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CircRNA_104293 Inhibits the mTOR/STAT3 Pathway and Alleviates Inflammation in Crohn's Disease by Adsorption of miR-497-5p

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ABSTRACT

The aim was to investigate the molecular mechanism of circRNA_104293 in CD, which may provide new therapeutic option for Crohn's disease (CD). The expression profiles of circRNAs from CD patients and healthy control (HC) samples were analyzed by sequencing. FHC cell line was treated by si-circRNA, miR-497-5p inhibitor or 100 ng/mL Rapamycin. The Q-PCR assay was utilized to identify the expression of miR-497-5p and CircRNA 104293. By using an ELISA assay, inflammatory factors were discovered in the cell lysates of each group. Apoptosis rate was detected by flow cytometry. CircRNA 104293 and miR-497-5p binding was forecast using Target Scan. Immunofluorescence assay was performed to detect dsDNA and ssDNA levels. Western blot was shown to monitor p-mTOR and p-STAT3 levels. Total 415 up-regulated circRNAs and 234 down-regulated circRNAs were obtained. CircRNA_104293 expressed higher in inflammatory model. Si-circRNA 104293 reduced the levels of all inflammatory cytokines. Expression of miR-497-5p was targeted by and negatively controlled by CircRNA 104293. Si-circRNA 104293 dramatically reduced the amounts of dsDNA and ssDNA, but its function was only partially restored by miR-497-5p inhibitor. While miR-497 inhibitor enhanced apoptosis rate, si-circRNA 104293 lowered it. Rapamycin and miR-497-5p mimics blocked the mTOR/STAT3 pathway. MiR-497-5p reduced CD inflammation by blocking the mTOR/STAT3 pathway. To concled CircRNA 104293/miR-497-5p, which may be a potential target for CD treatment, reduced inflammation levels in CD by blocking the mTOR/STAT3 pathway.

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Conceived and designed the study: YX, CR, HG. Performed the literature search and data extraction: YX, ZC, ZY. Analyzed the data: CR. Drafted the manuscript: YX, CR.

Key words
Crohn's disease, circRNA_104293, miR-497-5p, Inflammation, mTOR/ STAT3 pathway

INTRODUCTION

Crohn's disease (CD) is an inflammatory disease of the intestinal tract, mainly characterized by recurrent abdominal pain, diarrhea and weight loss. China has the highest incidence of Crohn's disease in Asia, with an incidence rate of 3/100,000 (Rickards *et al*., 1994). The incidence of CD-related morbidity have increased recently all across the world (Vanhove *et al*., 2016). It is well known that in addition to the genetic factors, CD is caused by an over-reactive immune response against microorganisms or antigens (Lomer *et al*., 2002). The mechanistic target of rapamycin, mTOR and STAT signaling pathways, which are associated to the inflammatory response, are involved in the onset and progression of CD (Long *et al*., 2013). It has been suggested that the mTOR/STAT3 pathway is crucial for both inflammation and apoptosis. Additionally, mTOR/STAT3 signaling is abnormally elevated in CD patients colonic epithelium, and blocking the mTOR/ STAT3 pathway alleviates CD in mice models of the condition (Li *et al*., 2018). Specifically, mTOR signaling has been implicated in modulating the immune response and intestinal epithelial cell function. Dysregulation of mTOR signaling can lead to aberrant immune cell activation, cytokine production, and disruption of the intestinal barrier, all of which contribute to the inflammatory milieu characteristic of CD. Studies have shown that inhibitors of mTOR, such as rapamycin, can ameliorate inflammation in experimental models of inflammatory bowel disease, highlighting the pathway's potential as a therapeutic target in CD (Wang *et al*., 2022). On the other hand, STAT3 activation has also been observed in immune cells and intestinal epithelial cells, driven by pro-inflammatory cytokines such as IL-6 and IL-21. This activation contributes to the pathogenesis of CD by promoting the survival and proliferation of T cells, inducing the production of pro-inflammatory mediators, and impairing the barrier function of the intestinal

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epithelium. The persistent activation of STAT3 signaling in CD suggests its role in maintaining chronic inflammation and offers a rationale for targeting STAT3 as a therapeutic strategy (Lovato *et al*., 2003). Consequently, a possible therapeutic target for the treatment of CD is the mTOR/STAT3 pathway. But the precise etiology is unknown.

