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Basic Study

SLC6A14 promotes ulcerative colitis progression by facilitating NLRP3 inflammasome-mediated pyroptosis

SLC6A1 regulates UC progression *via* pyroptosis

Abstract

BACKGROUND

Ulcerative colitis (UC) is an inflammatory condition with frequent relapse and recurrence. Evidence suggests the involvement of SLC6A14 in UC pathogenesis, but the central regulator remains unknown.

AIM

We aimed to explore the role of SLC6A14 in UC-associated pyroptosis.

METHODS

Quantitative Real-time PCR (qRT-PCR), immunoblotting, and IHC were used to assess SLC6A14 in human UC tissues. Lipopolysaccharide (LPS) was used to induce inflammation in FHC and NCM460 cells and model enteritis, and SLC6A14 Levels were assessed. Pyroptosis markers were quantified using ELISA, Western blotting, and qRT-PCR, and EdU incubation, CCK-8 assays and flow cytometry were used to examine proliferation and apoptosis. Mouse models of UC were used for verification.

RESULTS

SLC6A14 was increased and correlated with NLRP3 in UC tissues. LPS-induced FHC and NCM460 cells showed increased SLC6A14 Levels. Reducing SLC6A14 increased cell proliferation and suppressed apoptosis. Reducing SLC6A14 decreased pyroptosis-associated proteins (ASC, IL-1β, IL-18, NLRP3). NLRP3 overexpression counteracted the effects of sh-SLC6A14 on LPS-induced FHC and NCM460 cell pyroptosis. SLC6A14 improved the mucosa in mice with DSS-induced colitis.

CONCLUSION

SLC6A14 promotes UC pyroptosis by regulating NLRP3, suggesting the therapeutic potential of modulating the SLC6A14/NLRP3 axis.

Key Words: Ulcerative colitis; SLC6A1; NLRP3; Pyroptosis; Inflammasome

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Core Tip: Ulcerative colitis (UC) is an inflammatory condition associated with frequent relapse and recurrence. Dysregulation of intestinal epithelial cells (IECs) and mucosal barrier impairment contribute to sustained inflammation in UC. Hence, an in-depth exploration of the triggers and mechanisms of IEC death could result in efficacious therapeutic options for UC patients. Here, we demonstrated the close involvement of SLC6A14 in promoting pyroptosis in the context of UC by upregulating NLRP3 expression. These findings indicate the potential of targeting SLC6A14/NLRP3 axis-mediated pyroptosis as a promising therapeutic strategy for treating UC. Our research provides valuable insights into the mechanisms driving UC pathogenesis and offers a possible direction for developing innovative treatments to alleviate the impact of this chronic inflammatory disorder.

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory condition associated with frequent relapse and recurrence. Its prevalence is increasing in developing nations as a result of increased consumption of Western diets [1, 2]. UC is typified by ulceration and inflammation of the colonic and rectal mucosa, leading to symptoms such as pain, diarrhea, and bleeding [3, 4]. UC has a complex etiology involving genetic, environmental, and immunological elements [5]. Dysregulation of intestinal epithelial cells (IECs) and mucosal barrier impairment contribute to sustained inflammation [6, 7]. The death of IECs disrupts the balance between intestinal microorganisms and the host, the regulation of mucosal immunity, nutrient absorption, and the integrity of the mucosal barrier, culminating in recurrent, prolonged colitis [8, 9]. Hence, an in-depth exploration of the triggers and mechanisms of IEC death could result in efficacious therapeutic options for UC patients.

Recent investigations underscore the vital role of the NLR family pyrin domain containing 3 (NLRP3) inflammasome in UC [10, 11]. This is activated in conditions of tissue damage, pathogen infection, and oxidative stress [12-14]. NLRP3 interacts with ASC through its N-terminal PYD domain, upregulating pro-IL-1β and pro-IL-18 and thus augmenting inflammation[15, 16]. Consequently, NLRP3, in conjunction with IL-1β, IL-18, and ASC, has emerged as a prospective therapeutic target for ameliorating pyroptosis and mitigating inflammatory responses in UC.

