LncRNA-CBR3-AS1 promotes and enhances the malignancy of ulcerative colitis via targeting miRNA-145-5p/FN1

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ABSTRACT

Growing evidence suggested that long non-coding RNA (lncRNA) played a crucial role in the progression of ulcerative colitis (UC). Therefore, the purpose of this study is to understand how the lncRNA CBR3-AS1, which has been found to be up-regulated in UC, contributes to the bio-progression of the disease. To determine the concentration and relationship of the lncRNA CBR3-AS1, miRNA-145-5p, and FN1 in the LPS-induced Caco-2 model cells, qRTP-PCR was employed in this study. Starbase was used to predict the target sites of the lncRNA CBR3-AS1 and the miRNA-145-5p, and Targetsan was used to predict the probable linking points of the FN1 and the miRNA-145-5p, which was confirmed by a twofold luciferase reporter test. The vitality of Caco-2 cells was determined using the CCK-8 and FCM tests. Using the ELISA kit, TNF, IFN, IL-6, and IL-17 were identified. The results of the experiment show that in Caco-2 cells treated with 10 ng/mL LPS, LncRNA CBR3-AS1 was up-regulated. Additionally, Caco-2 cells' LPS-induced apoptosis and inflammatory response were inhibited by LncRNA CBR3-AS1 inhibition. Dual-luciferase reporter experiments demonstrated that miRNA-145-5p and LncRNA CBR3-AS1 might connect. Moreover, miRNA-145-5p, which was shown to be poorly expressed in UC, was found to suppress inflammatory and apoptotic responses in Caco-2 cells activated by LPS. It's significant that FN1 was confirmed to be miRNA-145-5p's downstream target. Sh-CBR3-AS1’s inhibitory effects were reversed by miRNA-145-5p knockdown, and the effects of the miRNA-145-5p inhibitor were reversed by sh-FN1. In conclusion, LncRNA CBR3-AS1 may offer a unique method for treating UC by suppressing the function of miRNA-145-5p, which is implicated in the development of UC.

Introduction

UC (ulcerative colitis) is a chronic, incapacitating inflammatory bowel disease that often lasts a lifetime (1). Abdominal discomfort and diarrhea with blood and mucus are the prototypical signs of UC. Despite efforts to address it, it is still unknown how UC develops (2,3). Despite advancements in UC diagnosis and therapy, the prognosis is still dismal. Biomarker-based diagnosis and therapy have received a lot of attention lately (4). Finding the pathogenic mechanism and useful biomarkers for UC is therefore urgently needed.

Long non-coding RNA (lncRNA) is a type of essential epigenetics-regulating gene that controls DNA methylation and genomic imprinting. LncRNAs, a kind of RNA molecule longer than 200 nt but lacking the capacity to translate, have been shown to regulate tumor bio-functions such as chemotherapeutic resistance, EMT, proliferation, and more (5-8). Certain lncRNAs have been linked in several studies to the etiology of ovarian cancer, such as LncRNA PTAR, which accelerated EMT in ovarian cancer via miR-101-3p and ZEB1 (9). It has been suggested that LncRNA-BCRT1 drives the development of breast cancer (10). LncRNA CASCI1 was up-regulated in bladder cancer (11). A brand-new lncRNA called CBR3-AS1 has been found to promote breast cancer cell metastasis (12).

Furthermore, through Wnt/beta-catenin signaling, LncRNA CBR3-AS1 enhanced NSCLC (13). According to LncRNA CBR3-AS1 involvement in osteosarcoma (14). Although LncRNA CBR3-AS1 has been linked to several types of human cancer, its exact method of control of UC is unknown, and this is why we are interested in understanding it.

MicroRNA has a length of around 18–20 bp and is a conserved microRNA (15). Certain miRNAs have been identified to have a role in the development of certain illnesses (16, 17). Recent research on miRNAs in UC has shown that some miRNAs, such as miR-31 and miR-223-3p, are crucial for the development of UC. Also, miRNA-145-5p played a role in glioma (20). However, it is yet unclear how miRNA-145-5p and associated pathways affect UC.

Extracellular matrix (ECM) protein with many domains is called fibronectin (FN1). The protein works by binding various ECM elements, including collagen, growth factors, and cell surface integrins, via its many domains (21). According to prior studies, FN1 is connected to the biological processes of several human illnesses, including head and neck squamous cell carcinoma (22), gastric adenocarcinoma (23), and others. The link between FN1 and UC has not, however, been found.

Therefore, it is beneficial to continue researching the
Materials and Methods

Cell line culture
The ATCC provided the 293T cells and Caco-2 human colorectal cancer cells used in the Dual-luciferase test. The cells were grown in Ham's F-12k medium from Vivacell in China along with 1% penicillin and streptomycin and 20% fetal bovine serum in a 5% CO\textsubscript{2} environment at 37\degree C.

