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CircRNA_104293 targets miR-497-5p to inhibit the mTOR/STAT3 pathway and mitigate inflammation in Crohn's disease.

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Keywords: Crohn Disease; mTOR/STAT3; Inflammation; circRNA_104293; miR-497-5p.

Abstract. Crohn's disease (CD) is a chronic inflammatory bowel disease driven in part by dysregulation of the mTOR/STAT3 signaling pathway, where mTOR activates STAT3 via the PI3K/AKT cascade. Circular RNAs (circRNAs) have recently emerged as essential regulators in inflammatory processes, although their specific roles in CD remain largely unexplored. In this study, circRNA expression profiles from CD patients and healthy controls were analyzed, revealing a significant upregulation of circRNA_104293 in CD tissues. Functional investigations demonstrated that knockdown of circRNA_104293 reduced inflammatory cytokine production and DNA damage markers, and decreased cell apoptosis. Bioinformatic analysis and experimental validation confirmed a direct interaction between circRNA_104293 and miR-497-5p. Furthermore, miR-497-5p inhibition reversed the anti-inflammatory effects of circRNA_104293 silencing. Notably, both rapamycin (an mTOR inhibitor) and miR-497-5p mimics suppressed the mTOR/STAT3 pathway and alleviated inflammatory responses. These findings suggest that the circRNA_104293/miR-497-5p axis contributes to CD progression by modulating the mTOR/STAT3 pathway, highlighting its potential as a novel therapeutic target for the treatment of Crohn's disease.

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CircRNA_104293 actúa sobre el miR-497-5p para inhibir la vía mTOR/STAT3 y mitigar la inflamación en la enfermedad de Crohn.

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Palabras clave: Enfermedad de Crohn; mTOR/STAT3; Inflamación; circRNA_104293; miR-497-5p.

Resumen. La enfermedad de Crohn (CD) es una enfermedad inflamatoria crónica del intestino cuya progresión está parcialmente mediada por la disfunción de la vía de señalización mTOR/STAT3, en la que mTOR activa a STAT3 a través de la cascada PI3K/AKT. Recientemente, los ARN circulares (circRNAs) han surgido como reguladores clave en procesos inflamatorios, aunque sus funciones específicas en la CD aún no están bien definidas. En este estudio se analizaron los perfiles de expresión de circRNAs en pacientes con CD y en controles sanos, y se identificó una sobreexpresión significativa de circRNA_104293 en tejidos de pacientes con CD. Los análisis funcionales mostraron que la reducción de la expresión de circRNA_104293 disminuyó la producción de citocinas inflamatorias y de marcadores de daño al ADN, así como la apoptosis celular. Mediante análisis bioinformático y validación experimental, se confirmó una interacción directa entre circRNA_104293 y miR-497-5p. Además, la inhibición de miR-497-5p revirtió los efectos antiinflamatorios inducidos por el silenciamiento de circRNA_104293. De manera destacada, tanto la rapamicina (un inhibidor de mTOR) como los miméticos de miR-497-5p suprimieron la activación de la vía mTOR/STAT3 y redujeron las respuestas inflamatorias. Estos hallazgos indican que el eje circRNA_104293/miR-497-5p participa en la progresión de la CD mediante la modulación de la vía mTOR/STAT3, lo que resalta su potencial como nuevo objetivo terapéutico para el tratamiento de la enfermedad de Crohn.

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INTRODUCTION

Crohn's Disease (CD) is characterized by chronic, repetitive inflammation of the gastrointestinal tract. Its etiology has not been fully clarified, but studies have shown that CD is closely associated with genetic, environmental, and immune factors, as well as changes in the gut microbiota[1]. Patients often present with abdominal pain, diarrhea, weight loss, fever, and other symptoms, which in severe cases can lead to complications such as intestinal obstruction and fistula formation [1, 2]. The mammalian target of rapamycin (mTOR) belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family and is mainly involved in processes such as cell growth, proliferation, metabolism, and autophagy [3]. Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that regulates cell survival, proliferation, and immune responses. In CD, aberrant activation of STAT3 is strongly associated with exacerbated inflammatory responses [4]. The mTOR/STAT3 pathway interacts in CD to co-regulate inflammatory

reactions. For example, mTOR activation has been found to promote STAT3 phosphorylation through the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) signaling pathway, thereby enhancing its transcriptional activity [5]. This interaction plays a key role in intestinal inflammation in Crohn's disease patients and may lead to its persistence and aggravation. But the specific molecular mechanism is unknown.

