miR-34a increases inflammation and oxidative stress levels in patients with necrotizing enterocolitis by downregulating SIRT1 expression

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Abstract. The miR-34a/SIRT1 signaling axis is an important signaling axis in tumors and diseases. Notably, low SIRT1 expression in the intestinal tissues of patients with necrotizing enterocolitis (NEC) has been reported. However, whether miR-34a/SIRT1 signaling as a target to protect the intestines during the NEC process is unclear and remains to be elucidated. Blood samples were collected from 30 patients with NEC, and an NEC rat model was used. The miR-34a and SIRT1 gene and protein expression levels were assayed by qPCR and Western blotting method. The inflammatory cytokine levels and oxidative stress levels were detected using the ELISA method. The results demonstrated that birth weight, albumin and glucose concentrations were significantly decreased in the NEC patient group compared with the control group, but the C-reactive protein (CRP) and procalcitonin (PCT) concentrations were significantly increased. The miR-34a expression level was notably increased in the NEC group, but the SIRT1 expression level was markedly decreased. Notably, the miR-34a was significantly correlated with NEC severity and the concentrations of CRP, PCT, IL-6, TNF-α, IL-1β, IL-8, MCP-1, VCAM1 and malondialdehyde (MDA), but was significantly negatively correlated with SIRT1 gene expression and the concentration of IL-10. Intestinal villi damage in NEC rats was decreased with miR-34a inhibition and SIRT1 activation treatment by decreasing the levels of inflammatory cytokines, including IL-6, TNF-α, IL-1β and IL-8, and oxidative stress proteins, including MCP-1, VCAM1, and MDA, as well as increasing the level of the anti-inflammatory cytokine IL-10. In addition, the results indicated that miR-34a inhibition and SIRT1 activation strongly protected the intestine and decreased the damage caused by NEC, not only by decreasing the protein levels of SIRT1, TNF-α, IL-1β, IL-6 and IL-8, but also by increasing the IL-10 protein levels. The miR-34a inhibition and SIRT1 activation may decrease the damage caused by NEC by decreasing proinflammatory cytokines and oxidative stress proteins and by increasing the anti-inflammatory cytokine pathway. Based on the aforementioned analysis, the miR-34a and SIRT1 proteins may be potential novel therapeutic targets in NEC.

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Introduction

Necrotizing enterocolitis (NEC) is a severe neonatal disease with a high mortality rate (1). A previous study reported that low SIRT1 expression in the intestinal tissues of patients with NEC, which may be due to the suppression of the SIRT1 signaling pathway, thereby promoting the occurrence of NEC (2). In addition, the serum SIRT1 level in children with NEC was low and continued to decrease with disease progression (2). According to previous results, serum SIRT1 is highly sensitive and specific to the diagnosis of NEC, which is of certain reference value for the early diagnosis of NEC (3). In a rat model, the results indicated that the expression level of SIRT1 was markedly decreased in the intestine of rats with NEC induced by LPS, but this effect was reversed with SIRT1 activator treatment to protect the intestine by inhibiting the inflammatory response (3,4). Therefore, SIRT1 may be a novel diagnostic biomarker of NEC and serves an important role in the development of NEC. However, the mechanism has not been fully elucidated.

In recent years, microRNA (miRNAs) has been revealed to serve an important regulatory role in the occurrence and development of NEC (5). The miRNAs are a class of small RNA molecules that regulate the translational level of genes. They can inhibit the translation of protein-coding gene expression to repress protein translation by binding to the target mRNAs (6-8). To date, it has been revealed that certain miRNAs are associated with the occurrence and development of NEC, including miR-429, miR-431 and miR-1290 (9,10). A previous study demonstrated that miRNAs are associated with cancer development, progression and treatment (11). The miR-34a is highly expressed and serves an important role

in a variety of tumor types and diseases (12-16). A previous study reported that the miR-34a and CSF1R inhibit each other and are lost in tumor cells, which may be associated with the treatment and prognosis of colorectal cancer (12). The miR-34a is an important small RNA molecule and is recognized as a master regulator of tumor suppression (13). Notably, the miR-34a expression is governed by p53, but it may be regulated by multiple p53-independent mechanisms (14-16). Another study demonstrated that propofol may induce apoptosis and inhibit the migration of PANC-1 cells in pancreatic cancer by promoting the upregulation of miR-34a-dependent LOC285194 and E-cadherin, respectively (17). In addition, miR-34a may target the WNT1 protein, and promote the proliferation and invasion of E-P cadherin through the WNT1 switch/catenin pathway in cervical squamous cell carcinoma cells (18).

Additionally, miR-34a regulation in inflammation is exemplified by direct repression of them iR-34a gene, which may provide a conserved STAT3-binding site in the first intron in colorectal cancer cells (19,20). Notably, the miR-34a is a crucial regulator of the suppression of tumor progression by inhibiting the IL-6/STAT3/miR-34a pathway (19). A previous study reported that melatonin may be effective in the hepatotoxicity induced by BAP through the miR-34a/Sirt1/autophagy molecular pathway (21). Additionally, CO decreases cell senescence and liver senescence by regulating miR-34a and Sirt1 expression (22). Another previous study indicated that miR-34a-5p may target SIRT1 and serves an important role in decreasing the damage of nucleus pulpoda cells induced by compression load (23). Serum miR-34a in patients with chronic hepatitis C (CHC) may promote liver fibrosis by mediating the Sirt1/P53 pathway, which may be a key biomarker for the prognosis and diagnosis of patients with CHC (16). These studies revealed an important function of miR-34a providing a potential therapeutic strategy for inflammation and also demonstrated that the miR-34a/SIRT1 signaling axis is important in tumors and diseases. However, the function of miR-34a and its mechanism in NEC remain unclear. Therefore, in the present study, the function of the miR-34a/SIRT1 signaling axis was evaluated in the NEC patient serum and NEC rat models.