Many malignancies, autoimmune conditions, and neurological illnesses develop and advance as a result of circular RNA (circRNA) (Yang and Chen, 2019). CircRNAs are highly stable in the cytoplasm and have evolved to be conserved because of their distinctive closure structure (Li *et al*., 2020). CircRNAs function as ceRNAs to separate miRNAs from their target genes, according to earlier research (Zhang *et al*., 2020). For instance, Pan *et al*. (2018) discovered that miR-7 sponging by ciRS-7 may play a crucial role in neuronal activity. Additionally, circHIPK3 is regarded as a colorectal cancer (CRC) predictive biomarker (Zhang *et al*., 2019). Through sponging the miR-30/DLL4 axis, CircRNA 103765 is a unique and significant regulator of CD pathogenesis (Ye *et al*., 2021). However, more research is required because it is unknown how circRNA 103765 affects the development and progression of CD.

A number of inflammatory illnesses, including CD, have been linked to deregulation of microRNAs (miRNAs), it should be noted (Wu *et al*., 2011; Feng *et al*., 2009). For instance, miR-497 is essential for controlling inflammation brought on by lipopolysaccharide (LPS) in mouse models of acute lung injury (ALI) and in human bronchial epithelium damaged by cigarette smoke extract (CSE) (HBE) (Ke *et al*., 2022; Ito *et al*., 2014). MiR-497 levels were reduced in inflamed mucosa of colitis mice and LPS-treated RAW264.7 cells (Zhang *et al*., 2021). The relationship between circRNA_103765 and miR-497 in CD was also detected in this study.

In this study, we sequenced the expression profiles of circRNAs from CD patient and healthy control (HC) samples in order to look into the therapeutic targets and molecular causes of CD. CircRNAs that were up-regulated and down-regulated were screened for and found. We used quantitative polymerase chain reaction (qPCR) to examine the expression of circRNA 104293 in CD patients, and we also looked into the molecular mechanism of circRNA 104293 in CD. The study might offer CD patients a new therapy alternative.

MATERIALS AND METHODS

Patients and genome sequencing analysis

For the sequencing phase, we included samples from 2 CD patients and 2 controls. The validation phase expanded to 48 CD patients and 40 controls. All patients who were 18-49 years of age, were newly diagnosed for inflammatory bowel disease were included in this study patients with previous IBD diagnosis with treatment those with co-existing conditions causing inflammatory changes in the intestines, such as CMV infection or *C. difficile*, those with complications like intestinal obstruction or cancer, and those with other chronic diseases, e.g., diabetes, hypertension were exclude from the study. High-throughput sequencing was performed on pathological and healthy tissues from CD patients who underwent surgery in our institution between June 2021 and March 2022. All patients signed informed consent forms after our hospital's ethics committee gave its approval for the study. Kang Cheng Bio-tech (Shanghai, China) supplied RNA high-throughput sequencing. Sample labeling and array hybridization were performed according to the manufacturer's protocol (Arraystar Inc.). Briefly, total RNAs were digested with RNase R (Epicentre, Inc.) to remove linear RNAs and enrich circRNAs. Then, the enriched circRNAs were amplified and transcribed into fluorescent cRNA utilizing a random priming method (Arraystar Super RNA Labeling Kit; Arraystar). The labeled cRNAs were purified by RNeasy Mini Kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol Cy3/μg cRNA) were measured by Nano Drop ND-1000. 1 μg of each labeled cRNA was fragmented by adding 5 μl 10 × blocking agent and 1 μl of 25 × fragmentation buffer, then heated the mixture at 60 °C for 30 min, finally 25 μl 2 × hybridization buffer was added to dilute the labeled cRNA. 50 μl of hybridization solution was dispensed into the gasket slide and assembled to the circRNA expression microarray slide. The slides were incubated for 17 h at 65°C in an Agilent hybridization oven. The hybridized arrays were washed, fixed and scanned using the Agilent scanner G2505C (Agilent Technologies, California, USA). Agilent feature extraction software (version 11.0.1.1; Agilent Technologies, California, USA) was used to analyze the acquired array images. Quantile normalization of raw data and subsequent data processing were performed using the R software limma package.

Cells and treatment

From Shanghai Institute of Life Sciences, Chinese Academy of Sciences cell bank, normal human colonic epithelial cells (FHC cell line) were purchased. In RPMI1640 media with 10% fetal bovine serum and 1% penicillin/streptomycin, FHC cells were grown. The cells underwent a 2-3 days transit. To create an inflammatory model, 500 ng/mL LPS solution was utilized, and serum-free medium was added to the control group. After being exposed to LPS, FHC cells were split into two groups, si-circRNA and siNC. Following the kit's instructions,

cells were transfected with Lipofectamine™ 2000 and collected 48 h later for further study. Moreover, Lipofectamine™ 2000 reagent was used to transfect inhibitors of miR-497-5p and NC. The mTOR-targeting inhibitor (Rapamycin, A606203) was purchased from Bioengineering Co. Cells in the rapamycin group was incubated with 100 ng/mL rapamycin.