Circular RNAs (circRNAs) are molecules with unique circular structures that play an essential role in a variety of diseases [6, 7]. In neurological diseases, circRNA is widely expressed in brain tissue and is closely related to neurodevelopment and signaling, showing abnormal expression in diseases such as cerebral palsy and stroke, and is considered a potential biomarker of neurological diseases due to its stable structure [8,9]. In malignant tumors, some circRNAs can adsorb microRNAs (miRNAs) as "molecular sponges", thereby relieving miRNA inhibition of oncogenes and promoting tumor development [10]. In CD, circRNA 103765 is reported to regulate disease progression [11]. At present, the study of circRNA in CD still needs to be further expanded to fully understand its effects on CD. MicroRNAs (miRNAs) are also closely associated with the progression of various inflammatory diseases [12, 13]. miR-497 has been reported to influence the progression of associated inflammatory diseases by modulating lipopolysaccharide (LPS) -induced inflammation in vivo [14, 15]. Furthermore, miR-497 expression was significantly decreased following LPS treatment of RAW264.7 cells and in a mouse colitis model [16].

Here, we performed sequencing analysis of circRNA expression profiles from CD patients to explore potential therapeutic targets and their molecular mechanisms. Through screening, we found circRNAs that were up- and down-regulated in CD. Further, qPCR was used to detect the expression level of circRNA 104293 in CD patients, and its mechanism of action in CD development was studied in depth. The results of this study are expected to provide a new treatment option for patients with CD.

METHODS

Patients and genome sequencing analysis

Between June 2021 and March 2022, we collected the inflamed colonic tissue samples from CD patients who underwent surgical treatment at our institution. The tissues were obtained from inflamed areas of the colon, confirmed by intraoperative findings and postoperative histopathological examination, and non-inflamed adjacent tissues served as control. Tissue total RNA isolation kit (RC101-01, Vazyme, Nanjing, China) was used for the RNA extraction, and RNA integrity was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). High-throughput circRNA microarray analysis was conducted by Aksomics Biotechnology (Shanghai, China). Sample processing was carried out according to the manufacturer's protocol. Briefly, the Seq-Star™ rRNA removal kit (AS-MB-001, Aksomics) was employed for the rRNA removal, and the circRNA purification and fluorescent cRNA transcription were performed using Seq-Star™ RNAClean and smallEnrich Beads (AS-MB-009) and rtStar™ First-Strand cDNA Synthesis Kit (AS-FS-001) from Aksomics. Labeled cRNA concentration and specific activity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). One microgram of labeled cRNA from each sample was fragmented, mixed with hybridization buffer, and loaded onto a Human circular RNA Array V2.0 (8 × 15K format, Aksomics). Hybridization was carried out at 65°C for 17 h in an Agilent Hybridization Oven (model G2545A). After hybridization, the microarrays were washed and fixed with Gene Expression Wash Buffers (Agilent Technologies, USA) and scanned with an Agilent DNA Microarray Scanner (model G2505C).

Differential expression analysis

Differential expression analysis was conducted using the DESeq2 R package. The fold change and statistical significance were calculated based on the log2|(fold change)|>1 and *p-adjust* <0.05.

Cells culture and treatment

In this study, Fetal Human Colon (FHC) cells (normal human colon epithelial cells) were purchased from the Cell Bank of Shanghai Institute of Life Sciences. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin-glutamine (PSG) and adapted for 2-3 days before use in experiments. To induce a general inflammatory response *in vitro*, FHC cells were treated with 500 ng/mL of lipopolysaccharide (LPS) and control cells were treated with serum-free medium. Treated cells were divided into two groups: small interfering RNA (si)-circRNA group and negative control group (siNC). Cells were transfected using Lipofectamine ™ 2000 following kit instructions and cells were collected 48 hours later for subsequent experiments. In addition, inhibitors of miR-497-5p and its negative control (NC) were transfected using Lipofectamine ™ 2000. The mTOR targeting inhibitor rapamycin (Bioengineering Co., A606203) was used for experiments and cells were co-incubated with 100 ng/mL rapamycin to investigate its effect.

Q-PCR assay

To determine the expression levels of miR-497-5p and circRNA 104293, we used qPCR. TRIZOL reagent (R0016, Beyotime, Shanghai, China) was used to extract total RNA and RNA was isolated through centrifugation. The RNA was then dissolved in RNase-free water. Then reverse transcription was performed to synthesize cDNA using BeyoRT™II First Strand cDNA Synthesis Kit (D7168S, Beyotime, Shanghai, China), which was subsequently used as the template for qPCR amplification following the instructions of the SYBR Green kit (D7260, Beyotime). The qPCR cycling conditions included initial denaturation at 95°C for 30s, followed by 39 cycles of 95°C for 5s, 60°C for 30s, and a final extension at 72°C for 5 seconds. The relative expression levels of circRNA_104293 were normalized to GAPDH as an internal control. Gene expression was calculated using the 2-ΔΔCt method.