Materials and methods

Blood samples. Blood samples were collected from control subjects and patients (Table I) with necrotizing enterocolitis (NEC) from January 2019 to January 2020. The present study was approved by the Fujian Provincial Hospital Ethics Committee (K2019-01-030). It was performed in accordance with the International Ethical Guidelines for Human Biomedical Research (2012). The information regarding the patients with NEC was provided by the guardians of the patients. Written informed consent was obtained from participants involved in the study. Study subjects (n=30 per group) comprised normal subjects and patients with NEC. Patient clinical information was collected, and the correlations were analyzed. The sample (1 ml of fasting peripheral venous blood) was collected from the radial vein of the newborn. The biochemical analyses were performed immediately after the sample collection, and the samples were stored at -80˚C for further analyses. Plasma albumin, glucose, C-reactive protein (CRP) and procalcitonin (PCT) were measured using an auto analyzer (Hitachi 912 Autoanalyser; Hitachi, Ltd.).

Construction and evaluation of the NEC rat model. Newborn SPF Sprague-Dawley (SD) rats that did not eat colostrum within 2 h of birth were used for NEC model construction. Newborn rats were placed in a self-made incubator (temperature 37˚C, humidity 45-55%), and artificially fed with milk substitutes, which were prepared according to previous studies (24,25). SD rats were purchased from Shanghai Slake Laboratory Animal Co., Ltd. (license no. SCXK 2017-0005). The 40SD rats (body weight, 5.6-8.5 g) were divided into five groups (n=8 per group), including control group, NEC model group, NEC+miR34a inhibitor group (inhibitor, ACAACCAGCUAAGACACUGCCA; provided by BD Biosciences, NEC+SIRT1 activator group, and NEC+miR34a+SIRT1 inhibitor group (Guangzhou Baisaike Biotechnology Co., Ltd.). The rat model was used according to the Animal Care and Use Committee of Provincial Clinical College of Fujian Medical University. The intestinal tissue and blood were sampled on the sixth day. The paraformaldehyde-fixed intestinal tissue was stained with hematoxylin for 8 min and 0.5% eosin for 2 min at 24˚C. Intestinal function was assessed by a double-blinded scoring method using the Shiou scoring standard (26). Morphologically, indicators of the integrity and tissue structure of intestinal mucosa villi were recorded the five fields each pathological tissue section and analyzed by a light microscope (Olympus Corporation, magnification, x40).

Analysis of inflammatory and oxidative stress cytokines. The serum concentrations of tumor necrosis factor (TNF) α, interleukin (IL) 1β, IL-6, IL-8, IL-10, monocyte chemoattractant protein (MCP) 1 and vascular cell adhesion molecule (VCAM) was determined using ELISA method and according to the manufacturer's protocols. The TNF-α (cat. no. ab181421), IL-1β (cat. no. ab214025), IL-6 (cat. no. ab46027), IL-8 (cat. no. ab214030), were purchased from Abcam, the rat IL-8 (cat. no. SEKR-0071), IL-10 (cat. no. SEKR-0006) and MCP-1 (cat. no. SEKR-0024) kits were purchased from Suolaibao Technology Co., Ltd. and the VCAM1 kits (cat. no. CSB-E07275r) were purchased from Wuhan Huamei Biological Engineering Co., Ltd.

The serum concentrations of superoxide dismutase (SOD) and malondialdehyde (MDA) were determined using commercial kits (Beijing Solarbio Science & Technology Co., Ltd.) according to the manufacturer's protocols.

Total RNA isolation and qPCR analysis. Total RNA was isolated from the serum of patients with NEC and normal controls using RNAiso Reagent (Takara Bio, Inc.) according to the manufacturer's protocols. Then, 2 μg of RNA was used as input material to synthesize cDNA. For qPCR, 2.0 μl cDNA was mixed with 12.5 μl qPCR SYBR® Green Master Mix (A6001; Promega Corporation), 2.0 μl primers and 8.5 μl nuclease-free water to a final volume of 25.0 μl. The miR-34a analysis was conducted in a final volume of 20.0 μl. The primers were designed and synthesized by Sangon Biotech Co., Ltd. and the primer sequences were as follows: SIRT1 forward, AGATCCTCAAGCCATGTTCG and reverse,

Table I. NEC clinical indices.

Characteristic	NEC group (n=30)	Control group (n=30)	Statistics	P-value
Gestation, weeks			χ^2=0.067	0.7952
<34	19	11		
≥34	11	19		
Birth weight, g			χ^2=4.267	0.0389[a]
<1,500	16	7.0		
≥1,500	14	23.0		
Sex			χ^2=5.711	0.0169[a]
Male	16	17		
Female	14	13		
Albumin, g/l	24.30±4.14	30.27±4.40	t=5.320	<0.001[c]
Glucose, mmol/l	2.68±2.80	3.85±1.52	z=-3.260	0.0011[b]
CRP, mg/l	32.50±17.81	4.89±2.20	z=-6.653	<0.001[c]
PCT, ng/ml	7.77±1.93	0.30±0.12	t=-20.811	<0.001[c]

[a]P<0.05, [b]P<0.01 and [c]P<0.001 indicate statistically significant differences. NEC, necrotizing enterocolitis; CRP, C-reactive protein; PCT, procalcitonin.

