Digestive Diseases and Sciences
https://doi.org/10.1007/s10620-021-07022-1

ORIGINAL ARTICLE

MiR155 Disrupts the Intestinal Barrier by Inducing Intestinal Inflammation and Altering the Intestinal Microecology in Severe Acute Pancreatitis

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Received: 13 December 2020 / Accepted: 21 April 2021

Abstract

Background Intestinal dysfunction is a common complication of acute pancreatitis. MiR155 may be involved in the occurrence and development of intestinal dysfunction mediated by acute pancreatitis, but the specific mechanism is not clear.

Aims To investigate the effect of miR155 on severe acute pancreatitis (SAP)-associated intestinal dysfunction and its possible mechanism in a mice model.

Methods In this study, SAP mice model was induced by intraperitoneal injection of caerulein and LPS in combination. Adeno-associated virus (AAV) was given by tail vein injection before the SAP model. The pancreatic and intestinal histopathology changes were analyzed. Cecal tissue was collected for 16S rRNA Gene Sequencing. Intestinal barrier proteins ZO-1 and E-cad were measured by Immunohistochemistry Staining and Western Blot, respectively. Intestinal tissue miR155 and inflammatory factors TNF-α, IL-1β, and IL-6 were detected by Q-PCR. The expression levels of protein associated with TNF-α and TLR4/MYD88 pathway in the intestinal were detected.

Results In miR155 overexpression SAP group, the levels of tissue inflammatory factor were significantly increased, intestinal barrier proteins were significantly decreased, and the injury of intestinal was aggravated. Bacterial 16S rRNA sequencing was performed, showing miR155 promotes gut microbiota dysbiosis. The levels of TNF-α, TLR4, and MYD88 in the intestinal were detected, suggesting that miR155 may regulate gut microbiota and activate the TLR4/MYD88 pathway, thereby affecting the release of inflammatory mediators and regulating SAP-related intestinal injury. After application of miR155-sponge, imbalance of intestinal flora and destruction of intestinal barrier-related proteins have been alleviated. The release of inflammatory mediators decreased, and the histopathology injury of intestinal was improved obviously.

Conclusion MiR155 may play an important role in SAP-associated intestinal dysfunction. MiR155 can significantly alter the intestinal microecology, aggravated intestinal inflammation through TLR4/MYD88 pathway, and disrupts the intestinal barrier in SAP mice.

Keywords MiR155 · Severe acute pancreatitis · Gut microbiology · Intestinal inflammation · Intestinal barrier

Abbreviations

SAP	Severe acute pancreatitis
TLR	Toll-like receptors
IP	Intraperitoneal
MYD88	Myeloid differentiation factor 88
AAV	Adeno-associated virus
CON	Control group
CAE-LPS	Caerulein + LPS treatment
CAE-LPS-miR155-O/E or CAE-LPS-miR155	Caerulein + LPS + miR155 overexpression treatment
CAE-LPS-miR155-sponge	Caerulein + LPS + miR155 sponge treatment

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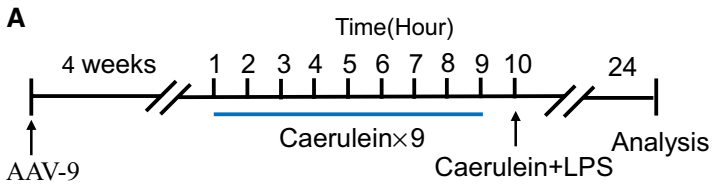
Published online: 02 August 2021



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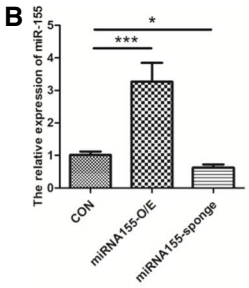

