Role of the PERK-eIF2 α -CHOP signaling axis in mucosal barrier injury caused by ulcerative colitis

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Abstract

The etiology and pathogenesis of ulcerative colitis (UC) remain unclear. Therefore, we observed the activation and transduction of the protein kinase RNA-like endoplasmic reticulum kinase (PERK)–eukaryotic initiation factor 2 α (eIF2 α)–C/EBP homologous protein (CHOP) signaling axis from the perspective of endoplasmic reticulum stress (ERS) and explored the pathogenesis of UC. Cell survival rate, apoptosis rate, cycle distribution, cell permeability, ultrastructural changes in the ER, and the expression and transcription of key genes and proteins were detected. A UC mouse model was induced using dextran sulfate sodium. The disease activity index, tissue damage index, intestinal epithelial cell apoptosis, serum D-lactate, and diamine oxidase contents were determined. Blocking the PERK and CHOP nodes in the PERK–eIF2 α –CHOP signaling axis increased stressed cell survival rate, G2 cell proportion, and monolayer cell transmembrane electrical impedance, but decreased the apoptosis rate and fluorescein isothiocyanate dextran content, so the ER structure could be alleviated. Treatment with 5-aminosalicylic acid significantly improved intestinal and systemic symptoms, and colonic mucosal damage was relieved. p-PERK, p-eIF2 α , activating transcription factor 4, and CHOP gene transcription levels were higher in stressed cells than in normal cells, and 5-aminosalicylic acid reduced the expression of these proteins. Intestinal epithelial cells experience excessive ERS during UC, and the stress signal is successively transmitted to the apoptosis marker protein CHOP along the signal axis. Targeted blockage of the PERK–eIF2 α –CHOP signaling axis may be an effective treatment strategy for UC.

Introduction

Ulcerative colitis (UC) is a nonspecific inflammatory bowel disease characterized by chronic inflammation and ulcer formation in the colonic and rectal mucosa and submucosa. The etiology and pathogenesis of UC remain unclear. Previous research has indicated that intestinal mucosa barrier injury in this disease occurs because of genetic disposition and the combined effects of the gut microbiota and environmental, psychological, and immunological factors [1, 2]. Intestinal epithelial cells (IECs) are key mechanical components of the intestinal mucosal barrier that endure damage, and the resulting abnormalities initiate the onset and progression of UC [3, 4]. Maintaining the structure and function of IECs is therefore key to prevent intestinal mucosal barrier injury and a potential approach for developing new UC treatment strategies. In recent years, the role of endoplasmic reticulum stress (ERS) in the onset of UC has received increasing attention. IECs that are continuously exposed to stressors in the intestinal lumen, such as pathogenic microbes, ischemia, and hypoxia, and inflammatory mediators, may be susceptible to excessive ERS, leading to excessive apoptosis and changes in epithelial permeability [5–7]. The protein kinase RNA-like endoplasmic reticulum kinase (PERK)–eukaryotic initiation factor 2 α (eIF2 α)–C/EBP homologous protein (CHOP) signaling axis is a transduction pathway triggered by ERS that can participate in the apoptosis of cells in various diseases such as cardiomyopathy and chronic kidney disease [8]. Our previous work demonstrated increased p-PERK and CHOP protein expression in both ERS-affected IECs and in the colonic mucosa of mice with UC. In this study, we investigated whether activation of the PERK–eIF2 α –CHOP signaling axis induced structural damage and dysfunction in IECs

and led to mucosal barrier injury to elucidate the possible mechanisms underlying UC onset from the hierarchical framework of “molecular mechanisms–cell behavior–overall effects.” This study may serve as an objective basis for the formulation of effective UC treatment strategies.

Materials

Experimental cell line

The human colorectal adenocarcinoma cell line HT29 was purchased from the Cell Bank of the Committee on Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

Experimental animals

Forty-five 8-week-old healthy male specific-pathogen-free grade BALB/c mice with body weights of 20 ± 2 g were purchased from Shanghai Silaike Experimental Animal Co., Ltd. (Shanghai, China) (Animal Production License No.: SCXK (Shanghai) 2017-0005, Certificate No.: 0345203).

Reagents and materials

Dextran sulfate sodium (DSS) was purchased from MP Biomedicals (USA); 5-aminosalicylic acid (5-ASA) extended-release tablets were purchased from Ferring Pharmaceuticals (Switzerland); tunicamycin (TM), fluorescein isothiocyanate (FITC)-conjugated dextran (FITC-dextran) solution, McCoy's 5A medium and D-lactate (D-LAC), and diamine oxidase (DAO) Enzyme-linked immunosorbent assay (ELISA) kits were purchased from Sigma-Aldrich (USA); PERK inhibitor GSK2606414 and CHOP inhibitor doxorubicin (DOX) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China); fetal bovine serum (FBS) and trypsin were purchased from Gibco (USA); Transwell inserts were purchased from Corning (USA); enhanced BCA protein assay kit was purchased from Beyotime (Shanghai, China); PVDF membranes were purchased from Millipore (USA); rabbit monoclonal anti-PERK antibody, rabbit polyclonal anti-phospho-PERK antibody, rabbit monoclonal anti-eIF2 α antibody, and rabbit monoclonal anti-phospho-eIF2 α antibody were purchased from Abcam (UK); rabbit monoclonal anti-activating transcription factor 4 (ATF4) antibody and mouse monoclonal anti-CHOP antibody were purchased from Cell Signaling Technology (USA); mouse monoclonal anti-GAPDH antibody, mouse monoclonal anti- β -actin antibody, horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody, HRP-conjugated goat anti-mouse IgG secondary antibody, CCK8 assay kits, apoptosis assay kits, and cell cycle assay kits were purchased from Multisciences (Lianke) Biotech (Shanghai, China); TUNEL in situ apoptosis detection kits were purchased from Roche (Shanghai branch, China); high-purity total RNA rapid extraction kits were purchased from Shanghai Generay Biotech Co., Ltd. (Shanghai, China); and HiScript-II Q RT SuperMix for qPCR was purchased from Vazyme Biotech Co., Ltd. (Nanjing, China).

Equipment

In this study, the ECLIPSE Ti-S inverted phase contrast microscope (Nikon, Japan); SpectraMax Plus 384 full spectral range microplate reader (Molecular Devices, USA); Accuri C6 flow cytometer (BD Biosciences,

USA); Millicell-ERS voltohmmeter (Millipore, USA); Tecnai 10 transmission electron microscope (Philips, The Netherlands); and Mini-PROTEAN Tetra electrophoresis system and ChemiDoc XRS + gel imaging system (Bio-RAD, USA) were used.