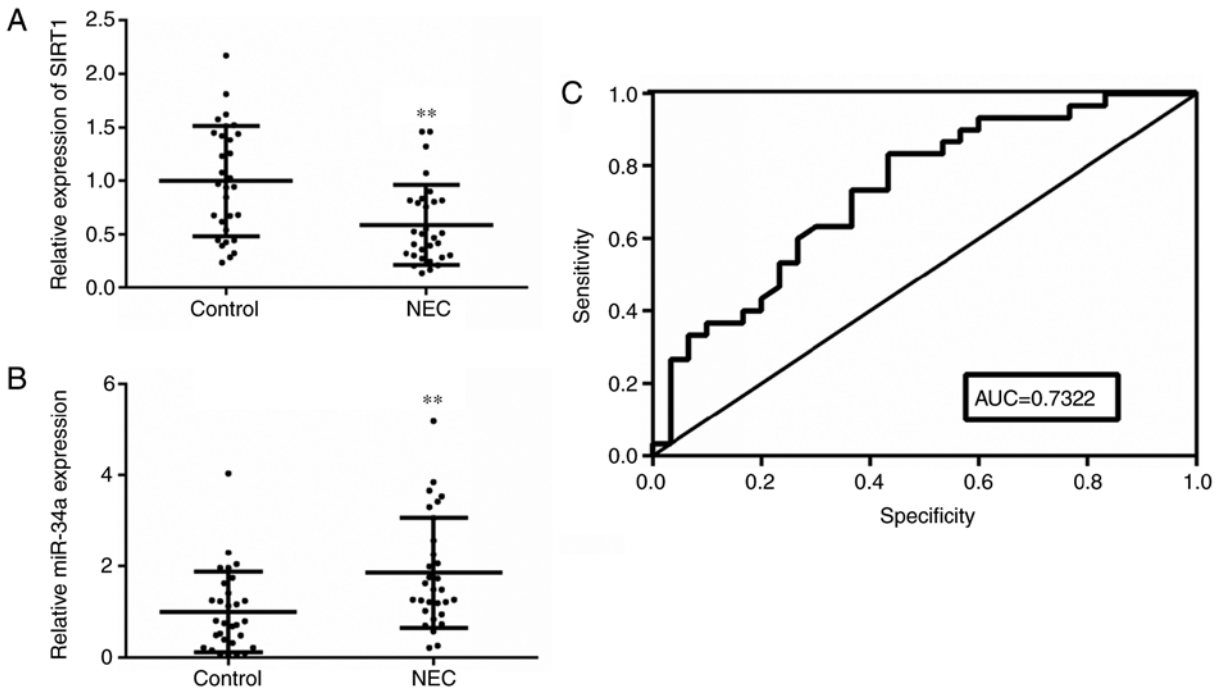

Figure 1. miR-34a and SIRT1 expression levels and diagnostic value in patients with NEC. (A) SIRT1 expression levels. (B) miR-34a expression levels. (C) Receiver operating characteristic analysis results. All the data are presented as the mean ± standard deviation. Compared with the control group, *P<0.05, **P<0.01. NEC, necrotizing enterocolitis; AUC, area under the curve.

CAGAGCTGCTCATGAATGCTG; GAPDH forward, GTC ATCCCTGAGCTGAACGG and reverse, CCACCTGGT GCTCAGTGTAG; miR-34a forward, CGCGTGGCAGTG TCTTAGCT and reverse, AGTGCAGGGTCCGAGGTATT; miR-34a RT, GTCGTATCCAGTGCAGGGTCCGAGGTA TTCGCACTGGATACGACACAACC; U6 forward, CTC GCTTCGGCAGCACATATACT and reverse, ACGCTTCAC GAATTTGCGTGTC; and U6 RT, AAAATATGGAACGCT TCACGAATTTG. The qPCR conditions were 95˚C for 5 min, followed by 40 cycles at 95˚C for 10 sec, 60˚C for 30 sec, 72˚C for 30 sec and 95˚C for 15 sec. According to a previous study, the mRNA expression was assayed using the ABI 7500 PCR system (ABI) and calculated using the $2^{-\Delta\Delta Cq}$ method of $\Delta\Delta Ct=(C_{t.Target}-C_{t.\ GAPDH})X-(C_{t.Target}-C_{t.GAPDH})_{Control}$ (27).

Western blot analysis. Total protein was isolated from cells using RIPA buffer (cat. no. R0278-50ML, Sigma-Aldrich; Merck KGaA) containing the PMSF. The protein sample

concentration was detected using a BCA Protein Assay Kit PC0020-500 (Beijing Solarbio Science & Technology Co., Ltd.). SDS-PAGE (12%) was performed to separate proteins (15 μg) according to a previous study (28). Following separation, the blots were blocked and transferred onto nitrocellulose membranes. The membranes were blocked with 5% bovine serum albumin (BSA) for 1 h at room temperature. Following washing with TBS, the membranes were incubated with the antibodies (Abcam) against SIRT1 (1:1,000 dilution; cat. no. ab189494), TNF-a (1:1,500 dilution; cat. no. ab205587), IL-1β (1:1,500 dilution; cat. no. ab205924), IL-6 (1:1,500 dilution; cat. no. ab9770), IL-8 (1:1,000 dilution; cat. no. ab170381) and IL-10 (1:1,000 dilution; cat. no. ab9969) overnight at 4˚C. The anti-mouse HRP-conjugated secondary antibody (1:1,000; cat. no. ab8227) was used and incubated for 2 h at room temperature. Next, the signal was detected by chemiluminescence with Thermo ECL (34080; Thermo Fisher Scientific, Inc.). Anti-β-actin was used as a loading control. All antibodies were purchased from Abcam. The results were collected by the Versa Doc imaging system (Shanghai Peiqing Science & Technology Co., Ltd.) and analyzed using ImageJ software (V1.8.0.112, National Institutes of Health).

