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Original Article

Notch/IL33/ST2 signaling was involved in the maintenance of intestinal epithelial barrier through regulating tight junction after LPS stimulation

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Abstract

Interleukin-33 (IL33), an alarm cytokine of the IL-1 family, is expressed mainly in epithelial cells of barrier tissues and is involved in the repair of epithelia to maintain barrier function. However, the mechanisms regulating IL33 expression and the mechanisms by which IL33 regulates the intestinal barrier function are not fully clarified. In this study, Caco-2 cells and siRNA were applied to investigate the role of Notch/IL33/ST2 Signaling in regulating intestinal epithelial barrier function, which was demonstrated by protein expression of tight junctions and trans-epithelial resistance (TER) assay. Our results revealed that Notch signaling pathway was activated and IL33 expression was up-regulated after LPS stimulation. After blocking Notch signaling with DPAT or siRNA for Notch1, IL33 expression was significantly down-regulated in Caco-2 cells. The protein expression of tight junctions (ZO-1, occludin, and claudin-1) was down-regulated after siRNA for IL33 in Caco-2 cells with LPS stimulation. Also, the intestinal epithelial TER was down-regulated after siRNA for IL33 with LPS stimulation or not. Exogeneous IL33 promoted the tight junction protein expression and increased the TER. Finally, our data further showed that IL33 regulates intestinal epithelial barrier function through the ST2 receptor. In conclusion, our results indicated that IL33/ST2 axis, which was activated by the Notch signaling, maintains intestinal epithelial barrier function through regulating tight junction protein expression under inflammatory conditions. This study provides a new therapeutic pathway for regulating intestinal epithelial barrier dysfunction.

Keywords: Intestinal epithelial barrier;,Notch signaling, IL33, Tight junction,TER.

1. Introduction

Inflammation is a natural defense mechanism of the body against foreign bacterial, viral and fungal infections [1]. Under inflammatory conditions, intestinal epithelium is a critical barrier to maintain intestinal homeostasis [2,3]. Increased intestinal permeability and barrier dysfunction could lead to systemic inflammatory responses, sepsis and multiple organ dysfunction syndrome-MODS [4]. Therefore, it is important to clarify the mechanism of intestinal epithelial barrier dysfunction under inflammatory conditions.

Interleukin 33 (IL33) of the IL1 family has been reported to play an important role in host defense against infections and is involved in the development of a variety of inflammatory diseases including asthma, rheumatoid arthritis, and anaphylaxis [5]. IL33 is widely expressed in a wide range of non-hematopoietic and inflammatory cell populations. Recent evidence suggested that IL33 acts as an “early warning” cytokine and was closely related to the regulation of intestinal homeostasis [6,7]. IL33 could also be released as a danger signal by damaged, stressed or necrotic cells to trigger local inflammatory responses [8]. Moreover, expression of IL33 has been detected in the intestinal epithelium of ulcerative colitis [5]. Importantly, IL33 was reported to protect against intestinal inflammation and was negatively correlated with pro-inflammatory gene expression in the intestinal epithelium [9]. However, the mechanisms regulating IL33 expression and how IL33 regulates intestinal epithelial barrier are not fully clarified.

Notch signaling is a highly conserved pathway with four Notch receptors (Notch 1 to 4) and five Notch ligands (Jagged1, Jagged2, DLL1, DLL3, DLL4) in mammals [10]. Notch-ligand binding leads to shedding of the Notch extracellular structural domain, followed by release of the Notch intracellular structural domain (NICD) via the γ-secretase complex. The NICD is translocated to the nucleus and binds to the transcription factor Rbp-jk, which triggers the activation of Notch downstream target genes [10,11]. Notch signaling in intestinal epithelial cells has been found to play an important role in intestinal epithelial proliferation and differentiation, tissue homeostasis, and organ size, which is a major regulator in determining cell fate during intestinal homeostasis [12]. It has been noted that Notch signaling can cooperate with IL10 to ensure microbiota homeostasis and regulate intestinal immune homeostasis [13]. Notch1 signaling is also reported to be involved in maintaining intestinal epithelial structure and barrier function [14,15]. Notch signaling was found to be positively correlated with IL33 in bronchial epithelial cells, vascular endothelial cells, endothelial colony-for-

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ming cells, and nasal epithelial cells [16-19]. However, whether IL33 is also regulated by Notch signaling in intestinal epithelial cells has not been reported.

