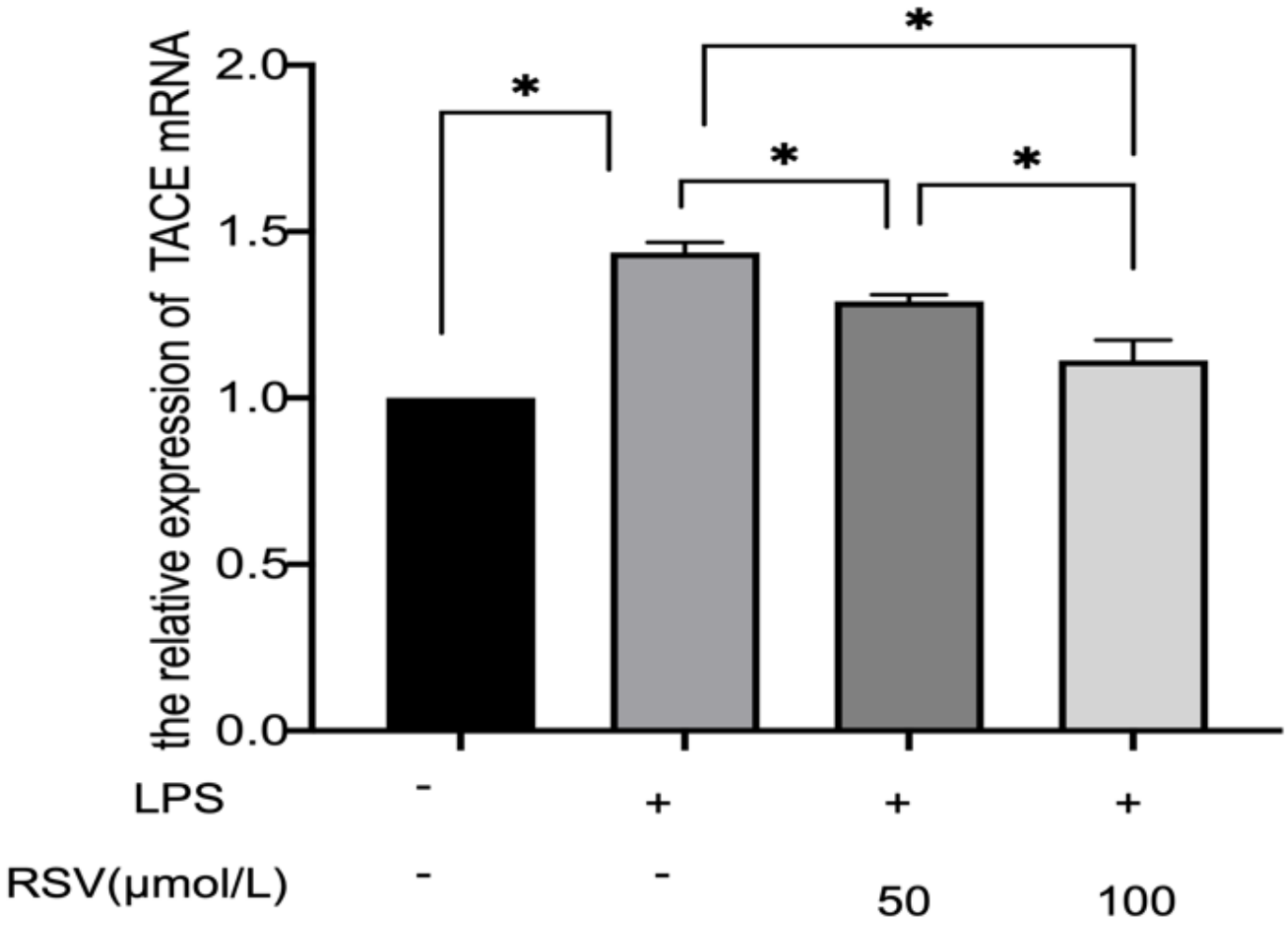

Figure 3

The effect of RSV on TACE in HT-29 cell inflammation model. The expression levels of TACE were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