Methods

Cell experiments

Cell culture, grouping, and treatment

HT29 cells were inoculated in a 50-mL culture flask with McCoy's 5A medium containing 10% inactivated FBS and 1X penicillin/streptomycin, and cultured in an incubator at 37°C with 5% CO₂ and saturating humidity. The culture medium was changed once every 1–2 days based on the cell growth. Cells were grown as adherent monolayers and passaged once every 2 to 3 days. The cultured HT29 cells were divided into four groups: the control group, ERS model group, GSK2606414 (PERK inhibitor) group, and DOX (CHOP inhibitor) group. A cell model replicating ERS was established using the TM induction method according to a previously described method [9]. Briefly, cells were treated with 10 µg/mL TM during 12 h.

Measurement of cell viability by CCK8 assay

HT29 cells in the logarithmic growth phase were harvested and digested with trypsin and 5×10^4 /mL cells were added to fresh medium. Cells were then inoculated on a 96-well plate (1×10^4 cells per well) and cultured for 24 h. The supernatant was removed, and the cells were subjected to the following interventions. (1) In the control group, cells were incubated in drug-free complete medium for 24 h. (2) In the ERS model group, cells were incubated in drug-free complete medium for 2 h, and subsequently in a complete medium containing 10 µg/mL TM for 22 h. (3) In the GSK2606414 group, cells were incubated first in a complete medium containing 10 µmol/L GSK2606414 for 2 h, and subsequently in a complete medium containing 10 µg/mL TM for 22 h. (4) In the DOX group, cells were first incubated in a complete medium containing 1 mg/L DOX for 2 h and subsequently in complete medium containing 10 µg/mL TM for 22 h. Following intervention, cells were washed with PBS, and cells were incubated with 10 µL of CCK8 working solution at 37°C for 2 h. The absorbance (A value) of each well at 450 nm was measured using a multimode plate reader and used to calculate the cell viability. The experiment was repeated three times for each group.

Measurement of apoptosis rate by flow cytometry

HT29 cells were obtained during the logarithmic growth phase, adjusted to a cell concentration of 1×10^5 cells/mL, inoculated onto a 6-well plate (2 mL/well), and cultured for 24 h. After the supernatant was removed, grouping and intervention were performed as described in the previous section. Following intervention, cells were digested with EDTA-free trypsin, harvested via centrifugation at $300 \times g$ for 5 min, and washed twice in 1X PBS. The cells were then suspended in 500 µL of binding buffer, 5 µL of Annexin

V-APC, and 10 μ L of 7-aminoactinomycin D (7-ADD), subjected to uniform mixing, and incubated at 25°C away from light during 5 min. The apoptosis rate was measured using flow cytometry (Annexin V-APC and 7-ADD measured in the FL4 and FL3 channels, respectively). The experiment was repeated three times for each group.

Measurement of cell cycle changes by flow cytometry

The cells were grouped and treated as described above. Subsequently, cells were washed twice with 1X PBS precooled to 4°C and centrifuged at $300 \times g$ for 5 min, mixed with 1 mL of DNA staining solution and 10 μ L permeabilization solution with a vortex mixer and incubated at 25°C away from light for 30 min before the cell cycle was assessed by flow cytometry. The experiment was repeated thrice for each group.

Measurement of cell monolayer transepithelial electrical resistance (TER) using a Millicell-ERS voltohmmeter

HT29 cells in the logarithmic growth phase were harvested and adjusted to a cell concentration of 5×10^4 cells/mL and inoculated in the upper compartment of the Transwell inserts (200 μ L/well) with 600 μ L of complete medium placed in the lower compartment. The cell growth was observed under a microscope until the cells were fully adherent at 12 h, after which they were grouped and subjected to intervention as described above. At the end of the intervention, the cell monolayer TER was measured using a Millicell-ERS voltohmmeter. The experiment was repeated three times for each group.

Measurement of paracellular permeability using the FITC-dextran method

The cells were placed into groups and subjected to intervention, as described in the previous section. After intervention, the cells were further incubated for 24 h, and the upper and lower compartments of the Transwell inserts were washed with Hanks' balanced salt solution. Subsequently, 100 μ L of 1 mg/mL FITC-dextran was added to the upper compartment and 500 μ L of Hanks' balanced salt solution was added to the lower compartment. The cells were then incubated at 37°C away from light for 2 h. The supernatant was collected from the base of each insert once every 30 min during incubation and placed in a 96-well plate for fluorescence intensity measurements using a fluorescence spectrophotometer at excitation and emission wavelengths of 480 and 520 nm, respectively. A standard curve was established for the calculation of the FITC-dextran concentrations, and the experiment was repeated three times for each group.

Observation of ultra-microstructural changes in the endoplasmic reticulum of cells using transmission electron microscopy

HT29 cells in the logarithmic growth phase were harvested and adjusted to a cell concentration of 1×10^5 cells/mL, inoculated on a 6-well plate (2 mL/well), and cultured for 24 h. After the supernatant was removed, grouping and intervention were performed as previously described. At the end of the intervention, cells were removed using a cell scraper, fixed sequentially in 2.5% glutaraldehyde solution for

4 h and in 1% osmic acid solution for 1 h, fixed and stained in uranyl acetate solution for 30 min, sequentially dehydrated with serial ethanol treatments (50%, 70%, 90%, and 100%) and 100% acetone, and embedded in resin. The resulting material was then subjected to polymerization and sliced into ultrathin sections. Ultra-microstructural changes in the endoplasmic reticulum of cells were observed using a transmission electron microscope.

Measurement of the expression levels of key proteins in the PERK–eIF2 α –CHOP signaling axis using western blotting

The cells were grouped and treated as described above. At the end of intervention, the cells were digested, lysed, and centrifuged, and the supernatant was collected. Protein concentration in the supernatant was measured using the bicinchoninic acid protein assay. Samples containing 20 μ g of protein from each group were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to a PVDF membrane, and blocked at 25°C for 1 h. Each membrane was incubated overnight in a shaking incubator at room temperature with anti-PERK, anti-phospho-PERK, anti-eIF2 α , anti-phospho-eIF2 α , anti-ATF4, and anti-CHOP primary antibodies diluted 1:1000. Subsequently, the membranes were washed three times with 1X TBST, incubated at 25°C for 1 h with the corresponding secondary antibodies diluted 1:5000, and washed three times with 1X TBST. The blots were visualized using electrochemical luminescence, and an image processing software was used to analyze the gray scale values of the bands. GAPDH was used as the internal loading control for the normalization of the sample loading.