Statistical analysis. The results were analyzed by SPSS 22.0 statistical software (IBM Corp.) using Student's t-test or one-way analysis of variance by the LSD model. P<0.05 was considered to indicate a statistically significant difference. All data are expressed as the mean ± standard deviation. The clinical index was analyzed using SPSS 22.0 statistical software using the models of Chi-square test, Z nonparametric test and T-test. Correlation analysis was performed between miR-34a and SIRT1, SOD, IL-10, TNF-a, IL-1β, IL-6, IL-8, MCP-1, MDA and VCAM-1 by SPSS 22.0 statistical software using Pearson's correlation model.

Results

Correlation between miR-34a and the NEC clinical index. The results of the NEC clinical index, including birth weight and the concentrations of albumin and glucose, were significantly decreased compared with the control group, but the CRP and PCT concentrations were significantly increased (Table I). The miR-34a and SIRT1 expression levels in the blood of patients with NEC are shown in Fig. 1. The SIRT1 gene expression level was markedly decreased in the NEC group, but the miR-34a gene expression level was notably increased in the NEC group (P<0.01; Fig. 1A and B). According to Table II, miR-34a was negatively correlated with albumin (P=0.0285) and glucose (P=0.0458), and positively correlated with CRP (P=0.0326) and PCT (P=0.0121). Notably, miR-34a showed a significant positive correlation with NEC severity (P=0.0034). ROC analysis results demonstrated that the AUC result was 0.7322, which indicated that miR-34a has a high accuracy in the diagnosis of patients with NEC (P<0.01; Fig. 1C).

Correlation between miR-34a and the inflammatory cytokine levels and oxidative stress in patients with NEC. The concentrations of IL-6, TNF-α, IL-1β and IL-8 were significantly increased in the blood of patients with NEC, compared with the blood of control patients, but the IL-10 was significantly decreased in the blood of patients with NEC (Fig. 2A). The MCP-1, VCAM-1 and MDA concentration were significantly increased in the blood of patients with NEC, compared with the control group, but the SOD concentration was significantly decreased in the blood of patients with NEC (Fig. 2B). Based on the blood of the patients with NEC, the correlation analysis results demonstrated that the miR-34a was significantly negatively correlated with SIRT1 gene expression and the concentrations of IL-10 and SOD but was significantly positively correlated with CRP (P=0.0326) and PCT (P=0.0121). Notably, the miR-34a and IL-6, TNF-α, IL-1β, IL-8, MCP-1, VCAM1 and MDA demonstrated significant positive correlations (Table III).

Table II. Correlation analysis between the miR-34a and clinical indicators.

Characteristic	has-miR-34a	*r*	P-value
Sex		-0.09755	0.6081
Male	16		
Female	13		
Gestational, weeks	33+1	-0.1118	0.5564
Birth weight, g	1,494.7±540.33	-0.0982	0.6057
Severity, bellstage		0.5169	0.0034[b]
Ia phase	19		
Ib phase	11		
Albumin, g/l	24.30±4.14	-0.3999	0.0285[a]
Glucose, mmol/l	2.68±2.80	-0.3674	0.0458[a]
CRP, mg/l	32.50±17.81	0.3911	0.0326[a]
PCT (ng/ml)	7.77±1.93	0.4523	0.0121[a]

[a]P<0.05 and [b]P<0.01 indicate statistically significant differences. CRP, C-reactive protein; CT, procalcitonin.

Table III. Correlation analysis among miR-34a and SIRT1, inflammatory factors and oxidative stress factors.

hsa-miR-34a	*r*	P-value
SIRT1	-0.4114	0.0239[a]
TNF-a	0.3907	0.0328[a]
IL-1β	0.4828	0.4606[b]
IL-6	0.4606	0.0104[a]
IL-8	0.5740	<0.001[c]
IL-10	-0.3781	0.0394[a]
MCP-1	0.5661	0.0011[b]
MDA	0.4029	0.0273[a]
SOD	-0.3936	0.0314[a]
VCAM-1	0.4553	0.0115[a]

[a]P<0.05, [b]P<0.01 and [c]P<0.001 indicate statistically significant differences. TNF, tumor necrosis factor; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1.