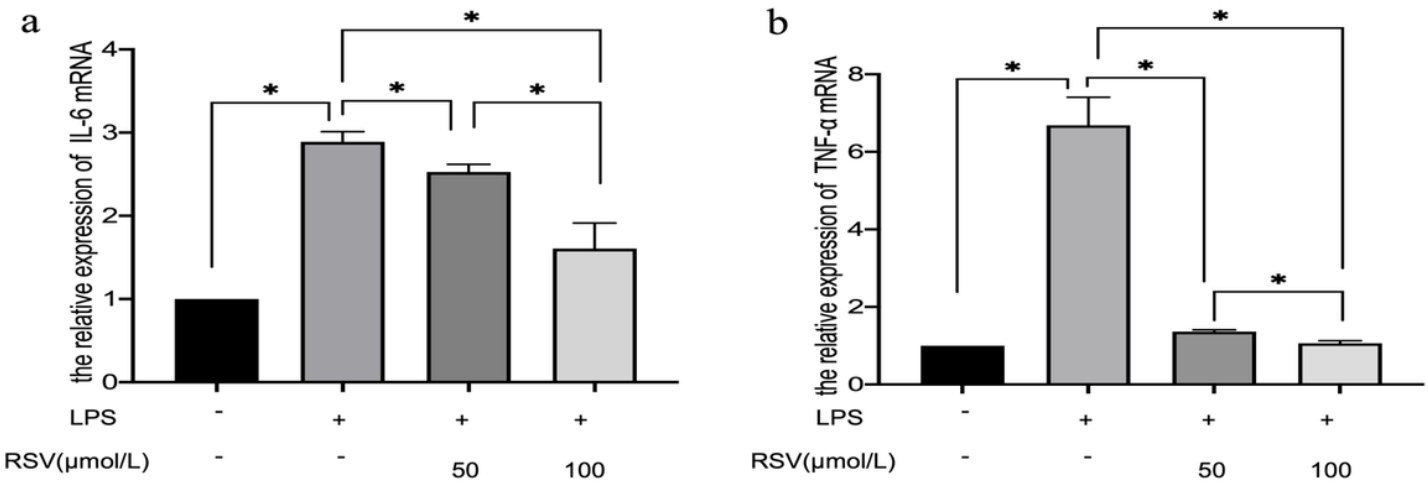

Figure 4

The effect of RSV on the inflammatory factors IL-6 and TNF-α in the HT-29 cell inflammation model. The expression levels of inflammatory factors IL-6 and TNF-α in the HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

Figure 5

The effect of RSV on tight junction proteins in HT-29 cell inflammation model. a-d The proteins expression levels of occludin, ZO-1, and claudin-1 in the HT-29 cell inflammation model were detected by Western Blot, e-g The mRNA expression levels of occludin, ZO-1, and claudin-1 in the HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

Figure 6

The effect of RSV on tight junction structure in HT-29 cell inflammation model. The tight junction structure between the HT-29 cell inflammation model was observed by transmission electron microscope, and the part indicated by the arrow was the tight junction structure (scale = 1 μm).

Figure 7

The effect of RSV on the activity of the Notch1 pathway in the HT-29 cell inflammation model. a-c The proteins expression levels of Notch1 and Hes1 in the HT-29 cell inflammation model were detected by Western Blot, d-e The mRNA expression levels of Notch1 and Hes1 in the HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

Figure 8

The effect of Jagged-1 on the activity of the Notch1 pathway in the RSV-intervened HT-29 cell inflammation model. a-c The proteins expression levels of Notch1 and Hes1 in the RSV-intervened HT-29 cell inflammation model were detected by Western Blot, d-e The mRNA expression levels of Notch1 and Hes1 in the RSV-intervened HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

Figure 9

The effects of Notch1 pathway activation on the expression levels of tight junction proteins in the RSV-intervened HT-29 cell inflammation model. a-d The proteins expression levels of occludin, ZO-1, and claudin-1 in the RSV-intervened HT-29 cell inflammation model were detected by Western Blot, e-g The mRNA expression levels of occludin, ZO- 1, and claudin-1 were detected by qRT-PCR in RSV-intervened HT-29 cell inflammation model. The value shown were mean ± SD, *P<0.05.

Figure 10

The effect of Notch1 pathway activation on tight junction structure in the RSV-intervened HT-29 cell inflammation model. The tight junction structure between the HT-29 cells was observed by transmission electron microscope, and the part indicated by the arrow was the tight junction structure (scale = 1 μm).