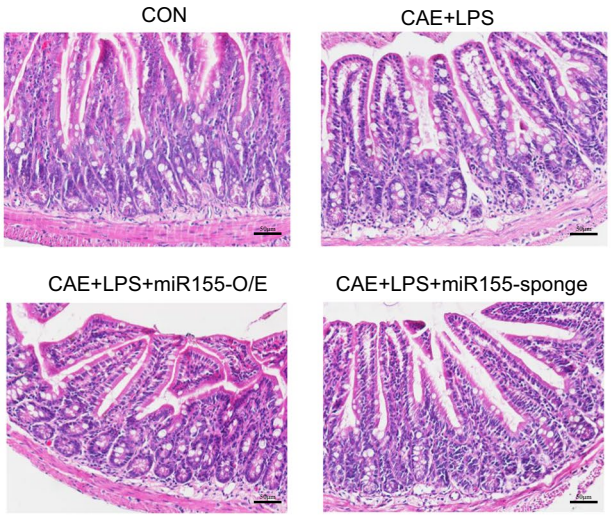

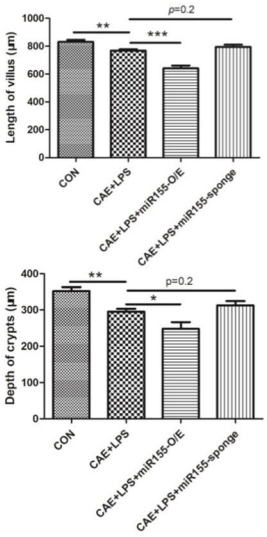

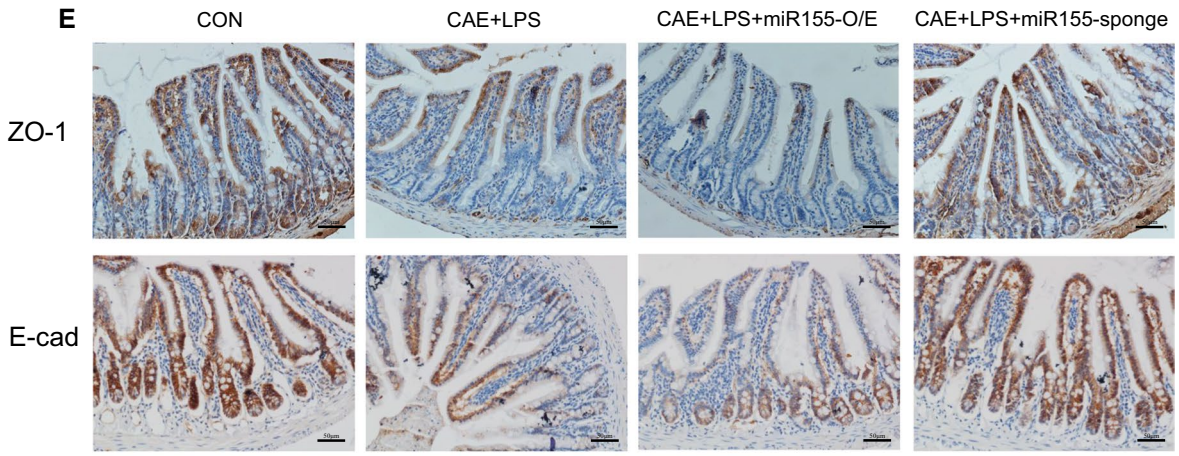

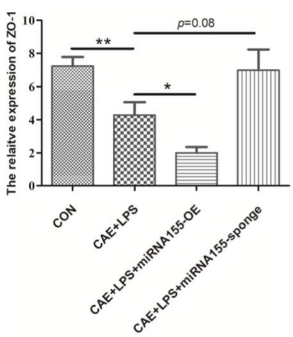

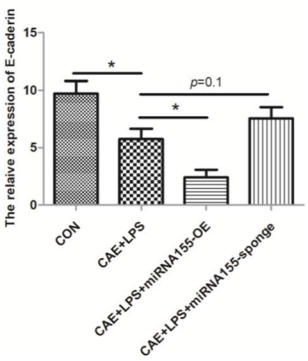

◂**Fig. 1** MiR155 aggravate the destruction of the intestinal barrier in SAP mice. **a** Design of experiment to mice (Con group $n=8$, CAE + LPS group $n=8$, CAE + LPS + miR155-O/E group $n=8$, CAE + LPS + miR155-sponge group $n=8$, Caerulein, 50 μg/kg, intraperitoneal for 10 times and LPS, 10 mg/kg, intraperitoneal for 1 time). **b** The expression of Micro-155 after interference with AAV. **c** Intestine histology in BALB/c mice and **d** pathology score of intestine was shown. **e** The expression of intestinal tight junction protein performed by immunohistochemistry. Control (CON): saline treatment. CAE + LPS: caerulein + LPS treatment. CAE + LPS + miR155-O/E: caerulein + LPS treatment and miR155 overexpression. CAE + LPS + miR155-sponge: caerulein + LPS treatment and miR155 inhibitor. Data shown are means ± SEM. $*P<0.05$, $**P<0.01$, $***P<0.001$

Introduction

Pancreatic infection is a common complication of advanced pancreatitis and is one of the leading causes of death from severe acute pancreatitis (SAP) [1–3]. It is currently believed that most of the bacteria in infection pancreatitis are derived from intestinal flora translocation caused by the destruction of the intestinal barrier [4, 5]. Recent studies have indicated that intestinal microecological disorders and intestinal inflammation are the main cause of intestinal barrier destruction in pancreatitis [5, 6]. However, the intestinal microecology is a complex ecological network that exists in symbiosis with the host. The specific mechanism of intestinal microecological dysfunction-mediated destruction of the intestinal barrier involves multiple factors.

MiRNAs are small noncoding RNAs that play key roles in gene expression regulation, affecting a range of host cell functions, most prominently the immune response [7, 8]. The inflammatory response in the gastrointestinal tract is the most common area of miRNA research. Recent studies have shown that miRNAs participate in the regulation of host immune function and inflammation by shaping the gut microbiota [9]. Furthermore, when the expression of miRNAs was compared between germ-free mice and pathogen-free mice, miRNAs were found to be differentially expressed in the ileum and colon [10], suggesting that miRNAs are closely associated with the intestinal flora. The dysregulation of the intestinal flora correlates with systematic inflammation and gut barrier dysfunction, which increases the severity of acute pancreatitis in patients and mice [11]. These studies indicate that miRNAs play an essential role in the flora-mediated intestinal immune response.

MicroRNA-155(MiR155) is mainly expressed in the thymus and spleen, indicating its unique ability to regulate immunomodulatory functions [12, 13]. An increasing number of studies have found that miR155 has an important effect on gastrointestinal inflammation [14]. Tian R et al. [15] observed that miR155 was associated with intestinal epithelial injure of SAP, which same as our result, the expression of miR155 is elevated (Supplemental Fig. 1). Their research was more focused on the phenomenon, the lack of interventions, and the mechanism of miR155 involved in SAP intestinal barrier disruption which have not been studied.

Interestingly, studies have found that miR155 affects Mycobacterium tuberculosis via specific T cell types and plays a negative regulatory role in the inflammatory response to Helicobacter pylori infection [16, 17]. MiR155 may participate in the flora-mediated intestinal immune response and intestinal barrier disruption. The TLR pathway plays a central role in microbiota-mediated inflammation [18] and antimicrobial defense in the intestine [19]. It has been reported that miR155 can affect the intestinal barrier through TLR. We hypothesize that miRNAs cause intestinal inflammation and intestinal barrier destruction by affecting the intestinal flora and thereby activating TLR signaling pathways.