ELISA assay

After transfection, the cells were washed three times with PBS. Subsequently, 0.5 ml of non-denaturing protein lysis buffer was added to each well and gently mixed to lyse the cells. The lysates were then incubated on ice for 20 minutes. The supernatants were immediately stored at -20°C. The tumor necrosis factor-alpha (TNF-α) ELISA Kit (PT518), interleukin-1 beta (IL-1β) ELISA Kit (PI305), IL-6 ELISA Kit (PI325) and IL-8 ELISA Kit (PI641) were purchased from the Beyotime biotechnology company (Shanghai, China). The levels of inflammatory cytokines in cell lysates were measured using ELISA assays according to the manufacturer's instructions.

Flow cytometry assay

A cell suspension of LPS-treated cells was prepared at a density of 5×10^5/mL and seeded into a 6-well plate. After 24 hours, the cells were transfected with si-circRNA 104293, or si-NC, followed by an additional 24-hour incubation. Post-transfection, the cells were harvested through digestion and centrifugation, then washed three times with ice-cold PBS. Apoptosis was assessed by staining the cells with 5 μL of Annexin V-FITC/PI. The apoptosis rate was subsequently quantified using flow cytometry. This protocol ensured consistent evaluation of cell viability under experimental conditions.

Dual-luciferase reporter gene assay

The TargetScan online tool was employed to predict potential miRNA binding sites on circRNA_104293, specifically identifying complementary sequences for

miR-497-5p. To validate this interaction, wild-type or mutant circRNA_104293 plasmids were constructed by GenePharma (Shanghai, China). The miR-497-5p mimic or negative control was co-transfected with either wild-type or mutant plasmids into FHC cells using Lipofectamine™ 2000 (Invitrogen, USA), with triplicate wells for each condition. After 24 h of post-transfection, the medium was discarded, and the cells were washed three times with PBS. Cell lysis was performed using 100 μL of lysis buffer (RG132S, Beyotime) at room temperature. Firefly and Renilla luciferase activities were measured sequentially using a Dual-Luciferase® Reporter Assay System (E1910, Promega, USA), including LARII reagent and Stop&Glo® reagent, according to the manufacturer's instructions. Luminescence was detected using a Glomax Multi+ Detection System (Promega, USA).

Immunofluorescence assay

Cells were cultured on sterile glass coverslips in 24-well plates and then treated with si-circRNA 104293 as the test and si-NC as the control. After treatment, the coverslips were washed three times with PBS and fixed with 37 g/L formaldehyde for 15 min. Permeabilization was performed using 0.5% Triton X-100 for 10 min, followed by blocking with 100 mL/L bovine serum albumin (BSA) for one h. Primary antibodies specific to dsDNA (ab27156, Abcam) and ssDNA (CBL407, Millipore) were applied at appropriate dilutions, and the samples were incubated overnight at 4°C. The coverslips were then washed three times with PBS and once with distilled water, then incubated with an FITC-conjugated secondary antibody (A-11001, Thermo Fisher Scientific) for one h at room temperature in the dark. Finally, the samples were mounted with glycerol and analyzed using fluorescence microscopy. Fluorescence intensity was quantified in ImageJ by measuring the mean fluorescence per cell and normalizing to control samples.

Western blot assay

Proteins were extracted from treated cells 48 h post-treatment using RIPA lysis buffer supplemented with protease and phosphatase inhibitors. The protein samples were quantified using a BCA protein assay kit (A65453, Thermo Fisher Scientific), and equal amounts of protein (30 μg/lane) were separated on an SDS-PAGE gel and subsequently transferred to a PVDF membrane. And then the membrane was blocked with 5% skim milk in TBST for one hour. After blocking, the membrane was incubated overnight at 4°C with primary antibodies targeting p-mTOR (ab109268, Abcam) and p-STAT3 (ab76315, Abcam). GAPDH (ab8245, Abcam) was used as the loading control. Following three washes with TBST, the sections were incubated with appropriate HRP-conjugated secondary antibodies (ab205719, Abcam). After additional TBST washes, the membrane was treated with a chemiluminiscent substrate (ab5801, Abcam), and protein bands were visualized using a Fusion Fx5 chemiluminescence detector (Vilber).