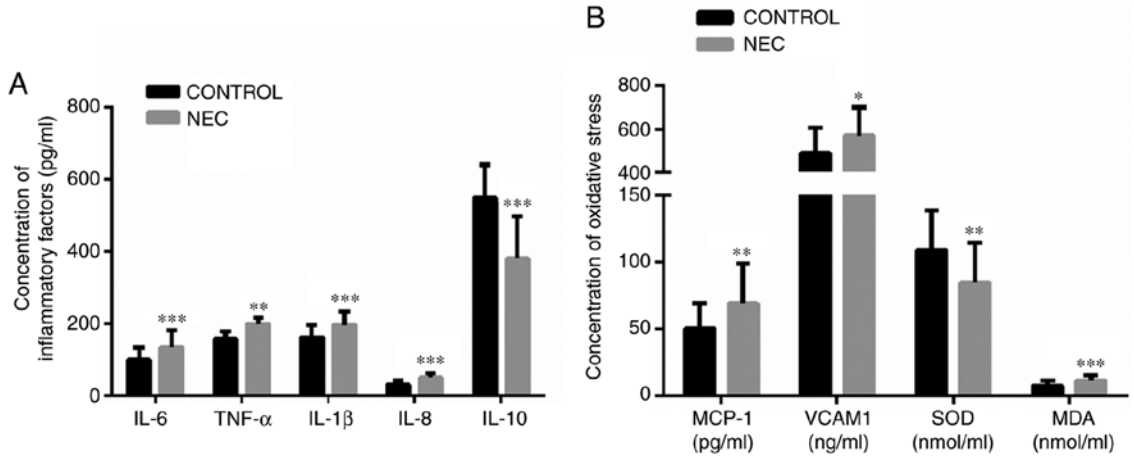

Figure 2. Correlation between miR-34a and the inflammatory cytokine levels and oxidative stress markers in patients with NEC. (A) The inflammatory cytokine level. (B) The oxidative stress factor level. All the data are presented as the mean ± standard deviation. Compared with the control group, *P<0.05, **P<0.01 and ***P<0.001. NEC, necrotizing enterocolitis; IL, interleukin; TNF, tumor necrosis factor; SOD, superoxide dismutase; MDA, malondialdehyde; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule 1.

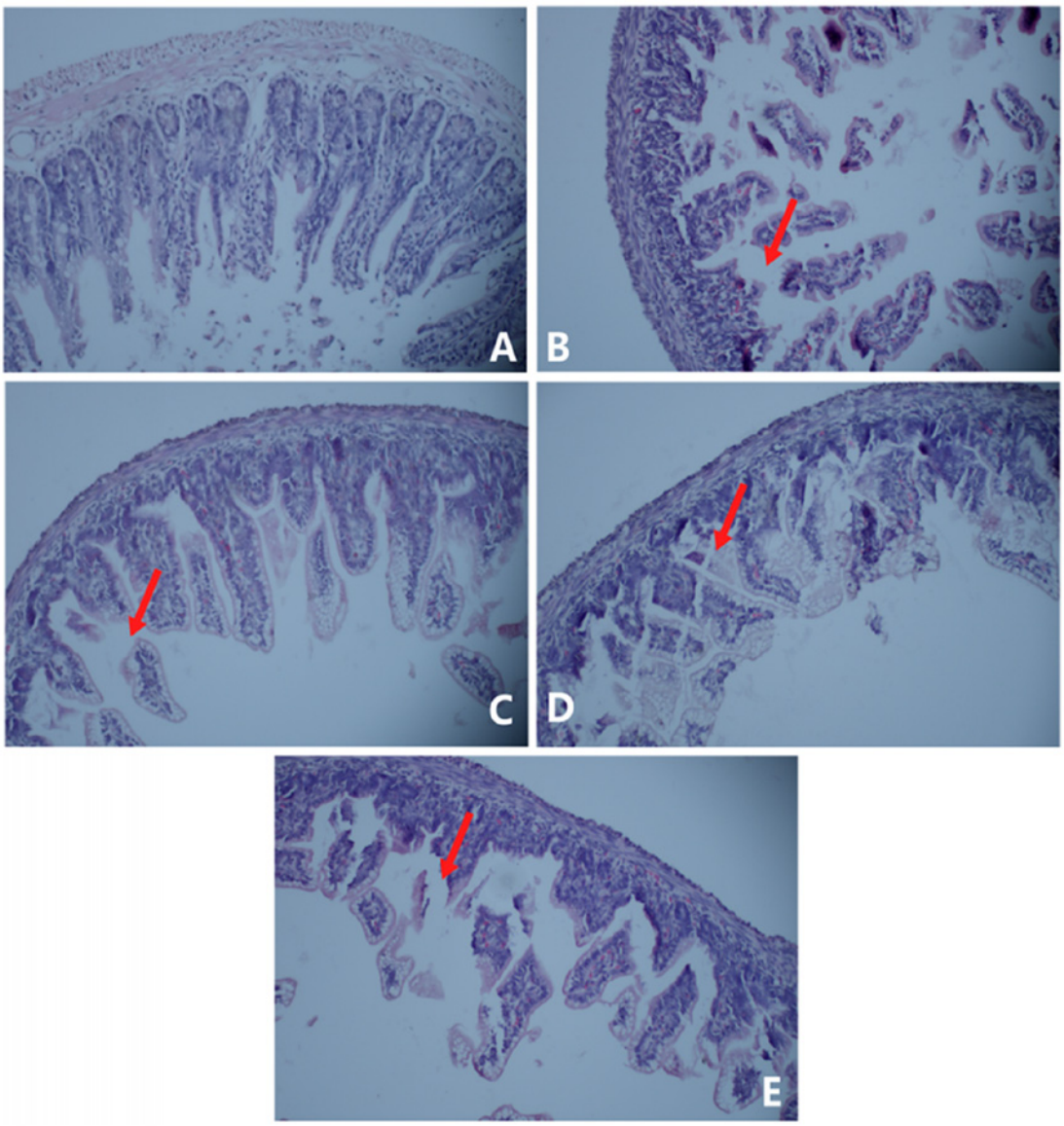

Figure 3. Histological examination of intestinal tissue (magnification, x40) of Sprague-Dawley rats following H&E staining. (A) Control group. (B) NEC model group. (C) NEC model group treated with miR-34a inhibitor. (D) NEC model group treated with SIRT1 activators. (E) NEC model group treated with miR-34a inhibitor and SIRT1 inhibitor. The red arrows represent the section of intestinal villus damage. NEC, necrotizing enterocolitis.