To find new targets for the treatment of intestinal flora-mediated intestinal inflammation and intestinal barrier destruction, the relationship between intestinal flora, miR155, and TLR pathways needs to be explored. In this study, we modulated the level of intestinal miR155 to observe the changes in the intestinal flora and destruction of the intestinal barrier.

Materials and Methods

Reagents and Lentivirus

Caerulein (catalog no. C9026) and LPS (catalog no. L4130) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The primary antibodies used for immunoblotting were as follows: GAPDH (CST, #2118), TNF-α (CST, catalog no, 11948), TLR4 (SANTA CRUZ, sc-293072), and MYD88 (CST, #4283). Immunohistochemistry (IHC) was performed using the following antibodies: E-cad (BD, catalog no. 610405) and ZO-1 (Invitrogen, catalog no. 40-2200). AAV-miR155 and AAV-miR155 sponges were obtained from Vigene Bioscience (Shandong, China).

Animals and Treatments

Six- to eight-week-old male BALB/c mice were purchased from Hunan SJA Laboratory Animal Co., Ltd. (HSLAC, Hunan, China). The mice were maintained in a temperature-controlled (21 ± 2 °C) environment under a 12 h light/dark cycle, provided water ad libitum, and fed a standard basal diet. The mice were allowed to acclimate for 1 week before surgery. The mice were randomly grouped and housed independently for each group. In studies of miR155 overexpression and silencing, mice were injected via the tail vein with adeno-associated virus (AAV) or control saline. The caerulein plus LPS-induced SAP model was established as

previously described (caerulein, 50 μg/kg intraperitoneally 10 times and 10 mg/kg LPS intraperitoneally once) [20] 4 weeks after AAV (1×10^{12}) injection. All animal-related protocols were approved by the Institutional Animal Ethics Committee of The First Affiliated Hospital of Nanchang University and carried out in compliance with guidelines for the Care and Use of Laboratory Animals.

Histological Evaluation

Freshly harvested pancreatic and distal ileal segments were fixed in 10% formalin overnight, dehydrated with an ethanol gradient, and embedded in paraffin blocks. Tissue sections (4 μm) were processed for hematoxylin and eosin (H&E) staining. Morphological changes, villus height, and crypt depth, a sign of barrier dysfunction, were examined using a light microscope (Nikon, Japan) at 400× magnification. The depths of crypts and the heights of villi were measured in each animal [21]. Image pro plus software 6.0 (Media Cybernetics, MD, USA) were used to measured. The heights of 5 villus and the depths of 5 crypts were measured for each animal.

RNA Extraction and Quantitative Reverse Transcription PCR (RT-PCR)

Total RNA was isolated from the small intestinal mucosa using TRIzol Reagent (Invitrogen, USA). Nucleic acid quantification was performed (NanoDrop 2.0; Thermo Fisher Scientific, Rockland, DE, USA). For miR155 expression analysis, a specific miRcute miRNA Isolation kit (TianGen, China) was used for miRNA isolation, and a miRcute Plus miRNA First-Strand cDNA kit (TianGen, China) was used for cDNA synthesis from the mRNA. The relative RNA levels were assessed using SYBR Green PCR reagents (Invitrogen, USA), and a miRcute Plus miRNA q-PCR kit (SYBR Green; TianGen, China) was used for miRNA according to the manufacturer's protocol. The relative expression ratios were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and 5S as the internal control gene for miRNA. Calculations were made based on the comparative cycle threshold method ($2 - \triangle\triangle Ct$). The primers are shown in Table 1. The primers used for miR155 (CD202-0165) and 5S snRNA (CD202-0012) were also obtained from TianGen.

Immunohistochemistry Staining

Paraffin-embedded tissues were used to analyze the expression of the intestinal barrier proteins ZO-1 and E-cad. After deparaffinization and hydration, the slides were heated in an autoclave with sodium citrate for antigen retrieval, followed by treatment with 3% hydrogen peroxide to abolish endogenous peroxidase activity. The slides were blocked with 2% goat serum and then incubated with primary antibodies included rabbit anti-ZO-1 (1:400) and mouse anti-E-cad (1:500). Then, the sections were incubated with secondary antibodies, developed with 3,3-diaminobenzidine (DAB) solution, and counterstained with hematoxylin. The sections were observed by light microscopy at a magnification of 200× (Nikon, Japan). The histological sections were evaluated by two experienced pathologists, and the degree of staining was scored as follows: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%) for positive staining; and 0 (normal), 1 (weak), 2 (medium), and 3 (strong) for the staining intensity.

Table 1 MIC and MBC of CST or TGC alone on planktonic and biofilm-dispersed MDRAB, and MBEC of CST or TGC alone on biofilm-embedded MDRAB

GAPDH-F (M)	TGATGACATCAAGAAGGTGGTGAAG
GAPDH-R (M)	TCCTTGGAGGCCATGTAGGCCAT
TNF-α-F (M)	GACGTGGAACTGGCAGAAGAG
TNF-α-R (M)	TTGGTGGTTTGTGAGTGTGAG
IL-1β-F (M)	GCCACCTTTTGACAGTGATGAG
IL-1β-R (M)	AAGGTCCACGGGAAAGACAC
IL-6-F (M)	TAGTCCTTCCTACCCCAATTTCC
IL-6-R (M)	TTGGTCCTTAGCCACTCCTTC

16S rRNA Gene Sequencing

Cecal tissue was collected from BALB/c mice into sterile tubes and promptly snap-frozen in liquid nitrogen. The frozen samples were stored at − 80 °C and transported on dry ice until analysis. DNA was isolated from the mouse cecal tissue and contents. The sequences of the primers were as follows: 515F (5′-GTGCCAGCMGCCGCG GTAA-3′), 806R (5′-GGACTACHVGGGTWTCTAAT-3′) as previously described. PCR products were purified with Agencourt AMPure XP (Beckman Coulter, USA) beads and pooled in equal concentrations. Then, the PCR products were sequenced in paired-end mode using an Illumina MiSeq instrument (Illumina, San Diego, California, USA).