The solute carrier (SLC) transporter family has been recognized as a significant regulator of ferroptosis and diverse cancers [17]. Solute Carrier Family 6 Member 14 (SLC6A14) belongs to the solute carrier (SLC) transporter family and may contribute to UC [18, 19]. In this study, we investigated SLC6A14, which is upregulated in various colonic disorders, including UC, to evaluate its role in modulating IEC pyroptosis within the context of UC by using human intestinal epithelial cells and murine models. Our findings revealed robust SLC6A14 expression in UC patients, experimental colitis models, and pyroptosis-induced cell models. Importantly, a positive correlation between SLC6A14 and NLRP3 expression was identified. Downregulating SLC6A14 reduced the levels of pyroptosis-associated proteins, including NLRP3, and the production of IL-18

and IL-1β. Mechanistically, SLC6A14 was a positive regulator of NLRP3, a central figure in inflammasome-driven pyroptosis. The promotion of pyroptosis by SLC6A14 by targeting NLRP3 was verified in LPS-treated IECs and a UC mouse model, indicating the potential of SLC6A14 in the treatment of UC.

MATERIALS AND METHODS

Subjects and Samples

Colorectal mucosal biopsies were procured from two groups: healthy individuals (n = 29) and patients diagnosed with ulcerative colitis (n = 55). These biopsies were obtained during endoscopic examinations conducted at the Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital (Lunshen (Yan)2022-380). The study protocol was granted ethics approval by the Ethics Committee of the same institution and was performed in compliance with the Declaration of Helsinki (2013 revision). Prior to the extraction of the tissue samples, written informed consent was provided by each participant. The collected tissue specimens were promptly cryopreserved using liquid nitrogen for subsequent analysis.

Histopathological Assessment

Hematoxylin and eosin (H&E) staining was performed using established protocols. In summary, murine and human colonic samples were initially fixed (4% paraformaldehyde) and embedded in paraffin before being sectioned (5 μm). The sections were stained with hematoxylin to visualize the nuclei, and eosin was used to reveal cytoplasmic structures.

Immunohistochemical (IHC) Staining

Immunohistochemical staining was performed as previously described using a DAB kit (Gene Tech, Shanghai, China). In summary, colonic tissue sections were treated overnight at 4 °Cwith an anti-SLC6A14 antibody (Abcam Cat# ab254786, RRID:AB_3073883) at a 1:200 dilution. Then, a subsequent incubation was conducted with an appropriate HRP-

conjugated secondary antibody for 1 h at 37 °C The reactions were visualized after incubation with DAB (brown) with hematoxylin counterstaining (purple). The prepared tissue sections were subsequently observed and imaged by phase-contrast microscopy (Leica, Germany). An evaluation of these images was performed by two independent blinded pathologists, and the slides were assessed by multiplying the staining intensity (ranging from grades 0 to 5, with 0 indicating negative and 5 indicating strong positivity) by the corresponding positivity score (ranging from 0 to 5, where 0% to 100% was indicated by the scores).

Cell Culture

The normal colon epithelial cell lines FHC and NCM460 were sourced from the National Collection of Authenticated Cell Cultures (Shanghai, China). FHC and NCM460 cells were grown in RPMI-1640 medium (Gibco, USA) with 10% fetal bovine serum (Gibco). To establish a cellular model of colitis, the cells were incubated with 10 ng/mL lipopolysaccharide (LPS) for 6 h [20, 21]. The cells were grown in 6-well plates at 37 °C and 5% CO_2.

Plasmid construction and cell transfection

To downregulate SLC6A14, shRNA sequences targeting SLC6A14 and the negative control (NC) shRNA were obtained from GenePharma (Shanghai, China). Additionally, the NLRP3 overexpression (OE) plasmid pcDNA4.0-NLRP3 and the empty pcDNA4.0 plasmid were procured from Synbio Technologies Co. Ltd.™ (Suzhou, China). Cells were grown to 70% confluence and transfected with the plasmids using Lipofectamine 2000 (Thermo Fisher Scientific, USA) according to the provided directions. RNA was isolated after 48 h, and protein was isolated after 72 h.