Measurement of the transcription levels of genes encoding key proteins in the PERK–eIF2 α –CHOP signaling axis by quantitative fluorescence PCR (QF-PCR)

The cells were grouped and treated as described above. At the end of intervention, the cells were digested and lysed, and total RNA was extracted using the TRIzol-spin column method. After the total RNA purity and content were measured, the RNA was reverse-transcribed into cDNA according to the instructions provided in the test kit. The obtained cDNA was then used as the template for QF-PCR to measure the mRNA expression levels of *PERK*, *eIF2 α* , *ATF4*, and *CHOP*. Table 1 shows the primer sequences used and the length of the amplified fragments. The reaction mix (20 μ L) comprised 10.0 μ L 2X ChamQ SYBR Color qPCR Master Mix, 0.6 μ L each of the forward and reverse primers (10 μ mol/L), and 8.8 μ L of cDNA. PCR was carried out at 95°C for 30 s, followed by 40 cycles at 95°C for 10 s and 60°C for 30 s, after which the temperature gradually decreased from 70°C to -95°C for generation of dissociation curves. Expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method, using the expression levels of the gene encoding actin as the internal control.

Table 1
Primer sequences and amplified fragments for quantitative fluorescence PCR.

Primer name	Primer sequence		No. of bases	Amplified fragment length (bp)
Actin	F	TGACGTGGACATCCGCAAAG	20	205
Actin	R	CTGGAAGGTGGACAGCGAGG	20	
PERK	F	ACGATGAGACAGAGTTGCGAC	21	80
PERK	R	ATCCAAGGCAGCAATTCTCCC	21	
eIF2α	F	TGGTGAATGTCAGATCCATTGC	22	114
eIF2α	R	TAGAACGGATACGCCTTCTGG	21	
ATF4	F	CCCTTCACCTTCTTACAACCTC	22	85
ATF4	R	TGCCCAGCTCTAAACTAAAGGA	22	
CHOP	F	GAACGGCTCAAGCAGGAAATC	21	80
CHOP	R	TTCACCATTCTGGTCAATCAGAG	22	
ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase.				

Animal experiments

Animal rearing, grouping, and treatment

BALB/c mice were acclimatized in a clean-grade animal room for 1 week with the temperature and relative humidity maintained at 20–25°C and 40–70%, respectively (Animal room license number: SYXK (Zhejiang) 2015-0008). All animal experiment protocols were conducted in accordance with the policies and regulations of the Animal Ethics Committee of Zhejiang Chinese Medical University (Zhejiang, China).

After acclimatization, the mice were randomly divided into three groups, which comprised 15 animals each: the control group, the UC model group, and the 5-ASA group. The UC mouse model was induced using DSS as previously described[10]. Mice in the UC model and in the 5-ASA groups were allowed to drink a 5% DSS solution (5 g of DSS dissolved in 100 mL of distilled water) ad libitum for seven consecutive days. In the control group, mice were allowed to drink distilled water instead of the DSS solution. Based on the human:mouse body surface area ratio, the clinically equivalent dose of 0.2 mL of 50 mg/L 5-ASA suspension was administered to the 5-ASA group by gavage once per day for 10 consecutive days while the model was established, whereas the control and UC model groups were given equivalent volumes of distilled water by gavage. On day 10, two mice in the UC group were subjected to laparotomy to remove the colon. After washing the colon, the absence or presence of hyperemia and edema was observed with the naked eye, and the distal colon at approximately 8 cm from the anus was

removed to prepare pathological sections. Mice in the UC group exhibited loose stools, bloody stools, reduced body weight, and pathological changes such as ulcers of the colonic mucosa, tissue hyperemia and edema, and infiltration of inflammatory cells (e.g., neutrophils) into the tissues, indicating that the UC model had been established successfully.

Clinical evaluation of the severity of colitis in mice

During the experimental period, the general health status (mental state, hair condition, and activity) of the mice in the experimental groups was observed daily, the intake of food and beverages was measured once every two days, body weight was measured simultaneously each day, and stool characteristics were observed and rated. Fecal occult blood tests (FOBT) were performed three times over 10 days. The disease activity index (DAI) for the mice was calculated using the following equation: $DAI = (\text{body weight reduction rate score} + \text{stool traits score} + \text{FOBT score})/3$, as previously described [11].

Pathological evaluation of colonic mucosal injury in mice

On day 10, the mice were sacrificed by cervical dislocation and colonic tissues were removed under aseptic conditions. The distal colon approximately 8 cm from the anus was obtained, trimmed to a size of 1.0 cm × 1.0 cm × 0.2 cm, fixed in formaldehyde for 4 days, embedded in paraffin, sectioned, and subjected to hematoxylin and eosin staining. Pathological presentations of colonic mucosal injury in the sections were observed under an optical microscope and 10 fields of view (FOVs) from each section were randomly selected for imaging at 200x magnification. The tissue damage index (TDI) was calculated using a previously reported method [12].

Measurement of IEC apoptosis in the colonic mucosa of mice via the TUNEL assay

Colonic tissues were trimmed, fixed, embedded, and sectioned according to the procedure described above. After TUNEL staining, the distribution of apoptotic (positively stained) IECs in the colonic mucosa was observed under an optical microscope. For each section, five FOVs were randomly observed at 400x magnification, and the number of TUNEL-positive cells was recorded. TUNEL-negative IECs were stained blue, whereas TUNEL-positive IECs were stained brown or dark brown.

Measurement of serum D-LAC and DAO levels using ELISA

Serum levels of D-LAC and DAO indicate the intestinal mucosal barrier permeability. On day 10, anticoagulated blood was collected by eye enucleation and centrifuged at $366 \times g$ for 10 min. The top serum layer was removed after centrifugation and subjected to ELISA following manufacturer's instructions.

Measurement of expression levels of key proteins in the PERK–eIF2 α –CHOP signaling axis using western blotting

Total protein was extracted from colon tissue samples of mice of all experimental groups, and the expression levels of PERK, p-PERK, eIF2 α , p-eIF2 α , ATF4, and CHOP were measured according to the procedure described in above.

Statistical analysis

Data were statistically analyzed using SPSS 20.0. Quantitative data were expressed as the mean $\pm$ standard deviation. Pairwise comparisons of the groups were performed using the independent samples t-test, and multiple groups were compared using one-way analysis of variance. Differences were considered statistically significant at $P < 0.05$.