The purpose of this study was to investigate the relationship between IL33 and Notch signaling pathways in Caco-2 cells after LPS stimulation. We further explored the mechanism by which IL33 regulates the intestinal epithelial barrier function.

2. Materials and Methods:

2.1. Cell culture and reagents

The human intestinal Caco-2 cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM/F12 medium containing 10% fetal bovine serum and 1% penicillin-streptomycin in an incubator at 37 ℃ and 5% CO2. When the cells reached 70%-80% fusion, the cells were cultured with or without the addition of LPS (Sigma-Aldrich, St. Louis, MO, USA) and with or without the addition of the γ-secretase inhibitor DAPT (Santa Cruz, Dallas, CA, USA). Through a series of concentration gradient and time gradient experiments, Caco-2 cells were finally incubated with LPS (4 μg/mL) for 24 h in the following experiments.

2.2. siRNA and *in Vitro* transfection

Plasmids were constructed by Ribobio (Guangzhou, China) The sequences of the siRNA targeting Notch1 were as follows: sense 5' CACCAGUUUGAAUGGUCAA dTdT3' and antisense 3' dTdT UUGACCAUUCAAACUGGUG'. The sequences of the siRNA targeting IL33 were as follows: sense 5' CCUUCAUAAUAUGCACUCCAATT dTdT3' and antisense 3' dTdT UUGGAGUGCAUAUUAUGAAGGTT'. The sequences of the siRNA targeting ST2 were as follows: sense 5' CGCAGGUGAUUACACCUGUAATT dTdT3' and antisense 3' dTdT UUACAGGUGUAAUCACCUGCGTT'. Caco-2 cells were cultured into 6-well plates at a density of 30%, and when they reached 70% fusion, the original medium was replaced with serum-free Opti-MEM and the siRNA was added to the cells to form a complex with PEI transfection reagent for subsequent experiments according to the instructions of manufacture.

2.3. Real-Time PCR analysis

RNA extraction and RT-PCR were performed according to previously published studies. Total RNA was extracted from the samples according to the instructions of the RNA extraction kit. Thereafter, the concentration, purity, and integrity of the RNA were determined. RNA was reverse transcribed to cDNA according to the manufacturer's instructions (Takara, Dalian, CHN), and gene expression levels were assessed using diluted cDNA as template DNA. Primers were designed using NCBI based on human gene sequences. Sample target fragments were then PCR amplified using the synthesized primers. RT-PCR was performed on a fluorescent quantitative gene amplifier using the UltraSYBR mixture, and each sample was repeated 3 times. GAPDH was used as a housekeeping gene to standardize the expression level of the target genes.DLL1 Forward primer: 5′-GGAGCCTAAGTTTGAGTTTGCTGTG-3′, reverse primer 5′-TGCAGCAGGTTGTCTTGGATG-3′; Jagged2 forward primer 5′-GGAGCCTAAGTTTGAGTTTGCTGTG-3′, reverse primer 5′-TGCAGCAGGTTGTCTTGGATG-3′; Notch1 forward primer 5′- TGAATGGCGGGAAGTGTGAAG-3′, reverse primer 5′-GGTTGGGGTCCTGGCATCG-3′; Hes3 forward primer 5′-GAGAAGCCTTCAGAACTCCTTGC-3′ , reverse primer 5′-CTGCCGACCTCATCTCCGCG-3′; IL33 forward primer 5′GTGGAAGAACACAGCAAGCA3′, reverse primer 5′AAGGCAAAGCACTCCACAGT3′; β-actin forward primer: 5′CTGGAACGGTGAAGGTGACA3′, reverse primer: 5′AAGGGACTTCCTGTAACAATGCA 3′.