Q-PCR assay

The Q-PCR assay was used to identify the expression of miR-497-5p and CircRNA 104293. The cells were lysed using the TRIZOL reagent. Centrifuging was used to obtain total RNA. RNase pyrophosphate was used to solubilize the total RNA in water, and a spectrophotometer was used to measure the concentration and purity of the total RNA (Nano-Drop 3000, Thermo). Reverse transcription was used to create the cDNA in accordance with the instructions provided in the kit, and the cDNA was then used as the template for PCR in accordance with the SYBR II kit's instructions. Pre-denaturation at 95ºC for 30 sec, 95 ºC for 5 sec, 60 ºC for 30 sec for 39 cycles, and 0.5 ºC for 5 sec were some of the reaction conditions. circRNA 104293's relative expression was measured using the 2^{-Ct} method.

ELISA assay

The cells were given three PBS washes after transfection. Non-denatured protein lysate was repeatedly blown into the cells after being supplied with 0.5 ml each well. After 20 min in an ice bath, the samples were moved to a centrifuge tube, centrifuged at 12000 rpm for 15 min, and the supernatant was immediately frozen at -20 °C. By using an ELISA assay, inflammatory factors (TNF-α, IL-1β, IL-6, and IL-8) were found in the cell lysates of each group. The expression was strictly adhering to the kit's directions.

Flow cytometry assay

A 5×10^5/mL cell suspension of the LPS-treated cells was made, and it was injected into a 6-well plate. On the second day, the cells were transfected with si-circRNA 104293, a miR-497-5p inhibitor, and si-NC before being incubated for another 24 h. The cells underwent digestion and centrifugation before being rinsed three times in pre-chilled PBS. The cells were then treated with 5 μL of Annexin V-FITC/PI for 15 min while avoiding light. Flow cytometry was used to measure the apoptosis rate.

Dual-luciferase reporter gene assay

An online tool named TargetScan can forecast the locations of miRNA binding sites. The database was used to estimate the binding location for circRNA 104293 and miR-497-5p. The wild-type plasmid and mutant luciferase reporter vector of circRNA 104293 were built based on the binding location. In 24-well plates, cells were inoculated and cultivated for 24 h. MiR-497-5p mimic was cotransfected with the CircRNA 104293 wild-type and mutant plasmids, and three duplicate wells were created. After the cells had been transfected for 24 h, the medium was removed, and the cells were washed three times in PBS before being thoroughly lysed at room temperature using 100 μl of lysis solution. LARII reagent was applied to test the activity of the firefly luciferase, and Stop and Glo reagent was utilized to find the activity of the renal luciferase. The ratio of renal luciferase to firefly luciferase was used to measure activity.

Immunofluorescence assay

DsDNA and ssDNA levels were determined using an immunofluorescence test. When the cells had grown and fused to about 70%, they were inoculated on coverslips and treated with si-circRNA 104293, miR-497-5p-inhibitor, or left untreated. After three thorough rinses, the coverslips were fixed for 15 min with 37 g/L of formaldehyde. The samples were treated with 0.5% Triton X-100 for 10 min. and 100 ml/l calf serum albumin for 1 h. after being rinsed with PBS. The dsDNA and ssDNA antibodies were added, and they were then incubated at 4 ºC overnight. The samples were treated with PBS, washed with distilled water, sealed with glycerol, and then incubated with FITC-labeled immunofluorescent antibody at 37 ºC for 1 h.

Western blot assay

The entire proteins were extracted from each group of cells after 48 h of treatment using RIPA lysis buffer and PMSF protease inhibitor, and the protein concentration was calculated using the BCA method. The BCA technique was used to calculate the protein concentration. The samples were wet transferred using a 0.45 μm PVDF membrane, coated with 12% separation gel, and sealed for one hour at 37 ºC in a shaker with 5% skim milk powder. Primary and secondary antibodies for p-mTOR and p-STAT3 were incubated on the membrane, and the bands were then washed with TBST and treated with chemiluminescent solution. A Fusion Fx5 chemiluminescence detector (Vilber) was used for color development, and Image J was used to quantify the bands.

Statistical analysis

The statistical analysis of the three repeated experiments was processed, and the data were then represented as $\bar{x}\pm s$. The data were analyzed using SPSS 21.0 statistical software. One-way ANOVA and the SNK-q test, respectively, were used to identify differences between

two groups and differences between several groups. A statistically significant difference was shown by P < 0. 05.