Statistical analysis

The data were processed using SPSS 21.0 software and R software (version 4.2.0). Differences between the two groups were evaluated using the independent samples t-test. A one-way ANOVA followed by the SNK-q test was used to compare multiple groups. A $p<0.05$ was statistically significant.

RESULTS

CircRNA_104293 was upregulated in CD patients

To identify differentially expressed circRNAs between the control and CD groups, genome-wide sequencing analysis was performed. A total of 415 up-regulated and 234 down-regulated circRNAs were identified (Fig. 1A). Among these, circRNA_103765 exhibited significantly elevated expression, highlighting its potential as a therapeutic

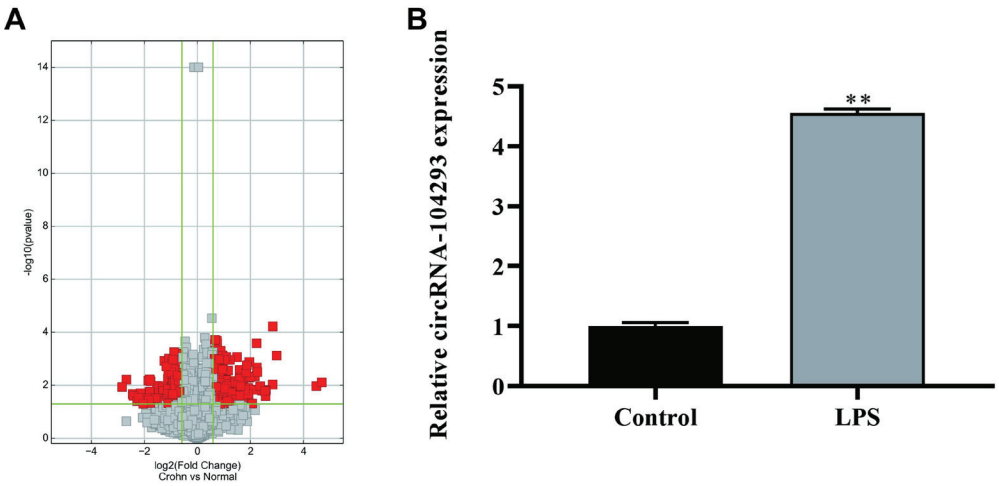

Fig. 1. Elevated circRNA_104293 expressions in Crohn's disease (CD) patients and lipopolysaccharide (LPS)-treated FHC cells. **A.** Volcano plot showing differentially expressed circRNAs in CD patients and controls based on $\log_2|\text{fold change}| > 1$ and an FDR-adjusted p-value < 0.05. **B.** Quantitative (q) PCR was used to compare the levels of circRNA_104293 in fetal human colon (FHC) cells under different treatments, using Student's t-test. (**p < 0.01).

target for CD. Further validation was conducted in LPS-treated FHC cells, where circRNA_104293 showed significantly higher expression than in controls (Fig. 1B). This finding underscores its potential role in inflammatory conditions.

Si-circRNA_104293 suppressed inflammation levels.

The transfection efficiency of si-circRNA_104293 and si-NC was evaluated using q-PCR. Results demonstrated that si-circRNA_104293 effectively reduced circRNA_104293 expression in LPS-treated FHC cells, confirming successful transfection (Fig. 2A). To assess the impact of si-circRNA_104293 on inflammation, ELISA was performed, revealing a significant decrease in pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8 (Fig. 2B). These findings suggest that si-circRNA_104293 effectively attenuates inflammatory responses. Additionally, flow cytometry indicated that si-circRNA_104293 markedly inhibited apoptosis in LPS-treated FHC cells (Fig. 2C), further supporting its role in mitigating cellular damage under inflammatory conditions.

CircRNA_104293 targeted miR-497-5p

TargetScan was used to identify the interaction between circRNA_104293 and miR-497-5p (Fig. 3A). A dual luciferase reporter assay confirmed this binding relationship, showing that the miR-497-5p mimic reduced luciferase activity in the circRNA_104293-WT group (Fig. 3B). This indicated that circRNA_104293 directly targets and negatively regulates miR-497-5p. Further validation using q-PCR revealed that miR-497-5p expression was lower in the LPS-treated group compared to controls (Fig. 3C). Additionally, silencing circRNA_104293 led to a notable increase in miR-497-5p levels (Fig. 3D), further supporting the regulatory role of circRNA_104293 in suppressing miR-497-5p expression. These findings collectively demonstrate that circRNA_104293 targets and downregulates miR-497-5p.