Results

Results of cell experiments

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the viability of stressed cells

According to the CCK8 assay, the cell viability of the control and ERS model groups was $95.22 \pm 6.05\%$ and $36.10 \pm 4.51\%$, respectively. Compared to the control group, the ERS model group showed a statistically significant reduction in cell viability ($P < 0.01$). The cell viabilities of the GSK2606414 and DOX groups were $79.63 \pm 6.58\%$ and $88.07 \pm 3.43\%$, respectively, and were significantly higher than those of the ERS model group ($P < 0.01$) (Fig. 1).

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the apoptosis rate and cell cycle distribution of stressed cells

According to flow cytometry results, the apoptosis rates of the control and ERS model groups were $5.47 \pm 0.18\%$ and $42.72 \pm 1.44\%$, respectively. The ERS model group showed a statistically significant increase in the apoptosis rate compared with the control group ($P < 0.01$). The apoptosis rates of the GSK2606414 and DOX groups were $19.43 \pm 1.44\%$ and $13.25 \pm 1.75\%$, respectively, both significantly lower than the rates observed for the ERS model group ($P < 0.01$) (Fig. 2).

Furthermore, flow cytometry results indicated that the percentages of cells in the G2 phase in the control and ERS model groups were $25.27 \pm 0.45\%$ and $4.29 \pm 0.60\%$, respectively. Compared to the control group, the ERS model group showed a statistically significant decrease in the percentage of cells in the G2 phase ($P < 0.01$). The percentages of cells in the G2 phase in the GSK2606414 and DOX groups were $13.60 \pm 0.60\%$ and $18.97 \pm 0.76\%$, respectively, significantly higher than those observed for the ERS model group ($P < 0.01$) (Fig. 3).

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the barrier function of stressed cells

The barrier function integrity and paracellular permeability of the cell monolayer indicate whether the homeostasis of the intestinal mucosal barrier is impaired. These two features were measured by TER and

by the amount of FITC-dextran that crosses the monolayer, respectively.

Measurements obtained with the Millicell-ERS voltohmmeter indicated TER values of $522.36 \pm 21.08 \Omega \cdot \text{cm}^2$ and $204.17 \pm 29.11 \Omega \cdot \text{cm}^2$ for the control and ERS model groups, respectively, indicating a statistically significant reduction in TER of the ERS model group compared to that of the control group ($P < 0.01$). The TER values of the GSK2606414 and DOX groups were $310.76 \pm 15.48 \Omega \cdot \text{cm}^2$ and $335.98 \pm 31.40 \Omega \cdot \text{cm}^2$, respectively, significantly higher than those obtained for the ERS model group ($P < 0.01$) (Fig. 4).

Results of the FITC-dextran assay indicated that the amount of FITC-dextran that crossed the cell monolayer 0.5 h after adding FITC-dextran did not differ significantly among the four groups ($P > 0.05$). However, at 1, 1.5, and 2 h, the FITC-dextran content was significantly higher in the ERS group than in the control group ($P < 0.01$). Compared with the ERS model group, the GSK2606414 and DOX groups showed significantly lower FITC-dextran contents at the three time points ($P < 0.01$) (Fig. 5).

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the endoplasmic reticulum structure of stressed cells

Observation of the ultramicroscopic structures of the cells from the groups using transmission electron microscopy revealed abundant membranous rough endoplasmic reticulum structures, non-enlarged endoplasmic reticulum lumen, large and clearly visible nuclei, and pronounced nucleoli in the control group. The rough endoplasmic reticulum structures in cells of the ERS model group were wide-ranging in terms of morphology and size, and evident swelling and vacuolar changes (vesiculation) were observed. This was accompanied by ribosomal detachment and pyknosis, indicating evident damage-related changes to the structure of the endoplasmic reticulum. Cells from the GSK2606414 and DOX groups exhibited less endoplasmic reticulum swelling and rupture, a decrease in the volume and number of vacuoles, and an absence of ribosomal detachment and pyknosis (Fig. 6).

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the expression levels of key pathway proteins

Western blotting indicated that regardless of blockage of upstream PERK signaling or downstream CHOP signaling, the total expression level of the PERK protein did not differ significantly between the four groups of cells ($P > 0.05$). Total eIF2 α protein expression levels were similar among the four groups ($P > 0.05$). Compared to the control group, the ERS model group showed a statistically significant increase in expression levels of the *p*-PERK, *p*-eIF2 α , ATF4, and CHOP proteins ($P < 0.01$). When intracellular PERK signaling was blocked with the specific inhibitor GSK2606414, expression levels of *p*-PERK, *p*-eIF2 α , ATF4, and CHOP proteins decreased significantly compared to those for the ERS model group ($P < 0.01$). When intracellular CHOP signaling was blocked with the specific inhibitor DOX, ATF4, and CHOP protein expression levels decreased significantly compared with those of the ERS model group ($P < 0.01$), whereas no significant difference was observed between the *p*-PERK and *p*-eIF2 α protein expression levels in the ERS model and DOX groups ($P > 0.05$) (Fig. 7).

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the gene transcription levels of key pathway proteins

The results of QF-PCR indicated that the ERS model group presented a statistically significant increase in the transcription levels of the genes encoding PERK, eIF2 α , ATF4, and CHOP compared with the control group ($P < 0.01$). When intracellular PERK signaling was blocked with GSK2606414, the expression levels of those genes decreased significantly compared to those of the ERS model group ($P < 0.01$). When intracellular CHOP signaling was blocked with DOX, the gene transcription level of the CHOP protein decreased significantly compared to that in the ERS model group ($P < 0.01$) (Fig. 8).

Results of animal experiments

Comparison of clinical manifestations of UC in mice

The main intestinal symptoms of UC include loose stools or diarrhea, muco-sanguineous stools, and abdominal pain. The persistence or recurrence of discomfort associated with these symptoms often leads to listlessness, dysphoria, and reduced appetite in patients [13]. During the experimental period, we observed that mice of the control group were mentally and physically active, had lustrous hair, and did not exhibit symptoms such as loose or bloody stools. From day 3 of drug administration, mice in the UC model group appeared mentally sluggish, had dark yellow messy hair, were physically inactive and exhibited loose stools, and blood could be observed by the naked eye in the stools of certain mice. These manifestations were most pronounced on day 7 of drug administration. Mice in the 5-ASA group had darker hair color and soft stools that were well formed in some mice and did not visibly contain blood. The food and water intake trends of the groups were as follows: (1) Control group: both food and water intake were stable throughout the entire experimental period; (2) UC model group: food and water intake decreased gradually and reached a minimum in day 7 to the end of the experimental period; (3) 5-ASA group: both food and water intake were significantly higher than those of the UC model group, and only slightly lower than those of the control group (Fig. 9).