2.4. Western blot assay

Cells were fully lysed as described in the previous cell spreading plate by waiting for the cells to have reached 80%-90% fusion, washing the cells with cold PBS and then adding a lysis buffer mixture containing protease inhibitors. The supernatant was centrifuged and stored after quantifying the protein concentration using the BCA protein assay kit. Equal amounts of proteins were separated by SDS-PAGE and transferred to PVDF membranes, which were subsequently blocked with 5% milk and incubated at 4 ℃ with the following antibodies:anti-NICD1 antibody (ab-52301, Abcam, UK), anti-IL33 (ab118503, Abcam, UK), anti-ZO-1 (21773-1-AP, Proteintech, USA), anti-Occludin (66378-1-Ig, Proteintech, USA), anti-Claudin-1 (28674-1-AP, Proteintech, USA), ST2 (ab317557, Abcam, UK) and GAPDH (sc-32233, Santa-Cruz, USA). The secondary antibodies were incubated after three washes with TBS-T, and the target proteins were detected and analyzed using the Super ECL detection reagent.

2.5. Trans-Epithelial Resistance measurement

Caco-2 monolayers were grown on 0.33 cm^2 Transwell supports (Millipore). TER was measured with a Millicell-ERS voltohmmeter (Millipore). TER measurements were calculated in ohms cm^2 after subtracting the blank value for the membrane insert. The TER values were normalized to the initial values and were expressed as percentages of the initial resistance values.

2.6. Statistical methods

The results were expressed as the mean ± standard deviation (SD). Analysis was performed using SPSS software (Statistical Package for the Social Sciences). Analysis of variance (ANOVA) was used for comparisons among 3 or more groups, and Student's t-test was used for comparisons between 2 groups. Differences were considered statistically significant at a $p<0.05$. If not otherwise stated, all experiments included three independent replicates and were performed in triplicate to ensure reproducibility.

3. Results

3.1. LPS induced activation of Notch signaling pathway and IL33 expression in Caco-2 cells

Previous studies have shown that the Notch signaling pathway was activated under inflammatory conditions [20,21]. In view of this, in our experiments, Caco-2 cells were firstly treated with different concentrations of LPS (0, 0.5, 1, 2, 4, and 8 μg/mL) for 6 h. The results showed that Jagged2 and notch1 mRNA expression was elevated after LPS stimulation dose-dependently ($p<0.05$), and the notch1 mRNA was significantly elevated under 4 μg / mL LPS stimulation ($p<0.01$) (Fig. 1A, B). Western blot analysis also showed that NICD-1 protein expression was elevated after LPS (4 μg/mL) stimulation ($p<0.01$) (Fig.

1C, D).

Then LPS (4 μg/mL) was used to treat Caco-2 cells and the total RNA and protein were harvested at indicated times (0, 3, 6, 12, and 24 h). The Realtime PCR results showed that DLL1, Jagged2, Notch1 and Hes3 mRNA levels were up-regulated in a time-dependent manner (p<0.05) (Fig. 1E-H). Then western blot results showed that NICD1 protein expression was remarkably elevated 24h after LPS (4 μg/mL) stimulation (p<0.01) (Fig. 1I, J), which was consistent with the PCR results. The above results indicated that the intestinal epithelial Notch signaling pathway was activated after LPS stimulation. In the following experiments, Caco-2 cells were incubated with LPS (4 μg/mL) for 24 h or not.

Under inflammatory conditions, intestinal epithelial cells can secrete IL33 to initiate self-protective regulation [13]. Therefore, we then detected the expression of IL33 by real-time PCR and Western blot. The results showed that mRNA expression of IL33 was slightly down-regulated at 3 h and 6 h, then it was significantly elevated at 12 h and 24 h (p<0.01) (Fig. 1K). In addition, the protein expression of IL33 was also up-regulated after LPS (4 μg/mL) stimulation for 24h, which was consistent with the mRNA results (p<0.01) (Fig. 1L, M).

3.2. DAPT and silencing RNA for Notch1 blocked Notch signaling and prevented LPS-induced expression of IL33

To further clarify the relationship between the Notch signaling pathway and IL33 expression, DAPT, one type of γ-secretase inhibitor, was used to block the activation of Notch signaling pathway [22]. Caco-2 cells were treated with 4 μg/ml LPS for 24 h, with or without DAPT pretreated for 12 h. Western blot results showed that NICD-1 protein expression was reduced in the DAPT group, indicating that DAPT inhibited the activation of the Notch1 signaling pathway in this experiment (p<0.01) (Fig. 2A, B). Then, we examined the effect of DAPT on IL33 expression.