Si-circRNA_104293 reduced inflammation

LPS-treated cells were divided into three groups: si-NC + NC-inhibitor, si-circRNA_104293 + miR-497-5p-inhibitor, and si-circRNA_104293 + NC-inhibitor, to investigate the roles of circRNA_104293 and

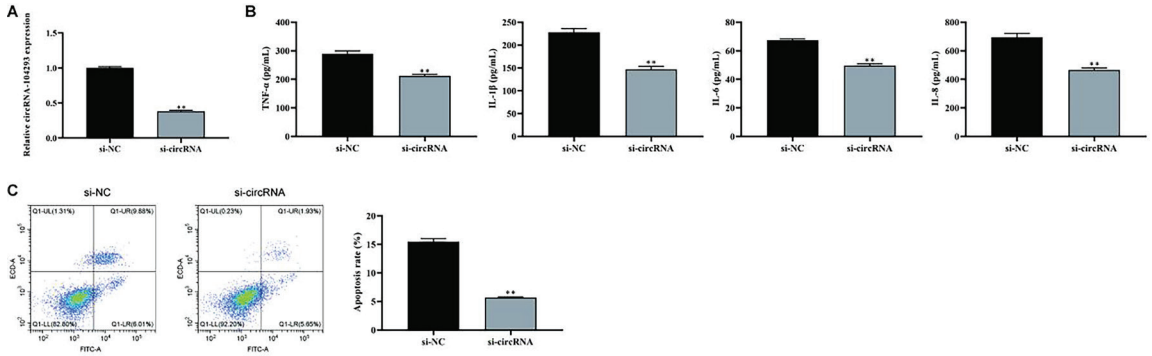

Fig. 2. Si-circRNA_104293 attenuates inflammation in LPS-treated FHC cells. **A.** Transfection efficiency of si-circRNA_104293 was confirmed by quantitative (q)PCR. **B.** ELISA measurements of TNF-α, IL-1β, IL-6, and IL-8 levels across different groups. **C.** Flow cytometry analysis of apoptosis in lipopolysaccharide (LPS)-treated fetal human colon (FHC) cells. (**p < 0.01 vs. si-NC group).

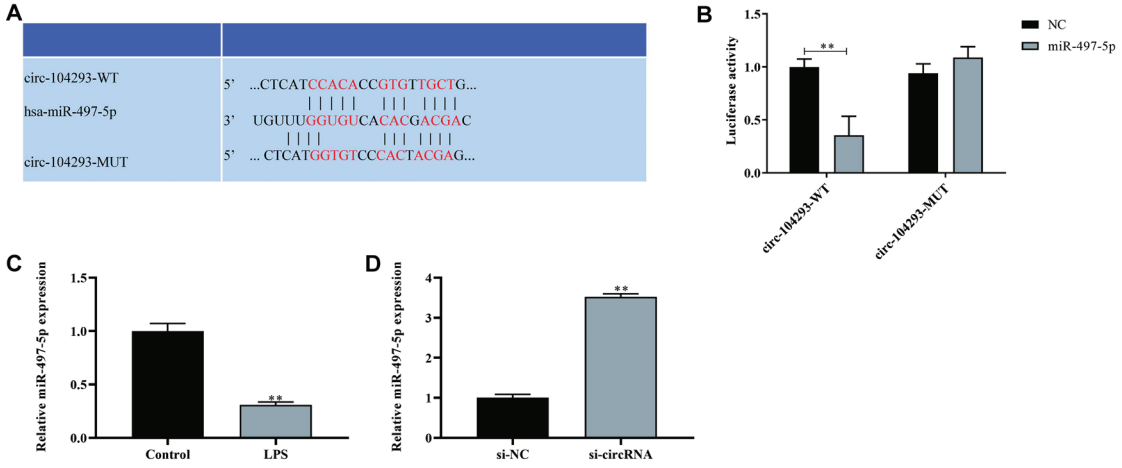

Fig. 3. circRNA_104293 targets miR-497-5p. **A.** Predicted binding site between circRNA_104293 and miR-497-5p. **B.** The dual luciferase assay confirmed the interaction between circRNA_104293 and miR-497-5p. **C.** qPCR analysis of miR-497-5p expression in control and lipopolysaccharide (LPS) groups. **D.** qPCR for miR-497-5p levels in cells treated with si-NC or si-circRNA_104293. (**p<0.01 vs. control or NC group).

miR-497-5p. The efficacy of the miR-497-5p inhibitor was confirmed via qPCR, showing a significant reduction in miR-497-5p levels (Fig. 4A). Immunofluorescence assays revealed that the miR-497-5p inhibitor partially reversed the effects of si-circRNA_104293, markedly decreasing dsDNA and ssDNA levels (Fig. 4B, C). Furthermore, si-circRNA_104293 downregulated inflammatory cytokines, while the miR-497-5p inhibitor partially counteracted this effect (Fig. 4D). Apoptosis assays demonstrated that si-circRNA_104293 reduced apoptosis rates. In contrast, the miR-497-5p inhibitor increased them (Fig. 4E). These findings indicate that circRNA_104293 mitigates inflammation and apoptosis by regulating miR-497-5p.