One of the main systemic symptoms of UC is the reduction in body weight. Analysis of the body weight of the mice during the experimental period revealed that the weight of the mice in the control group increased gradually, whereas those of the other two groups exhibited a tendency to decrease. In particular, the body weight of the UC model group decreased from day 4 onwards and reached a minimum by day 10. The body weight of the UC model group was significantly lower than the control group from day 4 to 10 ($P < 0.05$). Although mice in the 5-ASA group showed a decrease in body weight from day 6 onward, the trend of the fitted curve was not significantly different from that of the control group. The body weight of the mice in the 5-ASA group was also significantly lower than that of the mice in the control group from day 6 to 10 ($P < 0.05$), but significantly higher than that of the UC model group from day 9 to 10 ($P < 0.05$) (Fig. 10).

The DAI of the mice according to the reduction in body weight, stool characteristics, and FOBt results of the UC model group was significantly higher compared to that of the control group on days 3, 7, and 10

($P < 0.01$). At the same time points, the 5-ASA group had a DAI significantly lower than the UC model group ($P < 0.01$) (Fig. 11).

Comparison of pathological damage in the colonic mucosa of mice

The typical pathological manifestations of UC during the active phase include diffuse, acute, or chronic inflammatory cell (e.g., neutrophils, lymphocytes, plasma cells, and eosinophils) infiltration into the lamina propria, cryptitis, changes in the crypt structure, loss of crypts, and disorderly arrangement or massive damage and shedding of epithelial cells. Observations of the histological colonic mucosa of mice under an optical microscope revealed that the epithelium of the colonic mucosal tissue of mice in the control group was intact and smooth, with distinctly visible layered glands, and no erosion, ulcerations, or neutrophil infiltration could be observed. However, the colonic mucosa of the UC model group exhibited evident epithelial damage with widespread hyperemia and edema, erosion, and ulcerations in the tissues, partial collapses of the colonic wall, disorderly arrangement of epithelial cells, considerable cell damage, rupture, and shedding, clustered and blurred nuclei, submucosal crypt losses, and evident neutrophil and monocyte infiltration in the tissues. The colonic mucosal tissues of the mice in the 5-ASA group were largely smooth and intact, with a significantly lower extent of hyperemia and edema, a near-complete absence of erosion and ulcerations, orderly arrangement of the epithelial cells, a significant reduction in the number of ruptured cells, few crypt losses, and the absence of inflammatory cell infiltration. Furthermore, the TDI value was significantly higher in the UC model group than in the control group ($P < 0.01$), whereas the TDI value was significantly lower in the 5-ASA group than in the UC model group ($P < 0.01$) (Fig. 12).

Comparison of IEC apoptosis in the colonic mucosa of mice

A few apoptotic IECs were observed in the colonic tissue of the normal control group. The number of apoptotic IECs in the UC model group was significantly higher than that in the control group ($P < 0.01$), and the number of apoptotic IECs in the 5-ASA group was significantly lower than that in the UC model group ($P < 0.01$) (Fig. 13).

Comparison of serum D-LAC and DAO levels in mice

According to ELISA results, the serum D-LAC and DAO levels of the control group were 1.25 ± 0.31 $\mu\text{g/mL}$ and 1.13 ± 0.40 U/mL , respectively. The corresponding values for the ERS model group were 2.49 ± 0.79 $\mu\text{g/mL}$ and 4.55 ± 1.12 U/mL , respectively, which were significantly higher than those of the control group ($P < 0.01$). Serum levels of D-LAC and DAO of the 5-ASA group were 1.86 ± 0.60 $\mu\text{g/mL}$ and 2.58 ± 0.56 U/mL , respectively, and were significantly lower than those of the ERS model group ($P < 0.01$) (Fig. 14).

Comparison of the expression levels of key proteins on the PERK–eIF2 α –CHOP signaling axis in the colonic mucosa of mice

The results of western blotting did not indicate significant differences in the total protein expression levels of PERK and eIF2 α among the three groups ($P > 0.05$). However, the expression levels of the p -

PERK, *p*-eIF2 α , ATF4, and CHOP protein increased significantly increased in the ERS model group compared to those of the control group ($P < 0.01$) and with those of the 5-ASA group ($P < 0.01$) (Fig. 15).

Discussion

The global incidence of UC has grown in recent years. Due to its persistent nature, high recurrence, and high incidence among young adults, the disease has profoundly affected the social productivity and quality of life of many communities. Therefore, it is regarded as a major chronic disease that poses a threat to human health [14–16]. The intestinal mucosal barrier is a comprehensive defense system composed of mechanical, chemical, immune, and biological barriers that play a central role in the maintenance and execution of intestinal and systemic physiological functions and is therefore of immense importance to the body. With increasing depth of knowledge on the intestinal mucosal barrier, the basis of UC treatment has changed from inhibiting or downregulating the hyperactivity of immune-induced inflammatory cells to the novel concept of “barrier-based protection.” IECs are key structures in the mechanical barrier of the intestinal mucosa readily replenished upon physiological apoptosis through the differentiation of stem cells in the crypt bas [3, 4]. IECs are securely anchored to each other through closely linked intercellular structures, resulting in a stable structure that is the functional basis of the mechanical barrier. IECs exert their barrier effects mainly through highly selective epithelial permeability, allowing nutrients within the intestinal lumen to be absorbed into the submucosal blood flow via the transepithelial route on the one hand, while effectively preventing the free passage of pathogens and other exogenous antigens through the barrier to reach the lymphoid tissue of the intestinal lamina propria on the other, preventing the generation of abnormal immune responses. The abnormally excessive apoptosis of IECs associated with UC damages the mechanical barrier and increases the epithelial permeability of the intestine, which ultimately results in uncontrolled immune-induced inflammation and mucosal injury through the vicious cycle previously denoted as “local antigen exposure—release of inflammatory cytokines—networking communications among the cytokines—cascade amplification of the immune response” [17, 18]. Previous studies have demonstrated the presence of many apoptotic IECs and increased intestinal epithelial permeability in both animal models of UC and in the colonic mucosal tissues of patients with UC, indicating a role for the intestinal mucosal barrier in the onset and progression of UC [19–21].