Western Blotting

SLC6A14 protein levels in cells and patient biopsy tissues were analyzed by Western blotting. Proteins were extracted from the samples using RIPA buffer containing protease

and phosphatase inhibitors, and concentrations were assessed using a BCA kit (Thermo Fisher). Aliquots (30 μg) were separated on 10–12% SDS–PAGE gels and transferred to PVDF membranes (Millipore, Bedford, MA, USA). Primary antibodies against SLC6A14 (Thermo Fisher Scientific Cat# PA5-87998, RRID:AB_2804576, 1:1500), NLRP3 (Abcam Cat# ab263899, RRID:AB_2889890, 1:1000), ASC (Abcam Cat# ab283684, RRID:AB_3073880, 1:800), pro-IL-18 (Proteintech Cat# 10663-1-AP, RRID:AB_2123636, 1:1000), IL-1β (Abcam Cat# ab254360, RRID:AB_2936299, 1:1000), IL-18 (Abcam Cat# ab207324, RRID:AB_3073881, 1:1000), and GAPDH (Abcam Cat# ab9485, RRID:AB_307275, 1:1000) were used to probe the blots overnight at 4 °C, after which the blots were incubated with secondary antibodies for 60 min at room temperature. Bands were visualized using the ECL Western Blotting Detection System (Amersham, UK) and quantified using ImageJ. The internal reference was GAPDH. Three separate experiments were conducted.

Quantitative real-time PCR (qRT-PCR)

RNA was extracted from the samples using TRIzol reagent (Invitrogen, USA). cDNA was reverse transcribed using the Bestar qPCR RT kit (DBI Bioscience, #2220, Germany). To examine SLC6A14, the following primer sequences were used: forward primer 5′-TGCACCTGCTACCAGTCAAG-3′ and reverse primer 5′-GTCCATGGTTCACTCCCTCG-3′. The GAPDH primers were forward, 5′-GACAGTCAGCCGCATCTTCT-3′ and reverse, 5′-GCGCCCAATACGACCAAATC-3′. To assess SLC6A14 expression, Bestar qPCR MasterMix (DBI Bioscience, #2043) was used. GAPDH was used as the internal reference. The $2^{-\Delta\Delta Ct}$ method was used to quantify gene expression according to established procedures [22].

Cell Viability Assessment

Cell proliferation was assessed using CCK-8 assays (Dojindo Laboratories, Japan). Cells (2×10^3/well) were plated in 96-well plates and grown at 37 °C for 2 h. Absorbances at 450 nm were measured every 24 h for 72 h.

EdU Assay

Cellular proliferation was assessed using an EdU assay kit (Ribobio, China). Cells were seeded on confocal plates at 10×10^5 cells per well. Next, the cultures were treated with 50 µM EdU solution for 120 min at 37 °C, after which they were fixed (4% formaldehyde, 30 min) and permeabilized (0.1% Triton X-100, 20 min) before the nuclei were stained with Hoechst and the samples were evaluated by fluorescence microscopy.

Apoptosis assessment

Apoptosis was assessed using flow cytometry. FHC and NCM460 cells were transfected and grown to 90% confluence in 6-well plates. The cells were harvested and treated with 10 µL of reagent from the Annexin V-FITC/PI apoptosis kit (Lianke Biotech, China) (10–15 min, room temperature, away from light). The cells were evaluated on a FACSCalibur flow cytometer (BD Biosciences, USA).

Enzyme-linked Immunosorbent Assay (ELISA)

Culture supernatants and standards were incubated in precoated ELISA plates for 2 h at 37 °C. This was followed by two washes and probing with an HRP-conjugated secondary antibody (1 h, 37 °C). After further rinsing, the plates were incubated with the chromogenic solution (10–15 min, room temperature, away from light). After the addition of the stop solution, the absorbances at 450 nm were measured. The specific antibodies used were anti-human IL-1β (Abcam, ab214025), anti-human IL-18 (Abcam, ab215539), anti-mouse IL-1β (Abcam, ab197742), and anti-mouse IL-18 (Abcam, ab216165). All assays were performed in triplicate.