RESULTS

CircRNA_104293 expressed higher in CD patients and LPS-treated FHC cells

In order to screen different expressed circRNAs, genome sequencing analysis was undertaken. Total 415 up-regulated circRNAs and 234 down-regulated circRNAs were analyzed and shown in Figure 1A. In addition, it was discovered that circRNA 103765 was more highly expressed, suggesting a novel potential target for the therapy of CD. Then, the level of circRNA_103765 was detected in LPS-treated FHC cell models and controls. CircRNA_104293 expressed higher in inflammatory model (Fig. 1B).

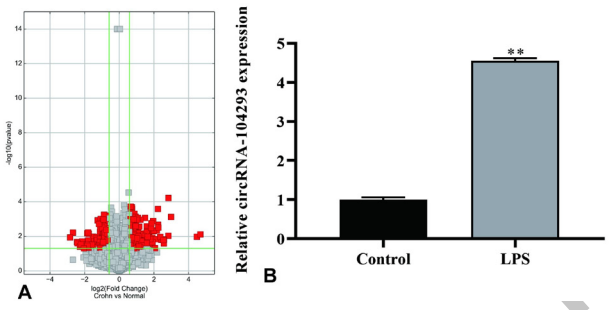

Fig. 1. CircRNA_104293 expressed higher in CD patients and LPS-treated FHC cells. (A) Volcano plot of screened circRNA. (B) The level of circRNA_104293 in LPS-treated FHC cell models and controls. vs control group, ** p < 0.01.

Si-circRNA_104293 suppressed inflammation levels in LPS-treated FHC cells

After si-circRNA_104293 and si-NC transfection, the transfection efficiency was detected by q-PCR assay. Si-circRNA_104293 significantly decreased circRNA_104293 expression in LPS-treated FHC cells, indicating that the transfection was successful (Fig. 2A). The effect of si-circRNA 104293 on inflammation levels in LPS-treated FHC cells was next examined using an ELISA technique. According to the findings in Figure 2B, si-circRNA 104293 reduced the levels of all inflammatory cytokines, including TNF-α, IL-1, IL-6, and IL-8. The results confirmed that si-circRNA_104293 suppresses inflammation levels in LPS-treated FHC cells. Moreover, si-circRNA_104293 significantly inhibited apoptosis of LPS-treated FHC cells (Fig. 2C).

CircRNA_104293 targeted miR-497-5p

TargetScan is an online software for predicting miRNA binding sites. The anticipated binding location for circRNA 104293 and miR-497-5p was based on the database and is depicted in Figure 3A. By using a dual luciferase reporter gene experiment, the binding interaction between circRNA 104293 and its target miR-497-5p was discovered. MiR-497-5p mimic significantly down-regulated circRNA_104293 expression in circRNA_104293-WT group, while no significant difference was shown in circRNA_104293-Mut groups (Fig. 3B). The outcomes demonstrated that miR-497-5p expression was targeted and negatively controlled by circRNA 104293. Then, the miR-497-5p expression qRT-PCR experiment was processed. MiR-497-5p in the LPS group was obviously lower than in the control group (Fig. 3C).