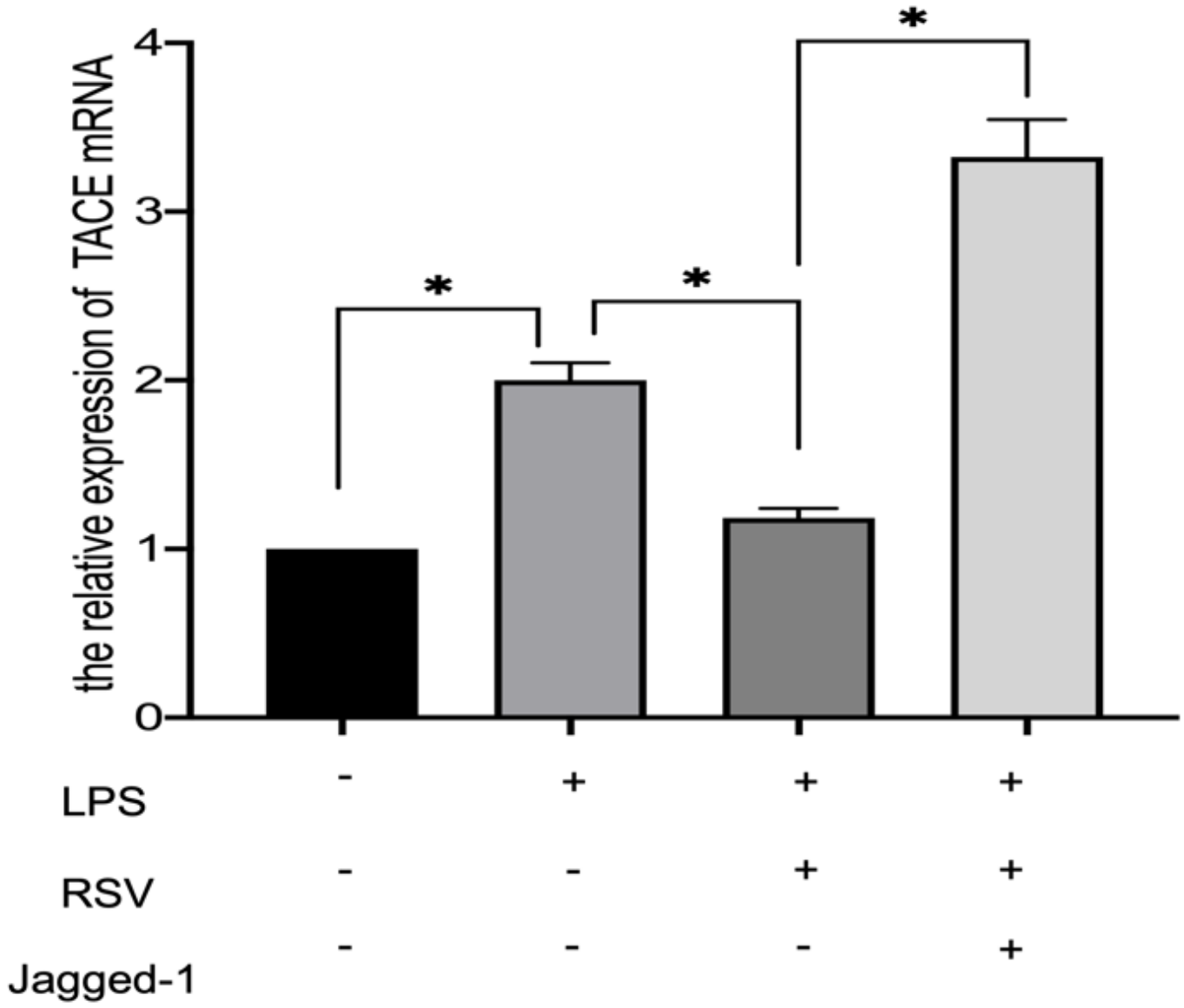

Figure 11

The effect of Notch1 pathway activation on expression levels of TACE in the RSV-intervened HT-29 cell inflammation model. The expression levels of TACE in the RSV-treated HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.

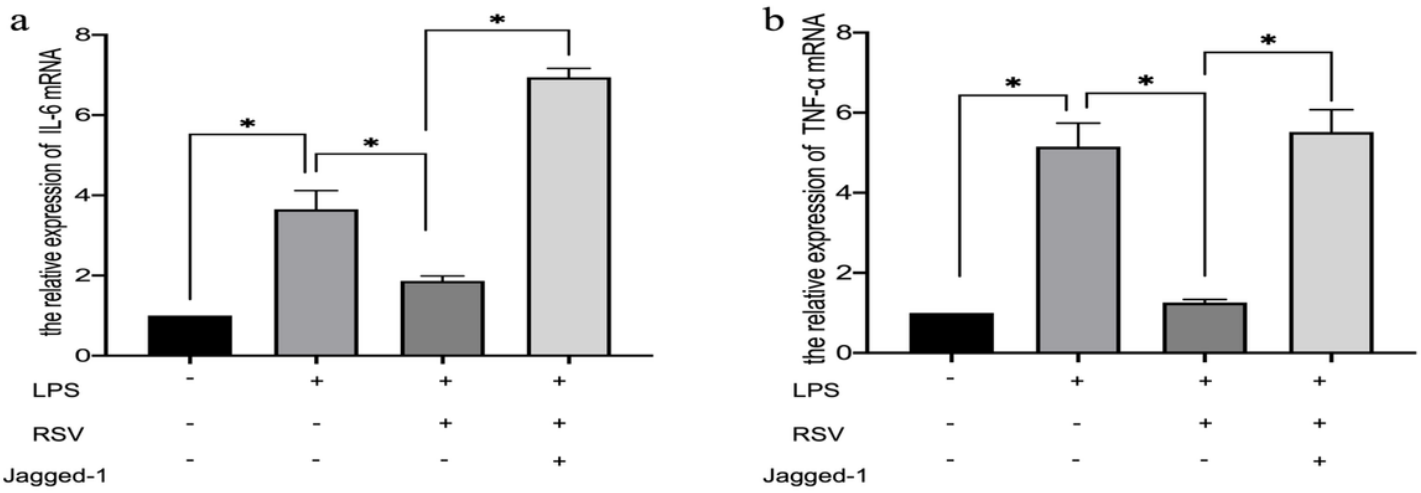

Figure 12

The effect of Notch1 pathway activation on the expression levels of IL-6 and TNF-α in the RSV-intervened HT-29 cell inflammation model. a-b The expression levels of inflammatory factors IL-6 and TNF-α in the RSV- intervened HT-29 cell inflammation model were detected by qRT-PCR. The values shown were mean ± SD, *P<0.05.