Animal Selection and Experimental Design

Kunming (KM) mice (male, 20 ± 2 g) were sourced from the Experimental Animal Center of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, China. All animal experiments were performed with the approval of the Animal Care and Use

Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital (Lunshen (Yan)2022-380). The animals were maintained at a temperature of 22–24 °C with 20% humidity and a 12-hour light/dark cycle. They were provided unrestricted access to standard feed and water. After one week of acclimatization, 48 mice were randomly assigned to four groups, with each group containing 12 mice. In Group 1, the mice were provided regular drinking water. Groups 2 to 4 were given drinking water containing 3% DSS (MW 36 to 50 kDa, MP Biomedicals LLC, Santa Ana, CA, USA) for seven days to induce UC. Group 3 received 100 µl of control lentivirus (LV-sh-CTRL, obtained from GenePharma, Suzhou, China) *via* tail vein injection twice per week, while Group 4 was injected with 100 µl of the SLC6A14 knockdown lentivirus (LV-sh-SLC6A14, obtained from GenePharma) *via* tail vein injection twice per week. The mice were euthanized using 1% sodium pentobarbital (administered *via* intraperitoneal injection) and sacrificed after a 14-day period. Colon lengths were measured, and the colons were immediately rinsed with chilled physiological saline. Colon samples were collected; some samples were immediately fixed (10% formalin), while other samples were frozen at -80 °C for further analysis.

Assessment of the Disease Activity Index

The animals were subjected to daily evaluations to gauge UC severity, and body weights, observable rectal bleeding, and stool consistency were assessed. A comprehensive disease activity index (DAI) score was assessed using an established method to quantify disease severity. The DAI score was determined by combining three individual scores: the weight loss rate score, the stool trait score, and the occult blood score. These three scores were added together and then divided by three to yield the final DAI score. This systematic approach provides an accurate representation of the overall disease severity experienced by the mice.

Colon Histopathology

Colon lengths were measured, and a sample (0.5 cm) was collected and fixed (10% formalin, 24 h). Following fixation, the tissue was paraffin-embedded and sectioned (5-μm sections) before undergoing H&E staining. Each sample was meticulously evaluated at a magnification of 100×.

Statistical Analysis

All statistical analyses were performed using GraphPad Prism 9.0 (GraphPad, USA). The data are presented as the mean ± standard deviation (SD). Group differences were analyzed by two-way analysis of variance (ANOVA), followed by Bonferroni post hoc test. For nonparametric data, the Kruskal–Wallis test was used, and continuous variables were analyzed using one-way ANOVA. P values <0.05 were considered statistically significant.

RESULTS

SLC6A14 is increased in UC

To examine the role of SLC6A14 as a diagnostic biomarker for UC, we examined SLC6A14 protein expression. Histopathological analysis revealed that UC samples showed typical colonic inflammation, which was particularly noticeable in UC biopsies compared to healthy adjacent tissue. Immunohistochemical (IHC) analysis of UC samples revealed increased SLC6A14 protein levels, which led to higher histological scores (Figure 1A, E). Additionally, a comprehensive investigation was performed using a dataset containing 55 UC tissues and 29 normal tissues, and SLC6A14 mRNA levels were quantified by qRT-PCR. Significant increases in the mRNA expression of SLC6A14 were observed in UC tissues relative to control tissues ($P < 0.01$, Figure 1B). Notably, these expression levels were further confirmed by Western blot analysis (Figure 1C and 1D).

SLC6A14 contributes to pyroptosis

To explore the correlation between SLC6A14 and pyroptosis, we examined NLRP3 protein levels in the tissues of UC patients. The results showed substantial upregulation

of the NLRP3 protein in UC tissues compared to their normal counterparts (Figure 1F and 1G). Moreover, our investigation revealed a positive correlation between SLC6A14 expression and NLRP3 expression (Figure 1H). These findings indicate a pronounced increase in SLC6A14 expression in the context of UC.

LPS induces pyroptosis in IECs

To mimic UC-induced inflammation, we treated FHC and NCM460 cells, which are normal IECs, with 10 ng/mL LPS. This stimulation reduced cell proliferation (Figure 2A-C). In parallel, flow cytometry (FCM) indicated an increase in cell death following LPS exposure (Figure 2D and 2E). Moreover, LPS increased inflammatory factor levels in the cells. Pyroptosis has been shown to enhance inflammation, and increased levels of IL-1β and IL-18 were observed after LPS exposure (Figure 2F and 2G). Subsequently, we used Western blot analysis to assess key effector proteins associated with pyroptotic pathways, including ASC, pro-IL-1β, pro-IL-18, IL-1β, IL-18, and NLRP3. Increases in these proteins were observed after LPS stimulation of FHC cells (Figure 2H and S1). Notably, SLC6A14 Levels in LPS-treated IECs were markedly increased relative to those in the controls (Figure 2I and 2J). These findings suggest that LPS induces pyroptosis in FHC and NCM460 cells, underscoring the potential involvement of SLC6A14 in this process.