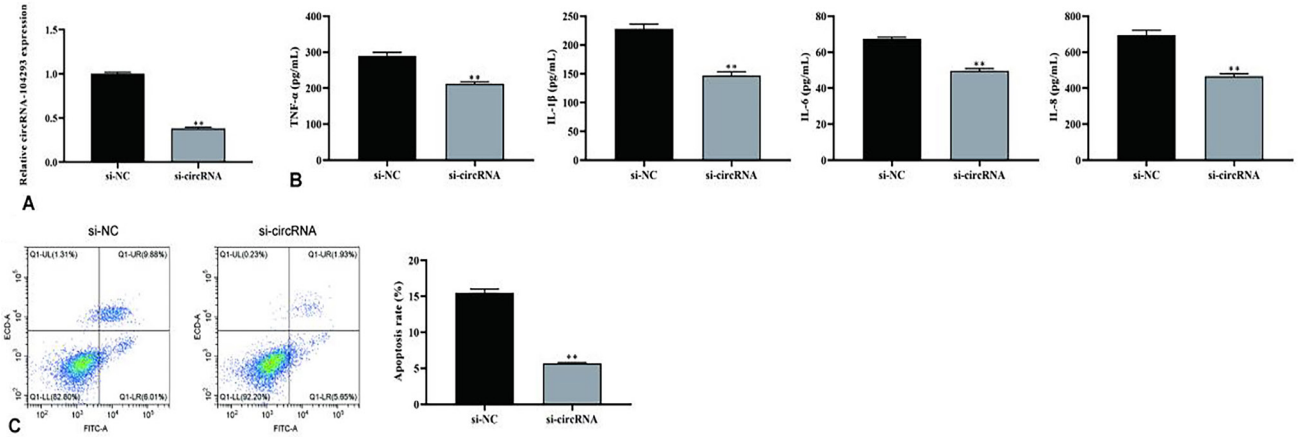

Fig. 2. Si-circRNA_104293 suppressed inflammation levels in LPS-treated FHC cells. (A) The transfection efficiency of si-circRNA_104293. (B) TNF-α, IL-1β, IL-6 and IL-8, were down-regulated by si-circRNA_104293. (C) C. si-circRNA_104293 significantly inhibited apoptosis of LPS-treated FHC cells. vs si-NC group, **p < 0.01.

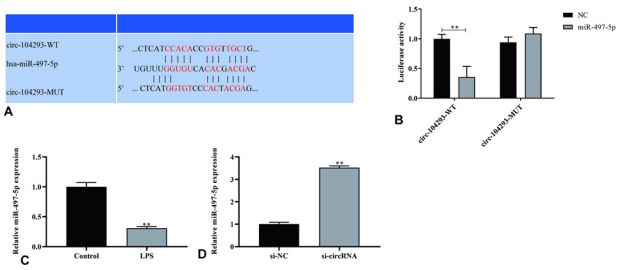

Fig. 3. CircRNA_104293 targeted miR-497-5p. (A) The binding site of circRNA_104293 and miR-497-5p. (B) The binding relationship of circRNA_104293 and its target miR-497-5p was detected by dual luciferase reporter gene assay. (C) LPS decreased miR-497-5p expression. (D) si-circRNA_104293 significantly increased miR-497-5p expression. vs control or NC group, **p < 0.01.

Additionally, a qRT-PCR test was used to identify the impact of circRNA 104293 on the expression of miR-497-5p. Interestingly, miR-497-5p expression was substantially higher under si-circRNA 104293 than under control (Fig. 3D). Based on these evidences, circRNA_104293 targeted and decreased miR-497-5p expression.

Si-circRNA_104293 reduced inflammation through miR-497-5p

LPS-treated cells were separated into 3 groups (si-NC+NC-inhibitor, si-circRNA 104293+miR-497-5p-inhibitor, and si-circRNA 104293+NC-inhibitor groups). These groups were used to detect the function of circRNA 104293 and miR-497-5p. By using qPCR test, the inhibitory effectiveness of the miR-497-5p-inhibitor on miR-497-5p was found. A miR-497-5p inhibitor clearly reduced the level of miR-497-5p, demonstrating the effectiveness of the inhibitory effect (Fig. 4A). Then, using an immunofluorescence assay, the amounts of dsDNA and ssDNA were found. Interestingly, miR-497-5p-inhibitor partially restored si-function circRNA 104293's while drastically lowering the amounts of dsDNA and ssDNA (Fig. 4B). TNF-α, IL-1, IL-6, and IL-8 were among the inflammatory cytokines that were down-regulated by si-circRNA 104293 while they were partially up-regulated by miR-497-5p-inhibitor (Fig. 4C). Additionally, miR-497 inhibitor enhanced apoptosis rate whereas si-circRNA 104293 decreased it (Fig. 4D). Thus, through miR-497-5p, si-circRNA 104293 decreased apoptosis and inflammation.