Western Blot

Tissues were mechanically homogenized in lysis buffer with a protease inhibitor cocktail (Roche, Amherst, CA, USA). The protein concentration was detected by a BCA assay. An equal amount of protein (25 μg) was separated on 10% SDS-PAGE and transferred to NC (nitrocellulose) membranes. The membranes were blocked in 5% skimmed milk at room temperature for 1 h and then incubated with

primary antibodies in 5% BSA-TBST at 4 °C overnight. Primary antibodies included rabbit anti-TNF-α (1:1000), rabbit anti-MYD88 (1:1000), mouse anti-TLR4 (1:1000). The membranes were then incubated with rabbit IgG-HRP and mouse IgG-HRP secondary antibodies at 4 °C for 4 h. The band intensity of the proteins of interest was normalized to that of mouse anti-actin (1:2000). The proteins were visualized by an enhanced chemiluminescence (ECL) system (Thermo Scientific). The intensity of the bands was quantified using ImageJ software.

Statistical Analysis

Data are expressed as the mean ± SEM. The parametric distribution of the data was confirmed using the Kolmogorov–Smirnov test. The statistical analysis between two groups was performed by independent t test or ANOVA with Tukey's post hoc test for multiple groups using GraphPad Prism 5 statistical software. $P < 0.05$ was considered indicative of a statistically significant difference.

Results

MiR155 Aggravates the Destruction of the Intestinal Barrier in SAP Mice

Previous studies have found that miR155 is highly expressed in the intestine of SAP mice. To examine the function of miR155 in the intestinal function destruction in mice with pancreatitis, two sets of studies were performed. First, we interfered with the levels of miR155 by AAV in the small intestine of SAP mice. After 4 weeks of injection of AAV with miR155 or miR155 sponges, SAP was induced in mice, and they were killed 24 h later (Fig. 1a). Treatment with AAV-miR155 resulted in the increased expression of miR155. Similarly, treatment with the AAV-miR155 sponge resulted in reduced miR155 expression in the small intestine (Fig. 1b). The histological damage in the small intestine increased significantly in mice with miR155 overexpression, as indicated by a decrease in the villus height and crypt depth (Fig. 1c, d). However, the inhibition of miR155 unefficient recovery for instine damage compared to the SAP group, which may result from unsatisfactory interference with miR155. Furthermore, ZO-1 and E-cad, two typical markers of the intestinal barrier, were detected by immunohistochemistry. The results showed that the expression levels of ZO-1 and E-cad were significantly lower in the CAE + LPS group than the control group; however, miR155 overexpression aggravated the downregulation of ZO-1 and E-cad expression (Fig. 1e). These various results signify that miR155 may aggravate the destruction of the intestinal barrier and that the downregulation of miR155 expression may protect the intestinal barrier.

Overexpression of miR155 Can Increase Intestinal Inflammation in SAP Mice

MiR155 overexpression is involved in many inflammatory diseases and involved in promoting inflammatory processes.

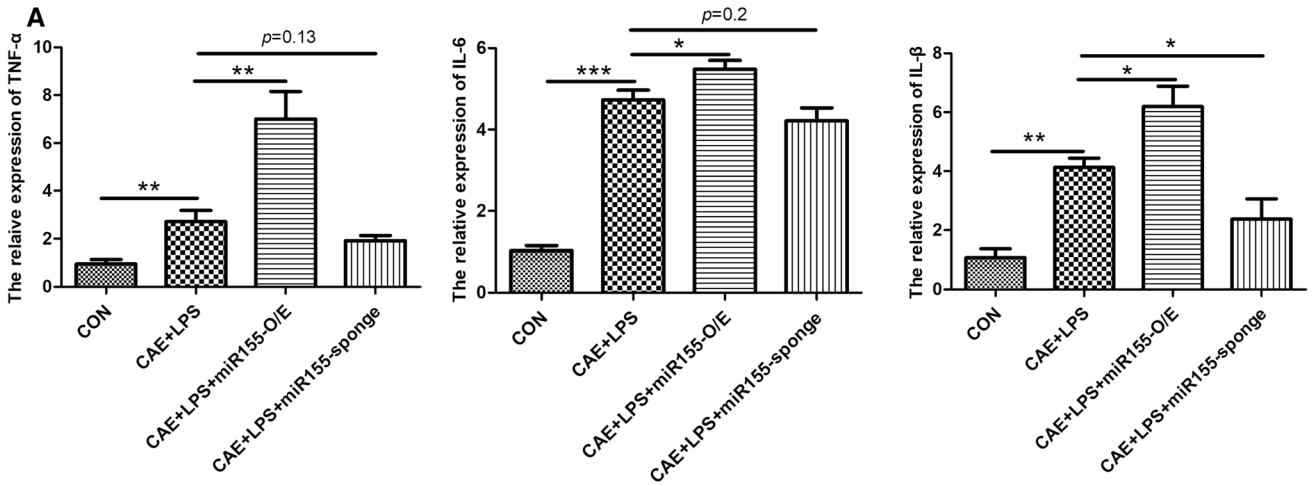

Fig. 2 MiR155 increases intestinal inflammation in SAP mice ($n = 8$ for each group). **a** The expression of inflammatory cytokines in intestinal tissue. Control (CON): saline treatment. CAE + LPS: caerulein + LPS treatment. CAE + LPS + miR155-O/E: caerulein + LPS treatment and miR155 overexpression. CAE + LPS + miR155-sponge: caerulein + LPS treatment and miR155 inhibitor. Data shown are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

Therefore, we observed the expression of inflammatory factors after the up- or downregulation of miR155 expression. After the induction of SAP, the mRNA levels of the cytokines TNF-α, IL-6, and IL-1β in the model group were significantly higher than those in the control group (Fig. 2a). Furthermore, the mRNA levels of TNF-α, IL-6, and IL-1β were markedly higher in the miR155 overexpression group than in the SAP group ($P<0.05$). Although the inhibition of miR155 inefficient reduced the levels of these inflammatory factors obviously, there has a tendency to decrease compared to the SAP group. Therefore, we speculate that the miR155 could aggravate inflammation in SAP mice.