ERS is a key response mechanism by which eukaryotes resist various stress-induced barriers to intracellular protein synthesis and processing [22]. Appropriate levels of ERS activate the unfolded protein response and protect cells from stress-induced injury or death; however, excessive ERS cannot be fully compensated for and results in cell injury. To maintain homeostasis within the body, a class of unique apoptotic signal transduction pathways is activated to eliminate dysfunctional stressed cells by inducing apoptosis. Of the three known ERS-specific apoptotic signal transduction routes, the PERK–eIF2 α –CHOP signaling axis is the most important and has been researched most extensively. Studies have shown that the dysregulation of this axis is an important feature of many diseases, including neurodegenerative diseases, cardiomyopathy, type 2 diabetes mellitus, chronic kidney disease, ischemia/reperfusion injury, and certain injuries associated with chemical poisoning [23]. Inhibited expression or activation of

components at key upstream or downstream nodes resist apoptosis, effectively preventing the onset and progression of the aforementioned diseases. Under conditions in which excessive ERS occurs, PERK is self-activated through autophosphorylation by homologous dimerization. The subsequent sequential translation and expression of eIF2 α , its phosphorylation, and the activation of ATF4 lead to the activation of the gene transcription of CHOP, which triggers ERS-induced apoptosis by inhibiting the anti-apoptotic protein Bcl-2 and promoting the expression of the pro-apoptotic protein Bax [24, 25]. Due to their unique physiological function, IECs are prone to excessive ERS. Previous research has shown that excessive ERS occurs in IECs of both animal models of UC and in the colonic mucosa of patients with UC [26–28]. Significant apoptosis has also been observed in in vitro models of stressed IECs [4, 5]. Therefore, investigating the relationship of the PERK–eIF2 α –CHOP signaling axis with abnormal IEC apoptosis and changes in epithelial permeability during UC could help elucidate the pathological and physiological mechanisms of intestinal mucosal barrier injury in UC.

In this study, a cell model of stressed IECs was first established to investigate the cellular behaviors of UC. The results of our cell experiments indicate using the ERS agonist TM for the in vitro induction of stress in HT29 cells resulted in reduced cell viability, increased apoptosis, decreased number of cells in the G2 phase, reduced cell monolayer TER, increased cell monolayer permeability to FITC-dextran, and reduced intracellular structural changes, such as swelling and vesiculation in the endoplasmic reticulum, ribosomal detachment, and pyknosis. These results demonstrate pronounced injury-induced changes in the endoplasmic reticulum structures of cells under ERS. Furthermore, the cell cycle was halted at the early mitotic phase in of cells, which increased the probability that cellular DNA was exposed to harmful exogenous stimuli. This led to severe injury and large-scale apoptosis, which impaired the integrity of the mechanical barrier and increased the epithelial permeability. Subsequently, we separately blocked PERK and CHOP signaling using specific inhibitors, and found that inhibition of either node of the PERK–eIF2 α –CHOP signaling axis caused an increase in cell viability under ERS, a reduction in the rate of apoptosis, an increase in the number of cells in the G2 phase, an increase in the cell monolayer TER, a decrease in the amount of FITC-dextran that crossed the cell monolayer, reduced swelling and vesiculation in the endoplasmic reticulum, and significantly alleviated ribosomal detachment and pyknosis. These results indicate that blocking PERK–eIF2 α –CHOP signaling effectively attenuates endoplasmic reticulum injury in cells and protects stressed cells against programmed cell death, thus maintaining the structural and functional stability of the intestinal epithelial barrier. Interestingly, the positive effects that blocking CHOP signaling had on the apoptosis rate, cell cycle, and the integrity of structures and functions of the epithelial barrier were more pronounced than those achieved by blocking PERK signaling. This may be partly because CHOP serves as a direct trigger for many downstream effectors that lead to apoptosis and because it can be activated by upstream signaling pathways other than the PERK–eIF2 α –CHOP signaling axis.

The effects of PERK–eIF2 α –CHOP signaling axis blockade were subsequently investigated using a mouse model of UC. The results of animal experiments indicated mice treated with DSS gradually exhibited typical intestinal symptoms of UC, such as loose and bloody stools, accompanied by systemic symptoms of UC such as listlessness, reduced food and water intake, and reduced body weight. Colonic

mucosal sections revealed the presence of typical UC-induced pathological changes, including epithelial damage, tissue erosion, ulceration, injury and shedding of epithelial cells, crypt loss, and acute inflammatory cell infiltration. The DAI, TDI, number of apoptotic colonic IECs, and serum D-LAC and DAO levels of the UC model group were also significantly higher than those of the control group, indicating the successful establishment of the DSS-induced UC mouse model. Our results also confirmed the presence of abnormal IEC apoptosis and an increase in the colonic epithelial permeability of the colonic mucosa in UC mice, in agreement with the results reported in previous studies. Subsequently, UC mice were treated with 5-ASA, which is the main medication used for the clinical treatment of UC currently. 5-ASA was found to significantly alleviate intestinal and systemic symptoms, attenuate colonic mucosal injury, reduce DAI and TDI values, serum levels of D-LAC and DAO levels, and the number of apoptotic colonic IECs in UC mice. This indicates that the protection of colonic mucosa from injury constitutes a mechanism by which 5-ASA exerts its therapeutic effects on UC.

Last, the protein expression and gene transcription levels of key proteins of the PERK–eIF2 α –CHOP signaling axis were measured. Our results indicate that the gene transcription levels of the four key node components PERK, eIF2 α , ATF4, and CHOP were lower in the cells of the control group than those of the two other groups. Both IECs subjected to ERS and the colonic mucosa of UC mice exhibited significantly higher p-PERK, p-eIF2 α , ATF4, and CHOP protein expression levels. This indicates that activation of the PERK–eIF2 α –CHOP signaling pathway under excessive ERS is the key mechanism by which intestinal mucosal barrier injury occurs in UC, mainly characterized by abnormal apoptosis of IECs and changes in intestinal epithelial permeability. We also observed that blocking the gene transcription and activating the protein expression of upstream PERK induced a reduction in the gene transcription and protein expression of the downstream components eIF2 α , ATF4, and CHOP. In contrast, blocking the signaling of downstream CHOP did not produce significant effects on the transcription and translation of the upstream components, except for decreasing the protein expression levels of ATF4. Compared with the indirect blocking of CHOP with a PERK inhibitor, directly blocking CHOP with DOX had a more pronounced effect on limiting CHOP signaling. Following treatment with 5-ASA, the UC mice also exhibited decreases in the p-PERK, p-eIF2 α , ATF4, and CHOP protein expression levels, indicating that inhibiting the PERK–eIF2 α –CHOP signaling axis may be a possible mechanism by which 5-ASA exerts its protective effects against UC-induced intestinal mucosal barrier injury.