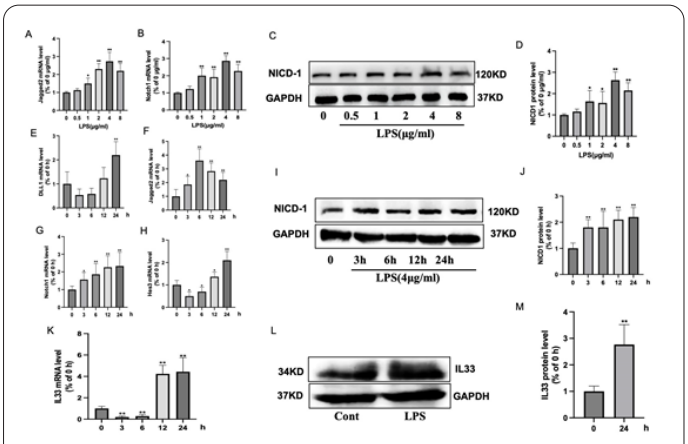

Fig. 1. Notch1 signaling pathway was activated after LPS stimulation in Caco-2 cells. (A-B)The mRNA expression of jagged2 and notch1 after LPS stimulation (6 h);(C-D) The protein expression of NICD1 after LPS stimulation (6 h); (E-H): The mRNA expression of DLL1/Jagged2/Notch1/Hes3 after LPS (4 μg/ml) stimulation;(I-J): NICD1 protein expression after LPS (4 μg/ml) stimulation; (k)The mRNA expression of IL33 was detected after LPS stimulation(4 μg/ml); (L-M) Protein expression of IL33 was detected by Western blot after LPS stimulation(4 μg/ml) for 24h. Data are shown as the means ± SDs (n= 3). ** p < 0.01 vs control group; * p < 0.05 vs control group. GAPDH was used as the loading control.

The Real-time PCR and Western blot results showed that DAPT inhibited the LPS-induced IL33 expression (p<0.01) (Fig. 2A, C, D). In order to further investigate the effect of Notch signaling activation on IL33 expression, Caco-2 cells were transfected with siRNA for Notch1. The siRNA for Notch1 down-regulated the NICD1 protein expression (p<0.01) (Fig. 2E, F). Simultaneously, the results showed that IL-33 expression was reduced after inhibiting Notch1 signaling through siRNA for Notch1 compared with control group with LPS stimulation or not (p<0.05) (Fig. 2E, G, H). The above results demonstrated that the Notch signaling pathway was involved in the LPS-induced IL33 expression in Caco-2 cells.

3.3. IL33 was involved in the intestinal epithelial Barrier protection after LPS stimulation

In order to investigate the effect of IL33 on intestinal epithelial barrier, we examined the protein expression of tight junctions (ZO-1, occludin, and claudin-1) with siRNA for IL33 or not. The Western blot results showed that pro-

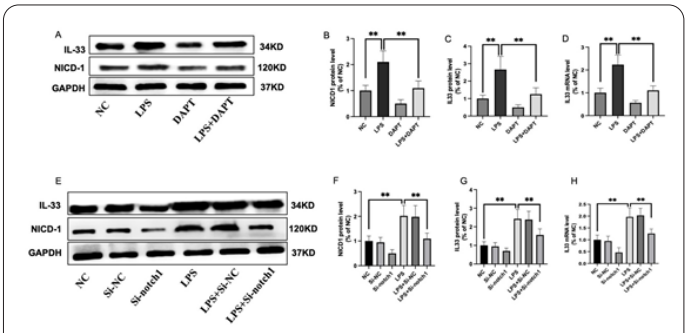

Fig. 2. Inhibition of Notch signaling by DAPT and siRNA for Notch1 inhibited LPS-induced IL33 expression. Caco-2 cells were treated with 4 μg/ml LPS for 24 h with or without 20 μM DAPT pretreated for 12 h. (A-B) The NICD1 protein expression was examined by Western blot; (A, C, D) Expression of IL33 was detected by Real time PCR and Western blot; (E, F) Inhibition of Notch1 with siRNA was carried out. After 48 h of culture, LPS was added for 24 h. NICD1 protein expression was detected by Western blot; (E, G, H) Expression of IL33 was detected by Real time PCR and Western blot. Data are shown as the means ± SDs (n = 3). ** p < 0.01 vs control group; * p < 0.05 vs control group. GAPDH was used as the loading control.