MiR155 Promotes Gut Microbiota Dysbiosis in Animal Model of SAP

The disruption of intestinal function in SAP patients may be related to intestinal inflammation caused by disruption of the intestinal microecology. MiRNAs have been found to participate in inflammation induced by pathogenic bacterial infections. Bacterial 16S rRNA sequencing was performed to examine the relationship between miRNA155 and the intestinal flora. The α diversity analysis based on the OTU level showed reduced bacterial diversity in the intestines of SAP mice and decreased rarefaction in miR155

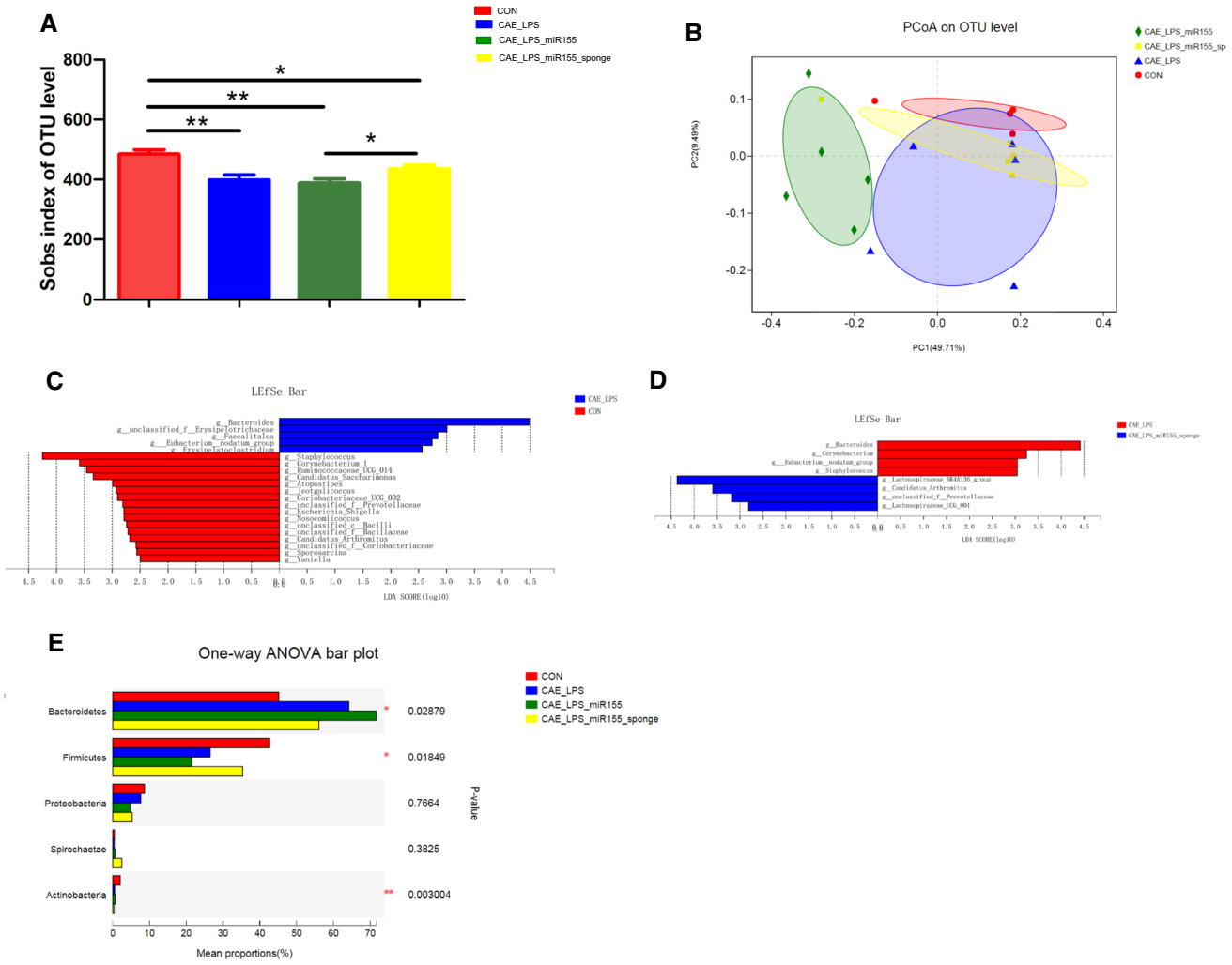

Fig. 3 MiR155 promotes gut microbiota dysbiosis in animal model of SAP ($n=5$ for each group). **a** Normally, alpha diversity was decreased significantly in CON compared with CAE-LPS and CAE-LPS-miR155 (**$P<0.01$), while the difference between CON and CAE-LPS-miR155-sponge (*$P<0.05$) was shrinking. **b** Principal coordinates analysis (PCoA) scores plot showed obvious distinctions between CAE-LPS (blue) and CAE-LPS-miR155 (green), while CAE-LPS-miR155-sponge (yellow) and CON (red) were the closest. **c** LEfSe analysis identified the most differentially abundant taxa between CON and CAE-LPS as well as CAE-LPS and CAE-LPS-miR155-sponge (**d**). **e** Analysis of bacterial differences between different groups. Species abundance was shown for the top five only. Control (CON): saline treatment. CAE-LPS: caerulein-LPS treatment. CAE-LPS-miR155: caerulein + LPS treatment and miR155 overexpression. CAE-LPS-miR155-sponge: caerulein + LPS treatment and miR155 inhibitor. Data shown are means ± SEM. *$P<0.05$, **$P<0.01$, ***$P<0.001$