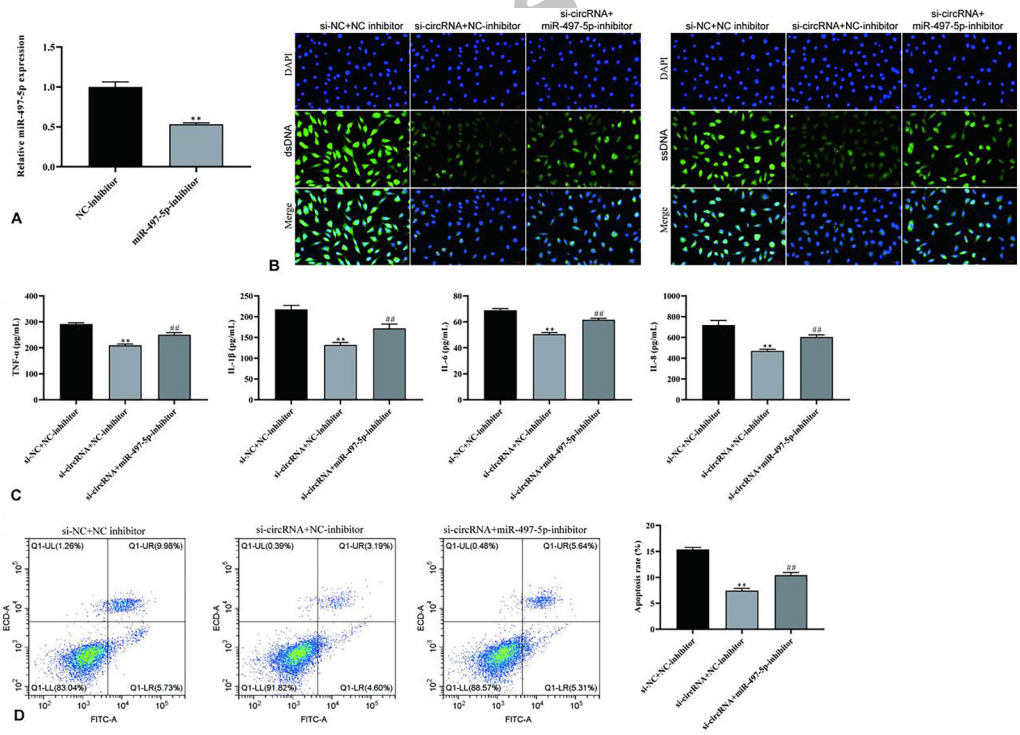

Fig. 4. Si-circRNA_104293 reduced inflammation through miR-497-5p. (A) The inhibitory efficiency of miR-497-5p-inhibitor on miR-497-5p. (B) si-circRNA_104293 significantly down-regulated the levels of dsDNA and ssDNA, while miR-497-5p-inhibitor partly rescued the function of si-circRNA_104293. (C) TNF-α, IL-1β, IL-6 and IL-8 were down-regulated by si-circRNA_104293, while they were partly up-regulated by miR-497-5p-inhibitor. (D) si-circRNA_104293 decreased apoptosis rate, while miR-497 inhibitor increased it. vs si-NC+NC inhibitor group, **p < 0.01, vs si-circRNA+NC inhibitor group, ##p < 0.01.