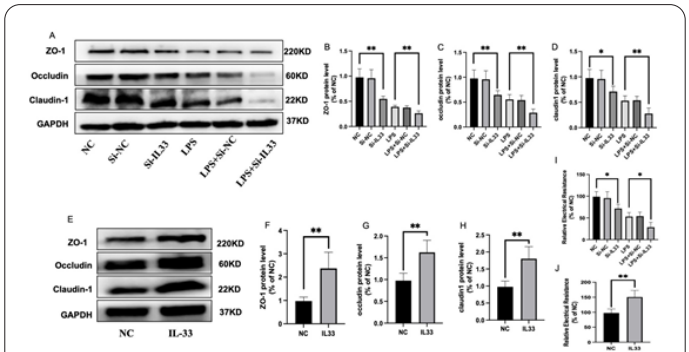

Fig. 3. IL33 was involved in the intestinal epithelial barrier protection after LPS stimulation (A-D) The protein expression of tight junction ZO-1, occludin, and claudin-1 in Caco-2 cells after siRNA for IL33 with LPS or not;(E-H): Protein expression of tight junction ZO-1, occludin, and claudin-1 after incubation with exogenous IL33;(I): TER of Caco-2 cells after siRNA for IL33 with LPS or not; (J) TER of Caco-2 cells after incubation with exogenous IL33. Data are shown as the means ± SDs (n = 3). ** p < 0.01 vs control group; * p < 0.05 vs control group. GAPDH was used as the loading control.

tein expression of tight junctions was down-regulated after siRNA for IL33 in Caco-2 cells ($p<0.05$) (Fig. 3 A-D). And importantly, after LPS stimulation, protein expression of tight junctions was further down-regulated in Caco-2 cells (Fig.3 A-D). However, the protein expression of the above-mentioned tight junction proteins increased after incubation with exogenous IL33 ($p<0.01$) (Fig. 3E-H). These results indicated that IL33 can improve the integrity of intestinal epithelial barrier by regulating tight junction protein expression.

To further evaluate the integrity of intestinal epithelial barrier function, TER of Caco-2 cells was examined. The results showed that TER values were significantly down-regulated in the LPS group compared with the control group, indicating that LPS disrupted intestinal epithelial barrier function ($p<0.05$) (Fig. 3I). Importantly, the intestinal epithelial TER was further down-regulated receiving siRNA for IL33 with LPS stimulation ($p<0.01$) (Fig. 3I). However, TER was elevated after incubation with exogenous IL33 in Caco-2 cells ($p<0.01$) (Fig. 3J). All of these results suggested that IL33 protected the intestinal barrier function by regulating tight junction protein expression under inflammatory conditions.

3.4. IL33/ST2 axis activated by Notch signaling pathway regulates intestinal epithelial barrier

IL33/ST2 axis was known as an important regulatory mechanism for maintaining intestinal homeostasis [23]. Therefore, we hypothesized that IL33 also regulates the intestinal epithelial barrier function via the downstream receptor ST2 after LPS stimulation. To clarify this hypothesis, firstly ST2 protein expression was examined after siRNA for Notch1 in Caco-2 cells. We found that ST2 protein expression in Caco-2 cells was down-regulated with siRNA for Notch1 compared with control group ($p<0.05$) (Fig. 4A, B). This result suggested that the protein expression of ST2 was regulated by the Notch-1 signaling.

In addition, we further investigated the role of the IL33/ST2 axis in the regulation of intestinal epithelial barrier after siRNA for ST2. The tight junction protein expression of ZO-1, occludin, and claudin-1 was down-regulated with LPS or not, when ST2 expression was inhibited by siRNA for ST2 ($p<0.05$) (Fig. 4 C-F). Meanwhile, TER was reduced when ST2 expression was inhibited by siRNA for ST2, compared with control group, with LPS or not ($p<0.05$) (Fig. 4G). Taken together, these results demonstrated that the IL33/ST2 axis, activated by Notch signaling pathway after LPS stimulation, could maintain the integrity of intestinal epithelial barrier function by regulating the expression of tight junction proteins.