overexpression, whereas the miR155 sponge treatment significantly increased the diversity (Fig. 3a). Principal coordinate analysis (PCoA) based on unweighted UniFrac distances revealed a significant distinction between the CAE-LPS and CAE-LPS-miR155 groups; reducing miR155 expression significantly reduced this difference (Fig. 3b). At the genus level, the CAE-LPS mice had a higher abundance of potentially harmful bacteria such as Bacteroides, Faecalitalea, and Eubacterium, than the CON mice The CAE-LPS-miR155-sponge mice had an increased abundance of *Candidatus arthromitus* and unclassified_f_Prevotellaceae, which was similar to CONT (Fig. 3c, d). At the phylum level, the abundance of Bacteroides, Firmicutes, and Proteobacteria, which are common intestinal bacteria, was relatively consistent between the CON and CAE-LPS-miR155-sponge mice, whereas the abundance of these phyla in the CAE-LPS and CAE-LPS-miR155 was confirmed to be different from that in the CON mice (Fig. 3e). Thus, silencing of miR155 can rescue the disruption of the intestinal flora toward that of the normal flora in SAP mice.

MiR155 Affects Intestinal Inflammation via the TLR4/MYD88 Pathway

Intestinal bacteria often mediate the downstream signaling pathways activated by TLR4/MYD88. To explore the

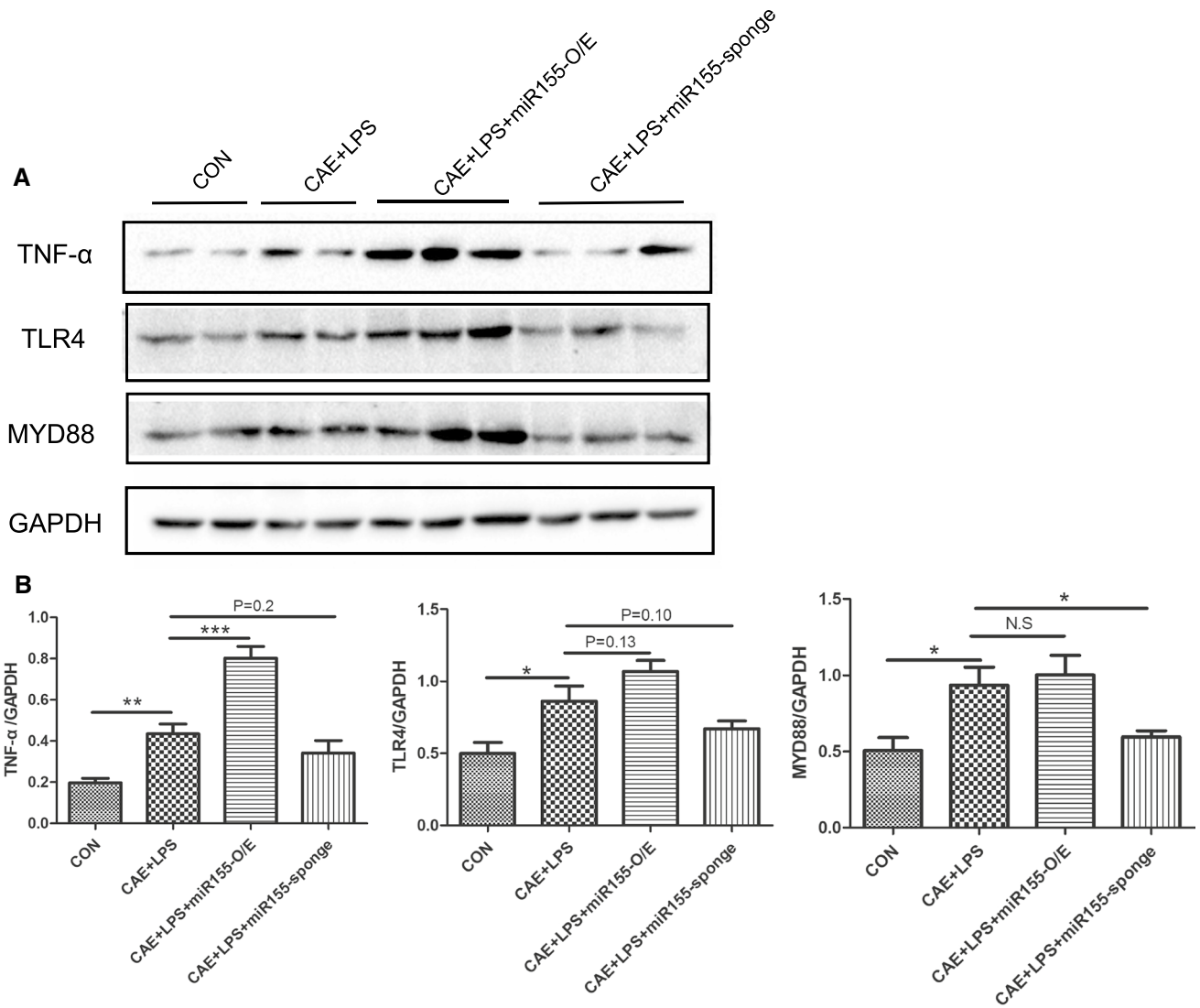

Fig. 4 MiR155 affects intestinal inflammation by TLR4/MYD88 pathway. WB analysis of TNF-α, TLR4, and MYD88 in mice at different groups (CON、CAE + LPS、CAE + LPS + miR155 and CAE + LPS + miR155-sponge). Data shown are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