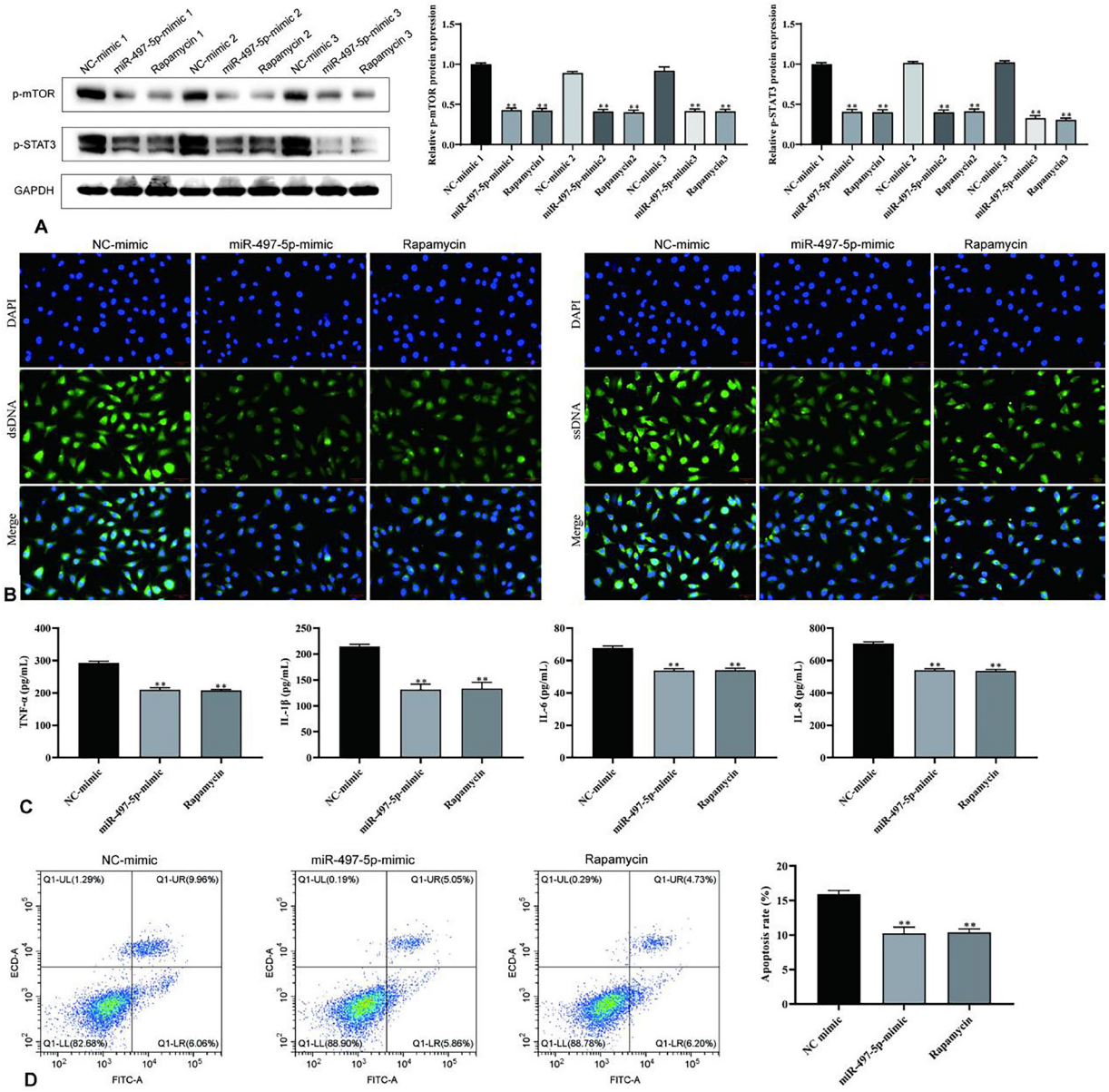

Fig. 5. CircRNA_104293/ miR-497-5p alleviated inflammation lev els in CD by inhibiting the mTOR/STAT3 pathway. (A) MiR-497-5p mimic and Rapamycin significantly decreased the expressions of p-mTOR and p-STAT3 than NC-mimic group. (B) miR-497-5p mimic and Rapamycin also decreased dsDNA and ssDNA levels. (C) MiR-497-5p mimic and Rapamycin down-regulated TNF-α, IL-1ß, IL-6 and IL-8 expressions. (D) Apoptosis rate was significantly lower in miR-497-5p-mimic and Rapamycin group. vs NC-mimic group, **p < 0.01

CircRNA_104293/miR-497-5p alleviated inflammation levels in CD by inhibiting the mTOR/STAT3 pathway

The downstream of circRNA_104293/miR-497-5p was researched by western blot, immunofluorescence assay, ELISA assay and apoptosis analysis. The experiments were divided into 3 groups, including NC-mimic, miR-497-5p-mimic and Rapamycin groups. Western blot identified proteins connected to the mTOR/STAT3 pathway. Rapamycin and MiR-497-5p mimics dramatically reduced the expression of p-mTOR and p-STAT3 compared to the NC-mimic group (Fig. 5A). The findings indicated that rapamycin and miR-497-5p mimics suppressed the mTOR/STAT3 pathway. Rapamycin and a miR-497-5p mimic also reduced the amounts of ssDNA

and dsDNA (Fig. 5B). Additionally, they reduced the expression of TNF-α, IL-1ß, IL-6, and IL-8 (Fig. 5C). In comparison to the NC-mimic group, the apoptosis rate was considerably reduced in the miR-497-5p-mimic and Rapamycin groups (Fig. 5D). These data suggest that miR-497-5p reduced CD inflammation by blocking the mTOR/STAT3 pathway.

DISCUSSION

CircRNA is more stable than linear RNA, indicating that circRNA is of great significance in the clinical diagnosis and prognosis evaluation of CD (Ma *et al*., 2021). In recent years, several circRNAs, such as circRNA_102610, circRNA_103516 and circRNA_102685, have been confirmed to participate in inflammatory processes in CD (Yin *et al*., 2019; Lu *et al*., 2021; Qiao *et al*., 2019). More circRNAs are desperately needed because it is still unclear how most of them affect the onset and course of CD. A new and crucial regulator of CD pathogenesis is circRNA 103765 (Ye *et al*., 2021). In this work, LPS-treated FHC cells and CD patients both had greater expression levels of circRNA 104293. In FHC cells exposed with LPS, Si-circRNA 104293 reduced the amount of inflammation. By using si-circRNA 104293, the inflammatory factors, such as TNF-α, IL-1, IL-6, and IL-8, were downregulated.