CircRNA_104293/miR-497-5p alleviated inflammation levels

Western blot analysis revealed that both miR-497-5p mimic and rapamycin downregulated the expression of p-mTOR and p-STAT3 compared to the NC-mimic group, indicating suppression of the mTOR/STAT3 pathway (Fig. 5A). Immunofluorescence as-

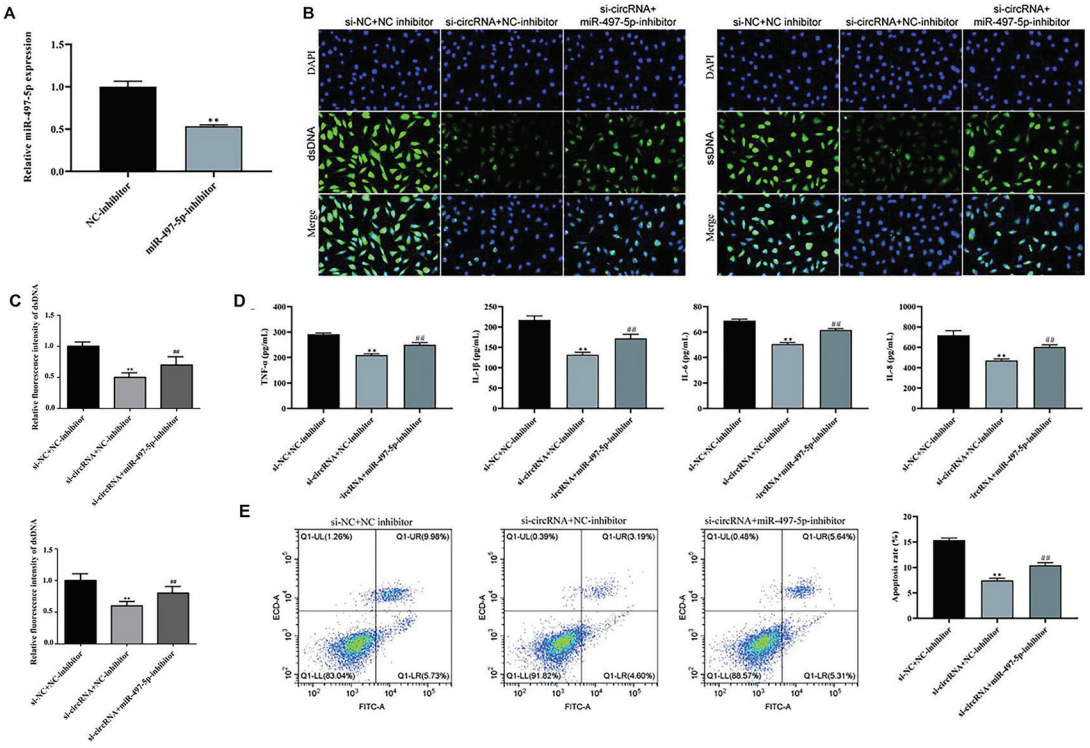

Fig. 4. Si-circRNA_104293 reduces inflammation via miR-497-5p. **A.** qPCR for the miR-497-5p levels in NC-inhibitor and miR-497-5p inhibitor. (**$p < 0.01$) **B-C.** Immunofluorescence analysis for the dsDNA and ssDNA levels in different treatment groups. (**p <0.01, si-circRNA+NC-inhibitor vs si-NC+NC-inhibitor, ## p <0.01, si-circRNA+miR-497-5p-inhibitor vs si-NC+NC-inhibitor). **D**. ELISA for the expression of inflammatory cytokines in different treatment groups. (**p<0.01, si-circRNA + NC-inhibitor vs si-NC + NC-inhibitor, ## p<0.01, si-circRNA + miR-497-5p-inhibitor vs si-circRNA + NC-inhibitor). **E.** Flow cytometry for the cell apoptosis in different groups. (**p<0.01, si-circRNA + NC-inhibitor vs si-NC + NC-inhibitor, ## p<0.01, si-circRNA + miR-497-5p-inhibitor vs si-circRNA + NC-inhibitor).

says showed reduced levels of ssDNA and dsDNA in the miR-497-5p-mimic and rapamycin groups (Fig. 5B, C). Additionally, ELISA results demonstrated decreased levels of inflammatory cytokines in these groups (Fig. 5D). Apoptosis analysis further confirmed that miR-497-5p mimic and rapamycin significantly lowered apoptosis rates compared to the NC-mimic group (Fig. 5E).