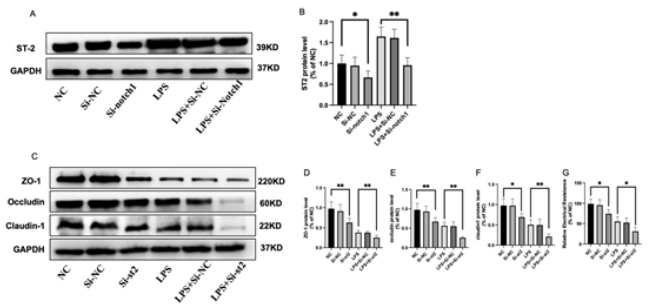

Fig. 4. IL33 regulates tight junction protein expression and maintains intestinal barrier function through ST2. (A-B) ST2 protein expression in Caco-2 cells after incubation with siRNA for Notch1 with LPS or not;(C-F): Protein expression of tight junction (ZO-1, occludin, and claudin-1) in Caco-2 cells after incubation with siRNA for ST2 with LPS or not;(G) TER of Caco-2 cells was examined after incubation with siRNA for ST2 with LPS or not. Data are shown as the means ± SDs ($n = 3$). ** $p < 0.01$ vs control group; * $p < 0.05$ vs control group. GAPDH was used as the loading control.

4. Discussion

The intestinal mucosa is a self-renewing tissue that ensures nutrient absorption and protects against invasion by external environmental factors such as viruses and bacteria [24]. During intestinal inflammation, the intestinal barrier is disrupted and a series of events occur to initiate the process of tissue repair and organ function recovery [25,26]. Exploring the mechanisms regulating intestinal barrier in inflammatory states can help to discover new therapeutic strategies to deal with these inflammatory diseases. IL33, a new member of the IL-1 cytokine family, could initiate a protective immune response under specific conditions [27,6]. Clinical retrospective studies found that intestinal IL-33 expression was increased in patients with inflammatory bowel disease after clinical treatment, and patients with higher mucosal expression of IL-33 tend to have a better response to clinical treatment[28]. However, the mechanisms regulating the IL33 signaling and by which IL33 regulates the intestinal barrier function are not fully understood.

Our previous studies have shown that Notch signaling serves as an important regulatory molecule for intestinal epithelial cell proliferation and apoptosis [29,30,31]. More importantly, our previous studies have observed that Notch signaling is involved in the regulation of intestinal barrier through tight junctions [14]. Therefore, in this study, we explored the relationship between Notch signaling pathway and the IL33 signaling in the Caco-2 cells with LPS stimulation or not. We also investigated whether IL33 could regulate intestinal barrier through tight junctions.

Our experiments showed that the expression of Jagged2, DLL1, notch1, and Hes3 was significantly altered after LPS treatment at different concentrations and time durations. These results confirmed that the Notch signaling pathway was significantly activated in intestinal epithelial cells under the LPS stimulation. Which was in accordance with our results, Fang *et al* also observed an increase in Notch/Hes1 expression in LS174T cells after LPS stimulation [32]. Similarly, Monsalve E *et al* found that Notch1 expression was upregulated in macrophages stimulated by LPS [33]. Simultaneously we found that IL33 expression was significantly elevated in intestinal epithelial cells after stimulation with LPS. Thereafter, in order to further investigate the relationship between Notch signaling and IL33 expression, DAPT and siRNA for Notch1 were used to inhibit the expression of Notch1. The results showed that IL-33 expression was reduced further after inhibiting Notch1 signaling. These results showed that IL33 is located downstream of the Notch1 signaling pathway in Caco-2 cells after LPS stimulation. Which was in accordance with our results, C. Di Sano *et al* found reduced IL33 expression in bronchial epithelial cells after DAPT treatment to inhibit Notch-1 activation [16]. Also, in vascular endothelial cells, recombinant Notch ligands could induce IL33 expression in the nucleus of cells *in vitro*, and the expression was reduced by inhibiting the classical Notch1 signa-

ling [17]. In nasal epithelial cells, Notch-1 expression was also significantly correlated with IL-33 expression [19]. These results are inconsistent with our observations.