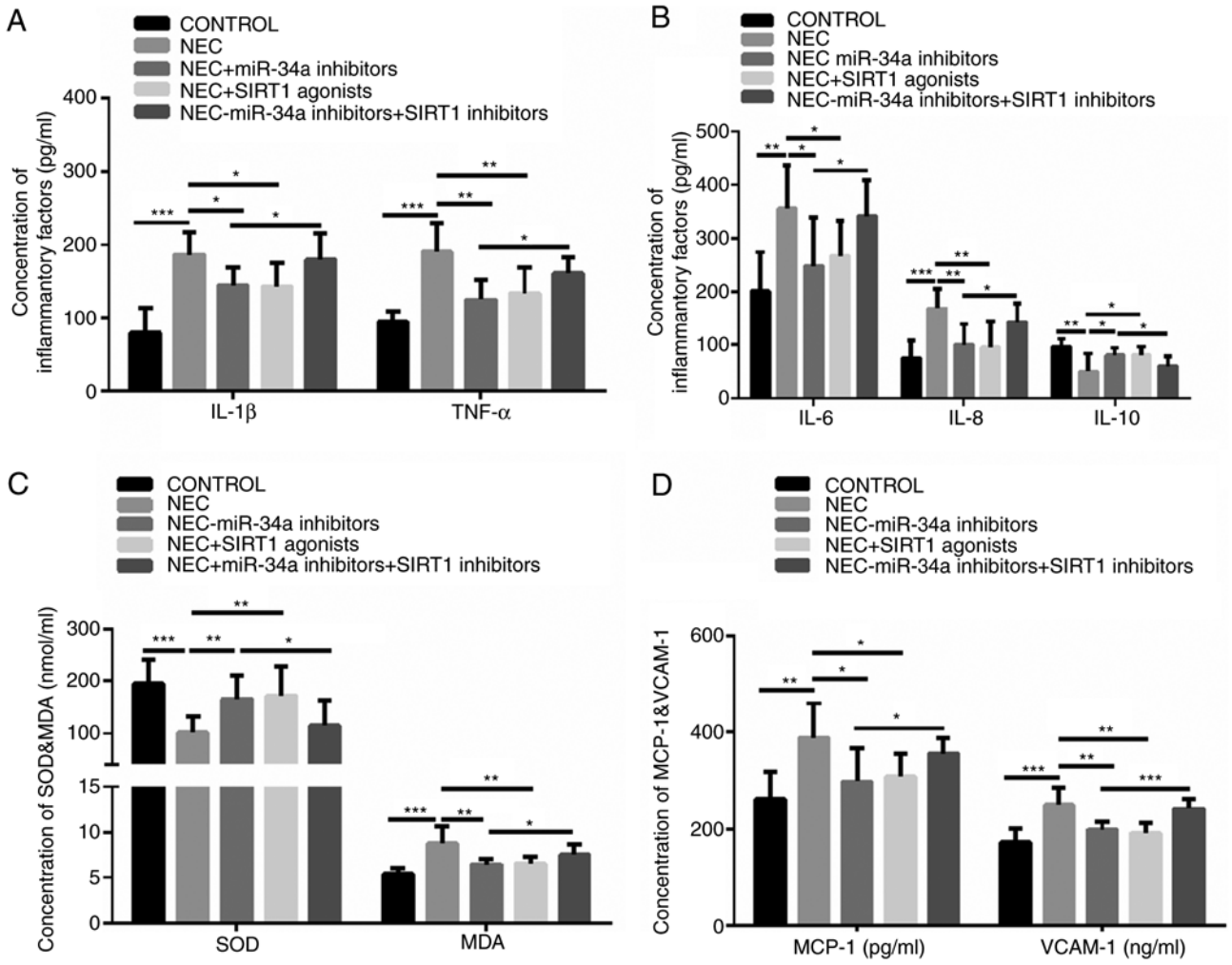

Figure 4. The inflammatory cytokine levels and oxidative stress markers in the NEC rat model. (A) IL-1β and TNF-α concentrations. (B) IL-6, IL-8 and IL-10 concentrations. (C) SOD and MDA concentrations. (D) MCP-1 and VCAM-1 concentrations. All the data are presented as the mean ± standard deviation. Compared with the control group, *P<0.05, **P<0.01 and ***P<0.001. NEC, necrotizing enterocolitis; IL, interleukin; TNF, tumor necrosis factor; SOD, superoxide dismutase; MDA, malondialdehyde; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule 1.

Pathological analysis of the rat NEC model. As shown in Fig. 3, compared with the control group (Fig. 3A), the intestinal tissue histological examination demonstrated that the intestinal villi were seriously damaged and shed in the NEC model group (Fig. 3B) and the double-blind score was 4. Based on the NEC group, miR-34a inhibitor and SIRT1 activator treatment decreased the double-blind score to 2 and demonstrated that the intestinal villus damage was decreased (Fig. 3C and D). However, the intestinal villus damage was significantly aggravated when the SIRT1 activators were replaced with SIRT1 inhibitors, and the double-blind score increased to 3 (Fig. 3E). These results indicated that miR-34a inhibitors and SIRT1 activators effectively improved NEC intestinal mucosal necrosis, and SIRT1 inhibitors reversed the efficacy of miR-34a inhibitors in an NEC rat model.