DISCUSSION

CircRNA is more stable than linear RNA, making it of great value for clinical diagnosis and prognostic evaluation of CD [17]. Recently, some studies have shown that circRNAs such as circRNA102610, circRNA103516, and circRNA102685 are involved in the inflammatory process of CD. However, the role of most circRNAs in CD pathogenesis remains elusive and therefore requires further exploration[18,20]. circRNA103765 has been identified as a key regulator of CD11 pathogenesis. In this study, significantly increased expression of circRNA104293 was observed in LPS-treated FHC cells and in CD patients. These results suggest a potential role for circRNA104293 in regulating inflammation in CD.

In IBD, TNF-α plays a critical role in the intestinal mucosa through autocrine and paracrine mechanisms [21]. LPS produced by the gut microbiota can directly activate macrophages in the intestinal lamina pro-

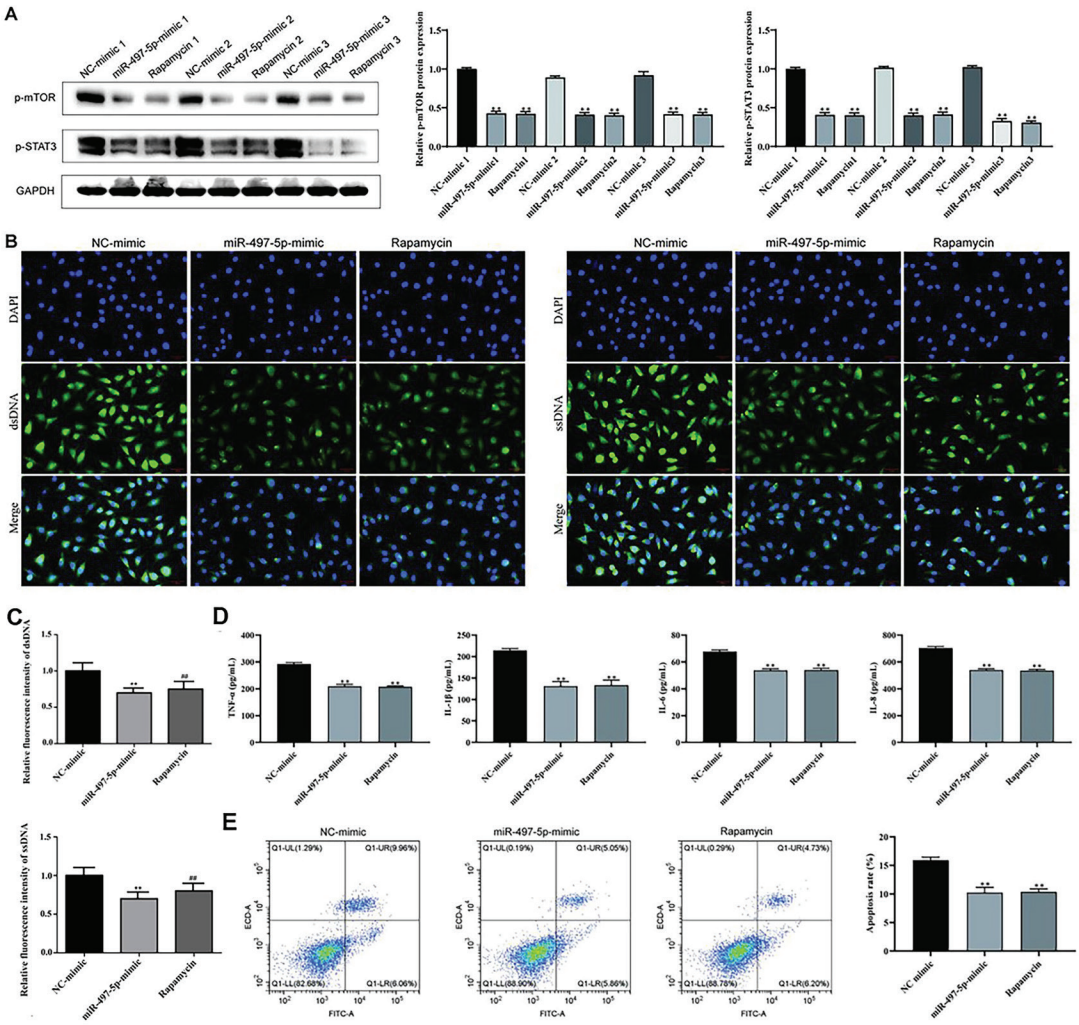

Fig. 5. circRNA_104293/miR-497-5p alleviates inflammation by inhibiting mTOR/STAT3 pathway. **A.** Western blot analysis of p-mTOR and p-STAT3 expression across different groups. **B-C.** Immunofluorescence analysis of dsDNA and ssDNA levels. (**p<0.01, miR-497-5p-mimic vs NC-mimic, ## p<0.01, rapamycin vs NC-mimic). **D.** ELISA measurements of TNF-α, IL-1β, IL-6, and IL-8 levels. **E.** Flow cytometry assessment of cell apoptosis in different groups. (**p<0.01 vs. NC-mimic group).

pria, promote their proliferation, and induce TNF-α release [22]. It has been shown that TNF-α and IL-1β expression are significantly abnormal in mucosal biopsies from pediatric CD patients [23]. In addition, the synergistic effect of TNF-α with interferon γ disrupts barrier function and alters the morphological structure of intestinal epithelial cells, resulting in increased permeability of the intestinal mucosa and vascular wall, ultimately triggering ulcer formation [24, 25]. In the pathogenesis of CD, inhibition of apoptosis leads to excessive accumulation of T cells, which in turn aggravates chronic mucosal inflammation, a process closely related to IL-6 signaling[26,27]. Notably, IL-8 expression is abnormal in the serum and intestinal tissues of immune CD patients, and its level not only reflects disease severity but also serves as an independent indicator of disease activity [28]. In this study, we found that si-circRNA104293 significantly reduced levels of inflammatory factors.