Suppressing SLC6A14 enhances proliferation and reduces apoptosis in LPS-induced epithelial cells

Given the close association between proinflammatory cytokine production and intestinal inflammation, we aimed to determine whether decreasing SLC6A14 expression could mitigate inflammation in the LPS-stimulated epithelial cell model. We introduced the sh-SLC6A14 plasmid into FHC and NCM460 cells to knockdown SLC6A14 expression. Subsequently, the cells were subjected to LPS stimulation (10 ng/mL) for 24 h. As shown in Figure 3A and 3B, there was a significant reduction in SLC6A14 expression in the LPS+sh-SLC6A14 group. This observation demonstrated the efficacy of the synthetic sh-SLC6A14 vector. Subsequently, we revealed that SLC6A14 downregulation increased cell

proliferation (Figure 3C-E). Moreover, flow cytometry (FCM) showed that suppressing SLC6A14 expression mitigated apoptosis induced by LPS (Figure 3F and 3G). Collectively, these findings suggested that suppressing SLC6A14 expression enhanced proliferation and reduced apoptosis in FHC and NCM460 cells subjected to LPS stimulation.

SLC6A14 enhances LPS-induced inflammatory cytokine secretion

Our subsequent experiments revealed that SLC6A14 could promote the secretion of the pyroptosis-associated proteins IL-1β and IL-18 by LPS-induced IECs (Figure 4A). In addition, Western blotting showed that reducing SLC6A14 expression inhibited key pyroptotic effector proteins (Figures 4B and S2). These findings suggest that SLC6A14 can amplify LPS-induced inflammatory cytokine secretion.

NLRP3 overexpression counteracts the inhibitory effect of SLC6A14 knockdown on LPS-induced pyroptosis

The EdU and CCK-8 assay results showed that upregulating NLRP3 counteracted the inhibitory effects of SLC6A14 knockdown induced by the sh-SLC6A14 plasmids on the proliferation of LPS-treated FHC and NCM460 cells (Figure 5A-C). Flow cytometry further revealed that NLRP3 upregulation mitigated the impact of SLC6A14 downregulation on apoptosis in LPS-induced FHC cells (Figure 5D and 5E). Moreover, ELISA revealed that NLRP3 overexpression reversed the SLC6A14-induced suppression of IL-18 and IL-1β production in both cell lines after LPS treatment (Figure 6A). Finally, Western blotting showed that increasing NLRP3 expression increased the levels of ASC, IL-1β, IL-18, and NLRP3. This effect abrogated the inhibitory effect of SLC6A14 downregulation of these protein levels (Figure 6B and S3). Taken together, these results demonstrate the involvement of SLC6A14 in NLRP3-mediated pyroptosis.

SLC6A14 downregulation attenuates DSS-induced UC in vivo

The colons of UC mice were markedly shorter than those of the controls. The downregulation of SLC6A14 mitigated the reduction in colon length induced by DSS (Figure 7A). DSS-treated animals and those that received a combination of DSS and the control vector exhibited greater fluctuations in weight relative to the controls. Furthermore, animals in the DSS and DSS plus vector groups experienced more substantial weight loss relative to those in the DSS plus SLC6A14 vector group (Figure 7B). An increase in the DAI was observed in the DSS-treated groups, as indicated by markedly increased DAI scores compared with those in the controls. Notably, lower DAI scores were observed in the DSS plus SLC6A14 vector group relative to the DSS-only group (Figure 7C). Additionally, histopathological analysis revealed that downregulating SLC6A14 significantly alleviated colonic inflammation induced by DSS and reduced epithelial crypt numbers, mucosal barrier disruption, and inflammatory cell infiltration (Figure 7D). Intriguingly, DSS dramatically induced SLC6A14 expression, while SLC6A14 expression was markedly decreased in DSS plus SLC6A14 knockdown mice compared to other DSS-treated mice (Figure 7E and 7F). It was also found that SLC6A14 downregulation reduced cytokine production. Thus, our results revealed that downregulating SLC6A14 ameliorated pyroptosis induced by activation of the NLRP3 inflammasome after DSS treatment (Figure 7G). Additionally, SLC6A14 transfection reduced NLRP3 Levels in the colons of mice following DSS treatment (Figures 7H and S4). In summary, our findings suggest that the downregulation of SLC6A14 effectively protected colon tissue integrity and mitigated the morphological changes caused by DSS, suggesting its potential for treating UC.