Based on the results of this study, we hypothesize that intestinal mucosal barrier injury in UC may be related to activated transduction by the PERK–eIF2 α –CHOP signaling axis. The onset of UC renders IECs in a state of excessive ERS, leading to the activation of the PERK–eIF2 α –CHOP signaling axis. Then, CHOP induces the abnormal apoptosis of IECs, which leads to increased intestinal epithelial permeability. Our results also indicate that PERK and CHOP are the key targets for upstream and downstream signal transduction of the PERK–eIF2 α –CHOP signaling axis, respectively. Given the complexity of the intestinal mucosal barrier and the diversity of ERS-related apoptotic signaling pathways, this study only partly elucidates the mechanisms of intestinal mucosal barrier injury in UC. Further research will be required to investigate issues such as the specific changes in the chemical, immune, and biological barrier components of the intestinal mucosal barrier under the pathological

conditions of UC, the presence or absence of interactive effects between these changes and mechanical barrier injury, other potential mechanisms of IEC injury, and whether the other two ERS-specific apoptotic signaling pathways participate in the onset of UC.

Declarations

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

Ethics approval: This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Experimental Animal Ethics Committee of Hangzhou Hebei Technology Co., LTD (July 7, 2020/HB2012030).

Consent to publish: Informed consent was obtained from all individual participants included in the study.

Abbreviations

5-aminosalicylic acid (5-ASA), 7-aminoactinomycin D (7-ADD), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), D-lactate (D-LAC), dextran sulfate sodium (DSS), diamine oxidase (DAO), disease activity index (DAI), doxorubicin (DOX), endoplasmic reticulum stress (ERS), eukaryotic initiation factor 2 α (eIF2 α), fecal occult blood testing (FOBT), fields of view (FOVs), fluorescein isothiocyanate (FITC), FITC-conjugated dextran (FITC-dextran), horseradish peroxidase (HRP), intestinal epithelial cells (IECs), protein kinase RNA-like endoplasmic reticulum kinase (PERK), quantitative fluorescence PCR (QF-PCR), tissue damage index (TDI), transepithelial electrical resistance (TER), tunicamycin (TM), ulcerative colitis (UC)

References

1. Nakase H, Uchino M, Shinzaki S et al (2021) Evidence-based clinical practice guidelines for inflammatory bowel disease 2020 [J]. J Gastroenterol 56(6):489–526

2. Ida Schoultz ÅsaV (2019) Keita Cellular and Molecular Therapeutic Targets in Inflammatory Bowel Disease—Focusing on Intestinal Barrier Function [J]. *Cells* 8(2):193
3. Kellie E, Cunningham EA, Novak G, Vincent et al (2019) Calcium/calmodulin-dependent protein kinase IV (CaMKIV) activation contributes to the pathogenesis of experimental colitis via inhibition of intestinal epithelial cell proliferation [J]. *FASEB J* 33(1):1330–1346
4. Pan H-H, Zhou X-X, Ma Y-Y et al (2020) Resveratrol alleviates intestinal mucosal barrier dysfunction in dextran sulfate sodium-induced colitis mice by enhancing autophagy [J]. *World J Gastroenterol* 26(33):4945–4959
5. Zeng LX, Tao J, Liu HL et al (2015) β -Arrestin2 encourages inflammation-induced epithelial apoptosis through ER stress/PUMA in colitis [J]. *Mucosal Immunol* 8(3):683–695
6. Dong Fu P, Li Q, Sheng (2019) Zhibao Lv. β -arrestin-2 enhances intestinal epithelial apoptosis in necrotizing enterocolitis [J]. *Aging* 11(19):8294–8312
7. Miao L, Chen X, Wang N et al (2018) Proanthocyanidins Protect Epithelial Cells from Zearalenone-Induced Apoptosis via Inhibition of Endoplasmic Reticulum Stress-Induced Apoptosis Pathways in Mouse Small Intestines [J]. *Molecules* 23(7):1508
8. Mahdi AA, Rizvi SH, Parveen A (2016) Role of Endoplasmic Reticulum Stress and Unfolded Protein Responses in Health and Diseases [J]. *Indian J Clin Biochem* 31(2):127–137
9. Takada K, Hirose J, Senba K et al (2011) Enhanced apoptotic and reduced protective response in chondrocytes following endoplasmic reticulum stress in osteoarthritic cartilage [J]. *Int J Exp Pathol* 92(4):232–242
10. Liljana Stevceva P, Pavli AJ, Husband, William F, Doe (2001) The inflammatory infiltrate in the acute stage of the dextran sulphate sodium induced colitis: B cell response differs depending on the percentage of DSS used to induce it [J]. *BMC Clin Pathol* 1:3
11. Hamamoto N, Maemura K, Hirata I, Murano M, Sasaki S, Katsu K (1999) Inhibition of dextran sulfate sodium (DSS)-induced colitis in mice by intracolonic administered antibodies against adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)) [J]. *Clin Exp Immunol* 117(3):462–468
12. Dieleman LA, Palmen MJHJ, Akol H, Bloemena E (1998) EPV Rees Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines [J]. *Clin Exp Immunol* 114(3):385–391
13. Bressler B, Marshall JK, Bernstein CN et al (2015) Clinical Practice Guidelines for the Medical Management of Nonhospitalized Ulcerative Colitis: The Toronto Consensus [J]. *Gastroenterology* 148(5):1035–1058e3. DOI: 10.1053/j.gastro.2015.03.001
14. Li X, Song P, Li J et al (2017) The Disease Burden and Clinical Characteristics of Inflammatory Bowel Disease in the Chinese Population: A Systematic Review and Meta-Analysis [J]. *Int J Environ Res Public Health*, 14(3). pii: E238
15. Bewtra M, Newcomb CW, Wu Q et al (2015) Mortality associated with medical therapy versus elective colectomy in ulcerative colitis: a cohort study [J]. *Ann Intern Med* 163(4):262–270