Inflammatory cytokine levels and oxidative stress in the NEC rat model. To evaluate the effect of miR-34a and SIRT1 on inflammatory cytokine levels and oxidative stress in the NEC rat model, the miR-34a inhibitors, the SIRT1 activators and SIRT1 inhibitors were used. The results demonstrated that the concentrations of IL-1β, TNF-α, IL-6 and IL-8 were significantly increased in the NEC group, compared with the control group, but they were significantly reversed in the groups treated with the miR-34a inhibitors and the SIRT1 activators (Fig. 4A and B). The IL-10 concentration was significantly decreased in the NEC group, compared with the control group, but it was significantly reversed in the groups treated with the miR-34a inhibitors and SIRT1 activators and the group treated with miR-34a inhibitors and the SIRT1 inhibitors (Fig. 4B). The MDA, MCP-1 and VCAM-1 concentrations were significantly increased in the NEC group, compared with the control group, but they were significantly reversed in the groups treated with the miR-34a inhibitors and SIRT1 activators (Fig. 4C and D). However, the SOD concentration was different from the MDA, MCP-1 and VCAM-1 results (Fig. 4A). In particular, miR-34a inhibitors and the SIRT1 treatment decreased the SOD concentration and increased MDA, MCP-1 and VCAM-1 concentrations, compared with the miR-34a inhibitor group. These data indicated that the miR-34a inhibitors and SIRT1 activators may decrease oxidative stress levels in the NEC rat model and that the SIRT1 inhibitors may reverse the effect of miR-34a inhibitors on the NEC-induced oxidative stress response.

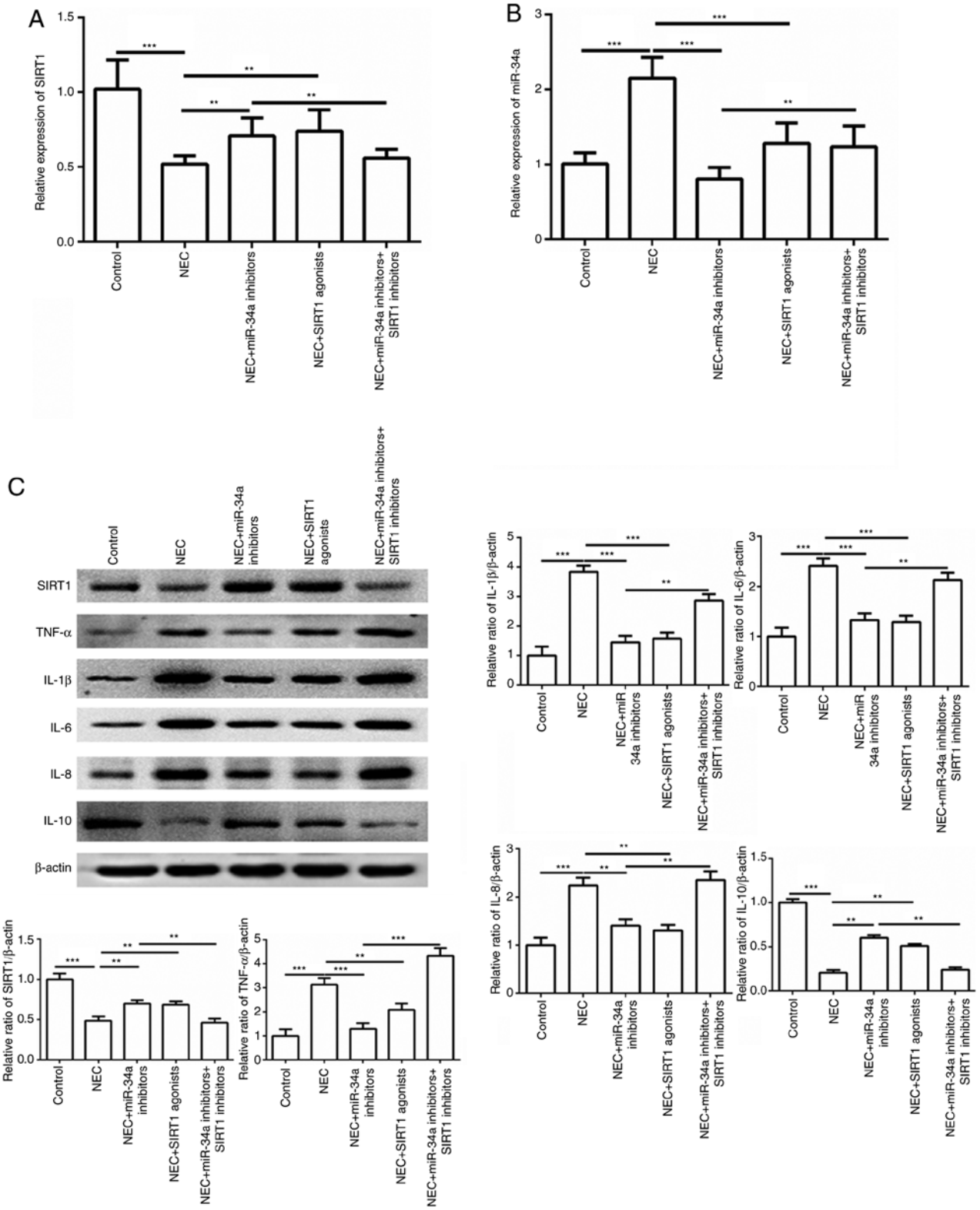

Figure 5. Effect of miR-34a and SIRT1 on the gene and protein expression levels of inflammatory cytokines and oxidative stress markers in a rat NEC model. (A) The SIRT1 expression level in the colon tissue. (B) The miR-34a expression level in the colon tissue. (C) Protein expression level in the colon tissue. All the data are presented as the mean ± standard deviation. Compared with the control group, **P<0.01 and ***P<0.001. NEC, necrotizing enterocolitis.