DISCUSSION

UC is associated with chronic inflammation in the colonic mucosa, and new forms of treatment are urgently needed [23-25]. Recent studies focusing on the pathogenesis of UC highlight the involvement of an aberrant immune response and dysregulated inflammation in UC development [26, 27]. Invading pathogens and threats are identified by PAMPs and DAMPs associated with the innate immune response [28, 29]. Intracellular

inflammasome complexes respond to PAMPs and DAMPs, triggering inflammation [30]. NLRP1, NLRP3, NLRC4, and AIM2 are well-documented inflammasome components and receptors. There is strong evidence of the involvement of the NLRP3 inflammasome in IBDs, including Crohn's disease and UC [31, 32].

Consistently, recent strategies to suppress chronic inflammation have targeted pyroptosis, offering a novel approach to managing IBD. For instance, L38 exerted positive therapeutic effects on a DSS-induced ulcerative colitis mouse model by inhibiting NLRP3 inflammasome activation and pyroptosis [33]. Similarly, mesalazine and corticosteroids have been shown to attenuate pyroptosis in IECs [34]. These approaches highlight the potential of targeting pyroptosis to alleviate inflammation in the context of IBD. There is a close link between UC progression and increased levels of IL-1β and IL-18, suggesting that overproduction of these cytokines by cells such as macrophages can worsen this condition [35]. However, NLRP3 activation stimulates IL-18 production and enhances intestinal barrier integrity. Intriguingly, the administration of exogenous recombinant IL-18 has been shown to alleviate the inflammatory symptoms of UC resulting from DSS administration [36]. This dual function of NLRP3 emphasizes the importance of selectively targeting macrophages rather than IECs for the effective management of UC [37].

Given the complex and sometimes contradictory aspects associated with NLRP3 activation in different cell types in the context of colitis, it is evident that further research is needed to elucidate the role of pyroptosis in UC. This would help guide the development of targeted therapeutic strategies to modulate the inflammatory response and effectively treat the disease. In our study, we treated IECs with LPS to create a model of intestinal inflammation. By analyzing the levels of key pyroptosis-associated factors, we observed the upregulation of these markers, indicating that LPS effectively induced pyroptosis in colonic epithelial cells. Additionally, our investigation of SLC6A14 expression in the context of LPS-induced colonic epithelial cells revealed the significant upregulation of SLC6A14. This finding is consistent with recent microarray expression data [38].

The SLC (solute carrier) transporter family, which includes proteins such as SLC7A11, SLC3A2, and SLC25A28, is linked with a variety of metabolic disorders, especially those of the liver. SLC6A14 in particular has been shown to be upregulated in different colonic diseases, including ulcerative colitis [18]. Studies using microarrays of colonic tissue from UC patients and normal tissue showed a noticeable increase in SLC6A14 mRNA expression in UC cases [39]. SLC6A14 is an efficient transporter of amino acids that is associated with various intracellular activities. Leucine, which is one of its substrates, is critical for activating the mTOR signaling pathway in tumor cells. Moreover, SLC6A14 contributes to cellular glutathione synthesis by using glycine as a substrate. Multiple studies have showed the critical involvement of SLC6A14 in ulcerative colitis. Zhang *et al* [40] suggested that SLC6A14 was a biomarker of UC in tissue biopsies and might offer a novel target for gene therapy in UC. Similarly, Li *et al* [41] identified a potential regulatory pathway involving NEAT1-miR-342-3p/miR-650-SLC6A14 in UC. More recently, Chen *et al* demonstrated that knockdown of SLC6A14 blocked ferroptosis and that SLC6A14 promoted ferroptosis in epithelial cells through C/EBPβ-PAK6 signaling in UC [18]. In the context of our study, we showed that downregulating SLC6A14 effectively blocked NLRP3 activation, resulting in notable alleviation of colitis. This suggests a potential therapeutic strategy for managing colitis by targeting SLC6A14 to modulate the NLRP3 pathway and pyroptosis. These findings highlight the complexity of SLC6A14 in inflammatory conditions and UC.