16. Ng WK, Wong SH, Ng SC (2016) Changing epidemiological trends of inflammatory bowel disease in Asia [J]. *Intest Res* 14(2):111–119
17. Vindigni SM, Zisman TL, Suskind DL, Damman CJ (2016) The intestinal microbiome, barrier function, and immune system in inflammatory bowel disease: a tripartite pathophysiological circuit with implications for new therapeutic directions [J]. *Th Adv Gastroenterol* 9(4):606–625
18. Zou Y, Lin J, Li W et al (2016) Huangqin-tang ameliorates dextran sodium sulphate-induced colitis by regulating intestinal epithelial cell homeostasis, inflammation and immune response [J]. *Sci Rep* 6:39299
19. Wang X, Li D, Zhang Y, Wu S, Tang F (2018) Costus root granules improve ulcerative colitis through regulation of TGF- β mediation of the PI3K/AKT signaling pathway [J]. *Exp Ther Med* 15(5):4477–4484
20. Wenyan Gao C, Wang L Yu, et al (2019) Chlorogenic Acid Attenuates Dextran Sodium Sulfate-Induced Ulcerative Colitis in Mice through MAPK/ERK/JNK Pathway [J]. *Biomed Res Int*, 2019: 6769789
21. Adolph TE, Tomczak MF, Niederreiter L et al (2013) Paneth cells as a site of origin for intestinal inflammation [J]. *Nature* 503:272–276
22. Margita Márton A, Kurucz B, Margittai L (2017) Gábor Bánhegyi, Orsolya Kapuy. A Systems Biological View of Life-and-Death Decision with Respect to Endoplasmic Reticulum Stress-The Role of PERK Pathway [J]. *Int J Mol Sci*, 18(1): 58
23. Mahdi AA, Rizvi SH, Parveen A (2016) Role of Endoplasmic Reticulum Stress and Unfolded Protein Responses in Health and Diseases [J]. *Indian J Clin Biochem* 31(2):127–137
24. Lei Y, Wang IS et al (2017) CHOP favors endoplasmic reticulum stress-induced apoptosis in hepatocellular carcinoma cells via inhibition of autophagy [J]. *PLoS ONE* 12(8):e0183680
25. Darling NJ, Balmanno K, Cook SJ (2017) ERK1/2 signalling protects against apoptosis following endoplasmic reticulum stress but cannot provide long-term protection against BAX/BAK-independent cell death [J]. *PLoS ONE* 12(9):e0184907
26. Wenzel UA, Magnusson MK, Rydström A et al (2014) Spontaneous colitis in Muc2-deficient mice reflects clinical and cellular features of active ulcerative colitis. *PLoS ONE* 9(6):e100217
27. Rainbolt TK, Saunders JM, Wiseman RL (2014) Stress-responsive regulation of mitochondria through the ER unfolded protein response. *Trends Endocrinol Metab* 25(10):528–537
28. Kim MH, Kim H (2017) The Roles of Glutamine in the Intestine and Its Implication in Intestinal Diseases. *Int J Mol Sci*. 18(5). pii: E1051

Figures

Figure 1

Figure 1

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the viability of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. **: $P < 0.01$. CHOP, C/EBP homologous protein; eIF2 α , eukaryotic initiation factor 2 α ; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 2

Figure 2

Effects of PERK-eIF2 α -CHOP signaling axis blockade on the apoptosis rate of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. **: $P < 0.01$. CHOP, C/EBP homologous protein; eIF2 α , eukaryotic initiation factor 2 α ; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 3

Figure 3

Effects of PERK-eIF2 α -CHOP signaling axis blockade on the cell cycle distribution of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. **: $P < 0.01$. CHOP, C/EBP homologous protein; eIF2 α , eukaryotic initiation factor 2 α ; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 4

Figure 4

Effects of PERK–eIF2 α –CHOP signaling axis blockade on the TER values of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group.**: $P < 0.01$. CHOP, C/EBP homologous protein; eIF2 α , eukaryotic initiation factor 2 α ; PERK, protein kinase RNA-like endoplasmic reticulum kinase; TER, transepithelial electrical resistance

Figure 5

Figure 5

Effects of PERK–eIF2α–CHOP signaling axis blockade on the FITC-dextran content of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; FITC, fluorescein isothiocyanate; FITC-dextran, FITC-conjugated dextran; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 6

Figure 6

Effects of PERK-eIF2α-CHOP signaling axis blockade on the endoplasmic reticulum structure of stressed cells. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 7

Figure 7

Effects of PERK-eIF2α-CHOP signaling axis blockade on the expression levels of key pathway proteins. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group. *: $P < 0.05$, **: $P < 0.01$. ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 8

Figure 8

Effects of PERK–eIF2α–CHOP signaling axis blockade on the gene transcription levels of key pathway proteins. A: control group; B: endoplasmic reticulum stress model group; C: GSK2606414 group; D: doxorubicin group.*: $P < 0.05$, **: $P < 0.01$. ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase

Figure 9

Figure 9

Comparison of food and water intake by the different groups of mice over time. A: control group; B: UC model group; C: 5-ASA group. ASA, aminosalicilyc acid; UC, ulcerative colitis

Figure 10

Figure 10

Comparison of body weights in the different groups of mice over time. A: control group; B: UC model group; C: 5-ASA group. ASA, aminosalicilic acid; UC, ulcerative colitis

Figure 11

Figure 11

Comparison of DAI values in the different groups of mice over time. A: control group; B: UC model group; C: 5-ASA group.**: $P < 0.01$. ASA, aminosalicylic acid; DAI, disease activity index; UC, ulcerative colitis

Figure 12

Figure 12

Comparison of pathological changes and TDI values of the colonic mucosa for different groups of mice. A: control group; B: ulcerative colitis model group; C: 5-aminosalicylic acid group.**: $P < 0.01$. TDI, tissue damage index

Figure 13

Figure 13

Comparison of intestinal epithelial cell apoptosis and the number of TUNEL-positive cells in different groups of mice. A: control group; B: ulcerative colitis model group; C: 5-aminosalicylic acid group.**: $P < 0.01$

Figure 14

Figure 14

Comparison of serum D-LAC and DAO levels in different groups of mice. A: control group; B: ulcerative colitis model group; C: 5-aminosalicylic acid group. **: $P < 0.01$. D-LAC, D-lactate; DAO, diamine oxidase

Figure 15

Figure 15

Comparison of expression levels of key proteins in the PERK-eIF2α-CHOP signaling axis of mouse colonic mucosa. A: control group; B: ulcerative colitis model group; C: 5-aminosalicylic acid group. **: $P < 0.01$. CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; PERK, protein kinase RNA-like endoplasmic reticulum kinase

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