Effect of miR-34a and SIRT1 on the gene and protein expression levels of inflammatory cytokines and oxidative stress markers in a rat NEC model. As shown in Fig. 5, the SIRT1 gene expression level was markedly decreased in the NEC group, but was significantly increased in the group treated with miR-34a inhibitors and the SIRT1 activators (P<0.001 or P<0.0001; Fig. 5A). The miR-34a gene expression level was the opposite of the SIRT1 expression (P<0.001 or P<0.0001; Fig. 5B). In particular, the expression of the SIRT1 gene was markedly decreased in the group treated with inhibitors of

miR-34a and the SIRT1 group, compared with the miR-34a inhibitor group. However, the miR-34a expression levels were significantly increased when the SIRT1 inhibitor supplementation was used. The protein expression results demonstrated that SIRT1, TNF-α, IL-1β, IL-6, IL-8 and IL-10 were consistent with the serum results. These data indicated that miR-34a and SIRT1 maybe an important index in regulating NEC inflammation (Fig. 5C).

Discussion

NEC is a severe neonatal gastrointestinal disease with a high mortality rate in preterm newborns (1). At present, the pathogenesis of NEC is considered to result from inappropriate proinflammation, prematurity and bacterial colonization (29). A previous study indicated that the function of microRNA is an important regulatory factor in the occurrence and development of NEC (5). Su *et al* (30) indicated that miR-874 may downregulate the aquaporin-3 expression in intestinal barrier dysfunction (30). In the present study, the miR-34a expression was markedly increased in the NEC group, compared with the control group. It was also demonstrated that inhibition of miR-34a expression may decrease intestinal damage in the NEC rat model according to the pathological results. Previous results have indicated that miR-7-5p, miR-181a-5p, miR-194-5p and miR-362-3p are associated with intestinal diseases (31-33).

NEC is also a severe inflammatory disorder in preterm infants based on severe gut barrier damage (34). In the blood of patients with NEC, the miR-34a was negatively correlated with albumin and glucose and positively correlated with CRP and PCT. Notably, miR-34a showed a significant positive correlation with NEC severity. Based on the ROC analysis results, miR-34a was highly accurate in the diagnosis of patients with NEC. Additionally, the concentrations of IL-6, TNF-α, IL-1β, IL-8, MCP-1, VCAM-1 and MDA were significantly increased in the blood of patients with NEC, but IL-10 was significantly decreased. This result is consistent with those of a previous study, which reported that miR-34a may direct the binding of the transcription factor NF-κB to regulate inflammation (35). Notably, the anti-inflammatory factor IL-10 was markedly decreased in the NEC group. Previous studies have demonstrated that miR-34a-overexpression inhibited cell proliferation in certain experimental models (36,37). In other words, inhibition of miRNA-34a lead to increased cell proliferation, which was accompanied by inflammatory suppression.

SIRT1 is an important apoptosis gene and a target gene of miR-34a. As in previous study, the histone deacetylase SIRT1 was repressed by the miR-34a, thereby transactivating its target genes (38). Recent studies suggested that SIRT1 is a potential treatment target for human degenerative intervertebral disc disease and myocardial infarction by inhibiting apoptosis (39,40). A previous study also indicated that activation of SIRT1 may suppress cellular senescence and promote cell proliferation (41). In the present study, SIRT1 expression was significantly downregulated in cells treated with miR-34a and the SIRT1 inhibitor, which was markedly reversed by inhibiting miR-34a. Furthermore, in the blood of patients with NEC, a significant downregulation of SIRT1 expression and significant upregulation of miR-34a expression was also observed. Notably, the negative correlation between miR-34a and SIRT1 mRNA expression was verified, which indicated that miR-34a upregulation may abrogate the repressive effect of the target gene SIRT1. These findings were also proven in the NEC rat model. Therefore, it was proposed that the miR-34a/SIRT1 axis serves a potential therapeutic role in NEC.

In conclusion, the results suggested that miR-34a may affect the occurrence of NEC by regulating the inflammatory factor balance and SIRT1 expression. Therefore, targeting the miR-34a/SIRT1 axis offers novel therapeutic opportunities for treating NEC.

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Availability of data and materials

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

Authors' contributions

HZ designed the research, analyzed and interpreted the patient data regarding the NEC disease. HZ, YLin and YLiu preformed the experimental analyses, and analyzed and interpreted the data in NEC rat model. YLiu performed the ELISA to detect the cytokine concentration. HZ was a major contributor in writing the manuscript. All authors read and approved the final manuscript. All authors confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Fujian Provincial Hospital Ethics Committee (K2019-01-030). It was performed in accordance with the International Ethical Guidelines for Human Biomedical Research (2012). The information of patients with NEC was provided by the guardians of the patient. Written informed consent was obtained from volunteers involved in the study.

Patient consent for publication

All patients provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

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