We also examined the role of SLC6A14 in UC pathogenesis using a UC mouse model. These mice showed severe damage to colonic tissue, increased oxidative stress and cytokine production, and NLRP3 inflammasome activation. These findings indicate that DSS-induced UC led to the activation of the inflammatory caspase-mediated NLRP3 inflammasome. NLRP3 activation increased cytokine production and induced pyroptosis. Notably, our findings suggest that DSS induced pyroptosis, which further aggravated colon tissue damage. Our investigations revealed that downregulating SLC6A14 inhibited IEC pyroptosis in UC, which was mediated by the NLRP3 pathway. Interestingly, our results also indicated a positive association between the increase in

SLC6A14 expression and pyroptosis in UC tissue samples. This evidence suggests that SLC6A14 actively promotes pyroptosis in the context of UC by regulating the NLRP3 pathway. Consequently, our findings highlight SLC6A14 as a prospective new therapeutic target that could be used to mitigate cellular damage during the course of ulcerative colitis. Our study contributes to our knowledge of UC pathogenesis and identifies a potential strategy for therapeutic intervention.

CONCLUSION

CONCLUSION

In summary, the results demonstrated the close involvement of SLC6A14 in promoting pyroptosis in the context of UC by upregulating NLRP3 expression. These findings indicate the potential of targeting SLC6A14/NLRP3 axis-mediated pyroptosis as a promising therapeutic strategy for treating UC. Our research provides valuable insights into the mechanisms driving UC pathogenesis and offers a possible direction for developing innovative treatments to alleviate the impact of this chronic inflammatory disorder.

ARTICLE HIGHLIGHTS

Research background

Ulcerative colitis (UC) is an inflammatory condition associated with frequent relapse and recurrence. Dysregulation of intestinal epithelial cells (IECs) and mucosal barrier impairment contribute to sustained inflammation in UC. Hence, an in-depth exploration of the triggers and mechanisms of IEC death could result in efficacious therapeutic options for UC patients.

Research motivation

Evidence suggests the involvement of SLC6A14 in UC pathogenesis, but the central regulator remains unknown.

Research objectives

We aimed to explore the role of SLC6A14 in UC-associated pyroptosis.

Research methods

qRT-PCR, immunoblotting, and IHC assessed SLC6A14 in human UC tissues. LPS induced FHC and NCM460 cell inflammation, modeling enteritis; SLC6A14 Levels were assessed. Pyroptosis markers were quantified using ELISA, Western blotting, and qRT-PCR, while EdU incubation, CCK-8 assay and flow cytometry examined proliferation and apoptosis, respectively. Mouse models of UC were used for verification.

Research results

SLC6A14 was elevated, correlating with NLRP3 in UC tissues. LPS-induced FHC and NCM460 cells showed increased SLC6A14. Reduced SLC6A14 boosted cell proliferation, suppressed apoptosis. Lower SLC6A14 Led to decreased pyroptosis-associated proteins (ASC, IL-1β, IL-18, NLRP3). NLRP3 overexpression counteracted sh-SLC6A14 effects on LPS-induced FHC and NCM460 cell pyroptosis. SLC6A14 improved murine DSS colitis mucosa.

Research conclusions

SLC6A14 promotes UC pyroptosis *via* NLRP3 upregulation, indicating therapeutic potential through SLC6A14/NLRP3 axis modulation.

Research perspectives

We demonstrated the close involvement of SLC6A14 in promoting pyroptosis in the context of UC by upregulating NLRP3 expression. These findings underline the potential significance of targeting the SLC6A14/NLRP3 axis-mediated pyroptosis as a promising therapeutic strategy for addressing UC.

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