In this study, circRNA104293 was found to play a regulatory role by targeting and inhibiting miR-497-5p expression. It has been shown that miR-497-5p can upregulate IL-6 expression, thereby inhibiting muscle cell atrophy, and affects the inflammatory process by participating in the NF-κB pathway and regulating T cell function [29]. The results of this work, consistently, shows that si-circRNA104293 significantly decreased the rate of apoptosis, whereas the miR-497-5p inhibitor increased it. In addition, si-circRNA104293 effectively reduced the inflammatory response by regulating miR-497-5p, indicating that the circRNA104293/miR-497-5p axis plays a vital role in regulating inflammation in CD. It was also found that si-circRNA104293 significantly reduced dsDNA and ssDNA levels, while miR-497-5p inhibitors partially reversed this effect, further confirming the key role of circRNA104293 in inflammation and cell damage.

In this study, we found that p-mTOR and p-STAT3 expression levels were significantly decreased in miR-497-5p mimic and rapamycin groups compared with NC mimic groups [30]. Previous studies have shown that p-mTOR levels are increased in colonic tissue of CD patients. mTOR, a key molecule regulating cellular energy metabolism, mitochondrial fusion, and glucose and lipid metabolism, has inhibitors that can effectively suppress the expression of inflammatory factors [31]. In addition, STAT3 acts as a multifunctional transcription factor and can activate inflammatory responses in T cells through IL-6-mediated JAK/STAT3 signaling pathway[32]. Blocking IL-6 signaling not only reduces STAT3 activation but also induces monocyte apoptosis, thereby relieving colitis triggered by IL-10 deficiency [33, 34]. In CD patients, total STAT3 and phosphorylated STAT3 levels were significantly increased in inflammatory intestinal mucosa, and phosphorylated STAT3 levels were positively correlated with the degree of inflammatory injury [35]. The results of this study further confirmed that circRNA104293/miR-497-5p significantly reduced CD inflammatory levels by inhibiting the mTOR/STAT3 pathway.

In summary, our findings suggest that the circRNA_104293/miR-497-5p axis regulates LPS-induced inflammatory responses by modulating the mTOR/STAT3 signaling pathway. These results indicate its potential as a therapeutic target in inflammatory processes.

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Conflict of interest

The authors declare that they have no conflict of interest regarding this study.

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki, and was conducted with approval from the Ethics Committee of The First People's Hospital of Shuangliu District/West China (Airport) Hospital, Sichuan University. Written informed consent was obtained from all participants.

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Author contributions

Conceived and designed the study: XY, RC, GH; performed the literature search and data extraction: XY, CZ, YZ; analyzed the data: RC; drafted the manuscript: XY, RC

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