detailed mechanism that contributes to intestinal damage during the modulation of miR155 expression, we examined the regulation of TLR4 and MYD88 in the small intestine (Fig. 4a, b). The results suggested that a basal level of TLR4/MYD88 signaling, stimulated by the microbiome, is required to mediate the intestinal inflammatory response. Additional evidence for the effect of miR155 is the finding that intestinal tight junction protein expression is reduced and intestinal inflammatory factor levels are significantly elevated in miR155-overexpressing mice. The inhibition of miR155 expression can preferentially alleviate intestinal damage. MiR155 affects inflammation, possibly through the activation of TLR4/MYD88 signaling in SAP mice.

Discussion

Intestinal injury is widely known to be a serious complication of AP [22] and can be explained by multiple factors. Intestinal flora, a major component of the intestine, are closely associated with intestinal immunity and play a central role in intestinal injury. The interaction between miR155 and the microbiota suggests that miR155 may play an important role in intestinal flora-mediated intestinal inflammation. Here, we altered the expression of intestinal miR155 and established an SAP mouse model to analyze the relationship among miR155, the intestinal flora, and the intestinal damage caused by pancreatitis. In the present study, our results suggest that miR155 increases the level of intestinal inflammation and aggravates the disruption of the intestinal barrier. Remarkably, we found that miR155 exhibits a strong effect on the intestinal flora and that the TLR4/MYD88 pathway is activated.

The intestine is an important barrier for host resistance to harmful foreign substances. Dysfunction of the intestinal barrier often occurs in SAP [23]. The most recent study implies that the destruction of the intestinal barrier accelerates the development of disease via the translocation and infection by intestinal bacteria [24]. It is becoming increasingly apparent that pancreatic infections are derived from the intestinal flora [5, 25]. By performing pathology in combination with a quantitative real-time PCR analysis, we found that the intestinal tissue was impaired in the SAP model mice. The destruction of the intestinal barrier potentially facilitates the subsequent intestinal flora translocation.

MiRNAs are increasingly recognized as playing an important role in many immune-related diseases. A recent study reported that miR155 regulates major immune cell types by binding to numerous targets [26] regulating the microenvironment [27]. The intestine is a vast immune organ and a complete and stable biological network [28]. The intestinal microenvironment is vital, and the intestinal flora is increasingly recognized as an important element of intestinal microenvironment; changes in the intestinal flora have been described in many diseases. Previous studies have reported that miRNAs participate in the regulation of immune function and inflammation in pathogenic bacterial infections [9], and Liu et al. reported that the growth of *E. coli* and *F. nucleatum* is regulated by synthetic miRNA mimics, which penetrate bacteria and consequently impact bacterial gene regulation [29].

MiR155 has complex biological functions and distinct expression profiles, which plays a crucial role in various physiological and pathological processes [30]. It is a widely studied miRNA that is closely associated with immunity and inflammation. Previous studies have confirmed that miR155 is highly expressed in blood samples from patients with severe acute pancreatitis or mouse models [31]. But there is a report showing miR155 was significantly decreased in MAP/SAP mice compared to controls [32], which may be related to different modeling methods. In the intestine, miR155 regulates the inflammatory response by affecting bacteria and dysregulates the intestinal epithelial [33, 34]. A previous study reported that expression of miR155 is elevated in intestinal epithelial injure of SAP [15]. In this study, we came to the same conclusion; furthermore, we employed AAV9, which can mediate targeted gene transfer in the intestine [35] to regulate the expression of miR155 in the intestine. We found that miR155 overexpressed aggravated the destruction of the intestinal barrier and the level of intestinal inflammation was consistent with the degree of intestinal barrier damage. Relief of the intestinal barrier damage was not significant after the inhibition of miR155, which probable due to miR155 inhibitory effect in intestinal is not obvious or the destruction of the intestinal barrier of SAP is multiple factors participation. So, we further proved that miR155 may play an important role in pancreatitis-mediated intestinal destruction.

The limitation of SAP animal model is a major obstacle to limiting the study of acute pancreatitis. We build SAP model by caerulein combined with LPS, which LPS can cause intestinal damage itself. According to our previous research, purely caerulein-mediated acute pancreatitis did not cause significant intestinal damage and the same dose of LPS alone could cause death in mice. No LPS only or caerulein only controls were used in this study. Excessive growth of intestinal flora in clinically SAP [36] may produce a large amount of LPS, leading to systemic inflammatory response syndrome (SIRS). Patients with persistent SIRS tend to develop systemic organ dysfunction including the intestine [37]. Therefore, we believe that the use of caerulein combined with LPS has certain advantages over other acute pancreatitis mice models in the construction of pancreatitis intestinal dysfunction models. MRL/Mp mice are a useful animal model for autoimmune pancreatitis, especially for chronic pancreatitis [38]. Autoreactive T cells are key

players in the pathogenesis of MRL/Mp to AIP [39]. The intestine is rich in lymphocytes, which play an important role in the intestinal injury mediated by pancreatitis. Therefore, MRL/Mp mice seem to be a good animal model in the study of pancreatitis-mediated intestinal injury.