In inflammatory bowel illness, TNF-α plays a significant function in the intestinal mucosa in both autocrine and paracrine mechanisms (Mosnier *et al*., 2009). The LPS produced by intestinal flora directly activated macrophages located in the lamina propria of intestinal mucosa to promote their proliferation and release TNF-α (Sun *et al*., 2020). In pediatric CD mucosal biopsies, TNF-α and IL-1β abnormally expressed (None, 2001). In addition, the combination of TNF-α and interferon γ changed the barrier function and morphological structure of intestinal epithelial cells (Van Winkle *et al*., 2020). Furthermore, the permeability of intestinal mucosa and blood vessel wall was increased, and the integrity of intestinal mucosa was destroyed eventually, and ulcers were formed (Coope *et al*., 2017). In the pathogenesis of CD, resistance of apoptosis leads to excessive accumulation of T cells and aggravation of chronic mucosal inflammation, which is related to IL-6 signal transduction (Nakano *et al*., 2011). In both ulcerative colitis and CD, the local role of CD4+ T cells in inflammation is dependent on anti-IL-6 signaling. Interestingly, IL-8 expression is abnormal in serum or intestinal tissues of patients with immune CD (Jones *et al*., 1993). Its level not only indicates the severity of the disease but also serves as a separate measure of the activity of CD. In this work, si-circRNA 104293 was found to reduce the levels of TNF-α, IL-1, IL-6, and IL-8, demonstrating that si-circRNA 104293 reduces inflammation in LPS-treated FHC cells.

It was noteworthy that in this study, circRNA 104293 targeted and suppressed miR-497-5p expression. Based on the findings of earlier research, miR-497-5p increased the expression of IL-6 and further prevented muscle cell atrophy (Freire *et al*., 2021). Besides, miR-497-5p also participated in inflammatory progress by involving in NF-κB pathway and regulating T cells (Wang *et al*., 2020). Similarly, results were also obtained in this study. Si-circRNA_104293 decreased apoptosis rate, while miR-497 inhibitor increased it. It was important to note that in this investigation, si-circRNA 104293 decreased inflammation via acting on miR-497-5p. In CD, the circRNA 104293/miR-497-5p axis was therefore crucial for inflammation. Additionally, si-circRNA 104293 considerably reduced the amounts of dsDNA and ssDNA in this study, while miR-497-5p-inhibitor partially restored si- circRNA 104293's functionality. While the general concept of circRNAs acting as miRNA sponges is well-established, our study is among the first to delineate this particular interaction and its biological implications in CD. The identification of circRNA_104293's role in sponging miR-497-5p represents a novel insight into the regulatory mechanisms governing inflammation in CD.

Furthermore, p-mTOR and p-STAT3 expression levels were considerably lower in the miR-497-5p mimic and rapamycin groups compared to the NC-mimetic group. In previous studies, p-mTOR levels were increased in colon tissue of CD patients (Brandt *et al*., 2017). MTOR is an important molecule that regulates cellular energy metabolism in the organism, regulating the process of mitochondrial fusion and the level of glycolipid metabolism (Luan *et al*., 2018). The use of mTOR inhibitors can inhibit the expression of inflammatory factors (Zhang *et al*., 2016). Besides, STAT3 is a multifunctional transcription factor. In previous studies, IL-6 activated STAT3 via the JAK/STAT3 signal transduction pathway, thereby activating the inflammatory pathway of T cells (Lieblein *et al*., 2007). Blocking IL-6 signal transduction reduced the activation of STAT3, induced the apoptosis of monocytes, and alleviated colitis caused by IL-10 deficiency (Akanda *et al*., 2018). In CD patients, the level of total STAT3 and phosphorylated STAT3 was consistently high in the inflammatory intestinal mucosa, and the elevated level of phosphorylated STAT3 was positively correlated with the degree of inflammatory damage in these tissues (Chao and Kuemmerle, 2018). Similarly, results were also obtained in this study. By blocking the mTOR/STAT3 pathway, CircRNA 104293/miR-497-5p reduced inflammatory levels in CD. The elucidation of a new regulatory axis involving circRNA_104293, miR-497-5p, and the mTOR/

STAT3 pathway opens up new avenues for therapeutic intervention in CD. By targeting this newly identified pathway, it may be possible to develop more effective treatments that can modulate the inflammatory response in CD patients more precisely.

Above all, circRNA 104293/miR-497-5p, a possible therapeutic target for CD, reduced inflammation levels in CD by blocking the mTOR/STAT3 pathway. The specific interaction between circRNA_104293 and miR-497-5p, and its role in regulating key signaling pathways involved in inflammation, offers new insights that could contribute to the development of novel diagnostic tools and therapeutic strategies.

DECLARATIONS

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IRB approval

This study has has been approved by the Medical Ethics Committee of the First People's Hospital of Shuangliu District, Chengdu (No:2021-5-05).

Ethics approval and consent to participate

This study was conducted in accordance with the declaration of Helsinki. This study 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.

Statement of conflict of interest

The authors have declared no conflict of interest.

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