A tight junction is very important for the integrity of the intestinal barrier. Precise regulation of tight junctions is necessary to maintain mucosal homeostasis. It was reported that the expression of IL33 was detected in intestinal epithelial cells under inflammatory conditions [5]. We further evaluated the effect of IL33 on the expression of tight junctions in Caco-2 cells with LPS stimulation. LPS, one of the most important inflammatory factors, could down-regulate tight junction protein expression and impair the integrity of the intestinal epithelial barrier. We also observed a more significant decrease in tight junction protein levels in response to LPS stimulation. More importantly, the expression of tight junction was further down-regulated after IL33 expression was inhibited. The expression of the tight junction was restored in Caco-2 cells after incubation with exogenous IL33. Then we further evaluated the effect of IL33 on the intestinal barrier function in Caco-2 cells by examining TER. The results showed that expression of IL33 was positively correlated with the integrity of intestinal barrier. Our results were in accordance with those of others. Lopetuso *et al* showed that exogenous administration of IL33 during recovery after DSS challenge enhances mucosal healing and the resolution of colitis [34]. Importantly, our results further showed that IL33 was related to the expression of tight junctions and could increase the TER of intestinal epithelial cells.

Then in order to explore the mechanism of IL33 regulating intestinal tight junction, siRNA for ST2, which acts as a receptor for IL33, was applied to inhibit the IL33 signaling. Prior to that we blocked Notch1 expression by siRNA and found that ST2 protein expression was downregulated simultaneously. This result implied that IL33 regulates the intestinal barrier through ST2 in our experiment. Thereafter, siRNA for ST2 was used to inhibit the IL33 signaling. And results exhibited a more significant down-regulation of ZO-1, occludin, and claudin-1 protein expression and a more severe reduction of TER after LPS stimulation. The above results suggested that ST2 was receptor of IL33 signaling in our experiment. This finding was also verified in experimental mouse model of colitis, where ST2-KO mice exhibited exacerbated colitis, which was ameliorated by the administration of recombinant IL-33, improving the disease activity index [35]. Clinically, compared to healthy controls, IBD patients had elevated serum concentrations of IL-33 and ST2 [36]. These facts suggested that the IL-33/ST-2 may act as a biomarker of disease severity and clinical treatment response, as well as a potential therapeutic target for the novel monoclonal antibodies.

However, this study also has limitations. Firstly, this study primarily utilized the Caco-2 cell line with LPS stimulation *in vitro*. In the following researches primary cultured intestinal epithelial cells and clinical specimens will be considered. The effect of Notch/IL33 signaling should also be further verified in animals and clinical patients. Moreover, the Notch signalig can also influence intestinal barrier function by interacting with other signaling pathways. For instance, Reiko *et al* have demonstrated that Notch signaling can synergize with TNF-α to promote mucosal regeneration[37]. Kawamoto *et al* also observed that expression of Notch-1 and TNF-α was upregulated simultaneously in IBD patients, promoting the expression of downstream genes [38]. In addition, in an allergic dermatitis animal model K. Taniguchi *et al* found that TNF-α induce the expression of IL-33 mRNA and protein[39]. Given this, Notch/IL-33, as a key regulator of intestinal homeostasis, is worth further investigation in future studies.

Taken together, the results of our study suggested that Notch signaling pathway, which was activated under LPS stimulation, regulates the expression of tight junctions through the IL33/ST2 axis, and consequently maintains the integrity of intestinal epithelial barrier function.

In conclusion, we demonstrated for the first time that the IL33/ST2 axis was involved in intestinal barrier maintenance by regulating tight junction expression in intestinal epithelial cells under inflammatory conditions. The activation of IL33/ST2 axis was dependent on the Notch signaling pathway. These findings may offer new strategies for the protection of intestinal barrier and provide theoretical basis for the research of clinical drugs or treatments.

Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YLZ and GQC designed the research study. YLZ, CX and FL performed the research. YLZ, FL and GQC analyzed the data. YLZ, CX and GQC drafted the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

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Ethics approval and consent to participate

No human participants or animals were involved in the present research.

Conflict of interests

The authors declare that they have no conflicts of interest.

Consent for publications

All authors have read and approved the final manuscript for publication.

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