Previous investigations have described that, during homeostasis, the gut microbiota is tolerated and establishes a relatively stable ecosystem [28]. The intestinal flora changes in response to the host state. On the contrary, the intestinal flora can be analyzed to evaluate host health to a certain extent [40]. The intestinal microenvironment changed in pancreatitis. After a natural selection-induced intestinal microecological change, we saw that the abundance of beneficial bacteria was reduced and accompanied by a rise in the abundance of harmful bacteria and activation of the inflammatory response in the intestine. In this study, we found intestinal microecological disorder in SAP mice by 16S RNA sequencing, and the level of intestinal inflammation was significantly increased. Interestingly, we found that mice overexpressing miR155 showed an apparent decline in intestinal flora diversity. In contrast, after the miR155 sponge treatment mice, which inhibits miR155 expression, mice showed an apparent restoration of the intestinal flora, suggesting that miR155 is essential for intestinal flora diversity.

Studies have found that miR155 regulates a variety of signaling pathways, including TLR-2/NF-kB [41], SHIP1/Akt [16], and autophagy [42], when bacterial infections occur. Our results revealed obvious changes in the protein levels of TLR4 and MYD88 in miR155-overexpressing mice. In contrast, inhibiting miR155 decreased TLR4 and MYD88 expression greatly. The Toll-like receptor signaling pathway plays an important role in bacterial-mediated intestinal immune regulation. TLR4 activation is fundamental for the development of immunity. Previous studies reported that miRNA modulation may affect TLR pathway activation [43] and miR155 regulates the TLR4 pathway [44]. Although we found that miR155 may activate the TLR4/MYD88 signaling pathway by regulating the intestinal flora, this requires further confirmation by a sterile mouse model, and we will further explore this aspect in the future. Therefore, we suggest that miR155 regulates inflammation associated with altered intestinal flora composition in the gut.

In conclusion, intestinal function is damaged in pancreatitis, and miR155 promotes intestinal epithelial destruction. MiR155 expression in the gut is strongly related to the critical role of the intestinal flora in mediating intestinal immunity. MiRNAs (including miR155) might directly regulate bacterial expression to modulate the host transcriptome. The pancreatitis-induced intestinal inflammation response increases intestinal barrier destruction and miR155 expression. MiR155 functions in inflammation, and miR155 disrupts the intestinal barrier by altering the intestinal microecology and activating the TLR4/MYD88 signaling pathway, as shown in in Fig. 5.

Supplement Figure

Clinically, many SAP patients have intestinal barrier destruction, which is the main cause of pancreatic infections. We also found intestinal barrier destruction in the SAP mouse model induced by caerulein combined with LPS. The length of the villi and crypts is commonly used to assess intestinal damage [21]. Pancreatitis caused bowel function impairment in mice, as indicated by a decrease in the lengths of villi and crypts (Supplement Fig. 1 A, B).The mRNA expression of inflammation indicators such as TNF-α and IL-6 was significantly elevated in the intestinal tracts of SAP mice (caerulein + LPS treatment; Supplement Fig. 1D). These results suggest that intestinal barrier destruction is accompanied by an increase in the level of inflammation. Furthermore, we examined the expression of miR155 in the mouse intestine

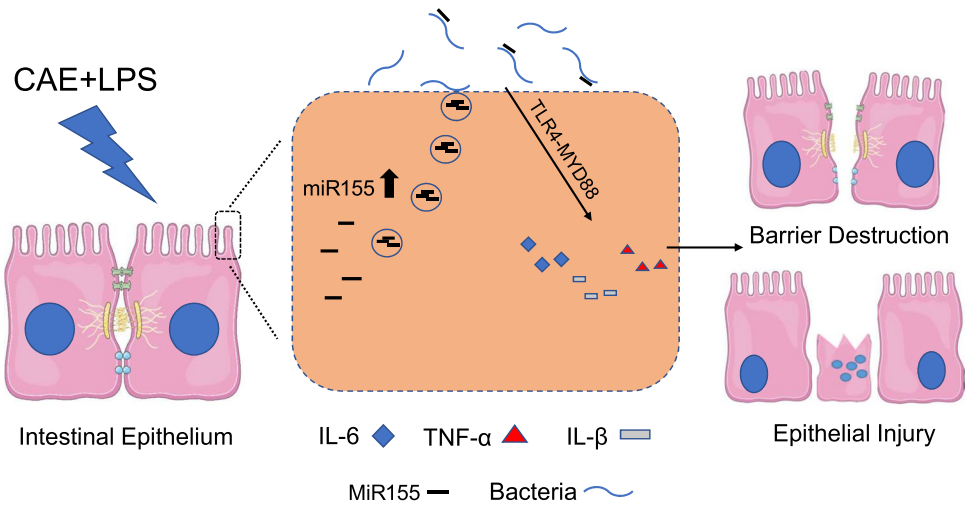

Fig. 5 The mechanism underlying miR155 regulation of intestinal barrier and intestinal flora changes in pancreatitis in SAP

and found that the expression of miR155 was significantly elevated in SAP mice compared with control mice (Supplement Fig. 1C).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10620-021-07022-1.

Acknowledgments The National Natural Science Foundation of China (Nos: 81460130 and 81760121), Funding scheme for outstanding young talents in Jiangxi province (20192BCB23021), and the Graduate Innovation Fund of Nanchang University (No. CX2018231) supported this study together.

Author's contribution JW, XY, and LX contributed to conception and design of the study; XY and JW performed experiment; NSL, CH, YZ, YR, XL, and YZ performed the data analysis; XY and JW drafted the manuscript; NHL and LX performed critical revision of the manuscript; JW, FL, and NHL contributed to reagents/materials/analysis tools; and all authors contributed to manuscript revision and read and approved the submitted version.

Declarations

Conflicts of interest The authors declare that they have no conflicts of interest.

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