LPS (L118716, Aladdin) was applied to Caco-2 to create an in vitro UC model. In a nutshell, Caco-2 cells were exposed to 10 ng/mL LPS for 24 hours before being collected for the tests that followed.

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Bioinformatics information collected
Starbase predicted the miRNA-145-5p binding sites on the lncRNA CBR3-AS1, while TargetScan 7.0 suggested FN1 fragments with miRNA-145-5p binding sites.

Relative Dual-Luciferase reporter gene assay
To increase luciferase activity, the 3'-UTR of the lncRNA CBR3-AS1 or the mutated lncRNA CBR3-AS1 3'-UTR sequence, as well as FN1, were synthesized into a pGL3-U6-purumycin vector (Tisingke, China). After that, the 293T cells were co-transfected using jetPRIME following the supplier's instructions (Polyplus, France) with WT pGL3-lncRNA CBR3-AS1 (or FN1)-3'UTR or mutative pGL3-lncRNA CBR3-AS1 (or FN1)-3'UTR with NC mimic and mimic of miRNA-145-5p. 24 hours after infection, the activity was measured using a reporter system (Promega, USA). The outcomes have to do with Renilla luciferase.

Cell transfection
For control of expression of miRNA-145-5p, an inhibitor of miRNA-145-5p and NC inhibitor (miRNA-145-5p inhibitor: 5'-GGGCUUCCUCGUAGCUAGA-3' and NC inhibitor: 5'-CAAUACACUUUGUGAGAAC-3'), the mimic of miRNA-145-5p (5'-GUCCAGTTTTCCGCTCAT-3' (F) and 5'-GAGGATCAGTATTATCTA-3' (R); β-actin: 5'-CTGTCGCCAAGCTGGGAGTGC-3' (F) and 5'-GAGACCGCTGGTCTCTTCTCATC-3' (R); miRNA-145-5p: 5'-GUCCAGTTTTCCGCTCAT-3' (F) and 5'-ACCTTCTCGGC-GCCCTCCGA-3' (R)).

Enzyme-linked immunosorbent analysis
The levels of the inflammatory cytokines TNF-α, IFN-γ, IL-6, as well as IL-17 were measured in the supernatant. R&D (Bio-techne, USA) provided the ELISA kit.

Statistical analysis
The results from the triple experiments are shown as the average ± standard deviation (SD). Tukey's multiple-comparison test was used for multi-group data, whereas the student t-test is used for two groups. Statistics is considered significant if P< 0.05.

Results
CBR3-AS1 is down-regulated in UC
The study investigated CBR3-AS1's impact on UC because of its important role in a variety of disorders. We used RT-qPCR to examine the amount of CBR3-AS1 expression in UC. Results showed that CBR3-AS1 was highly expressed in Caco-2 cells treated with 10 ng/ml LPS (Figure 1A). In contrast to the sh-NC group, CBR3-AS1 was expressed at a lower level in Caco-2 cells trans-
sh-CBR3-AS1 transfection of Caco-2 cells increased the amount of miRNA-145-5p (Figure 2C). Therefore, these findings suggest that CBR3-AS1 controls miRNA-145-5p in Caco-2 cells in an opposing manner.

miRNA-145-5p is down-regulated in UC

By using RT-qPCR to determine the amount of miRNA-145-5p, it was shown that LPS-treated Caco-2 cells had lower levels of miRNA-142-5p (Figure 3A). MiRNA-145-5p mimic transfected into Caco-2 cells led to greater expression of miRNA-145-5p compared to the NC mimic group (Figure 3B). CCK-8 was used to measure viability, and the findings showed that LPS greatly inhibited cell growth, whereas transfection of a miRNA-145-5p mimic could partially restore cell growth (Figure 3C). The Tunel experiment, in the meantime, demonstrated that up-regulated miRNA-145-5p unmistakably decreased the cell apoptosis ratio that was amplified by LPS (Figure 3D). TNF-α, IFN-γ, IL-6, and IL-17 levels were all significantly elevated by LPS and decreased by the transfected miRNA-145-5p mimic (Figure 3E).

FN1 is downstream of miRNA-145-5p

The possible miRNA-145-5p bind sites in FN1 were discovered when we utilized Targetscan 7.0 to query the

Figure 1. The amplification of LncRNA CBR3-AS1 in UC. (A) LncRNA CBR3-AS1 levels in control and LPS-treated Caco-2 cells. (B) The efficacy rate of transfecting sh-LncRNA with CBR3-AS1. (C) CCK-8 was employed to calculate cell viability. (D) Tunel was applied to determine the Caco-2 cells’ apoptosis ratio. (E) An ELISA kit was implemented to quantify the cell inflammatory cytokines. Data are presented as average ± SD of triple experiments.

Figure 2. The miRNA-145-5p target of lncRNA CBR3-AS1. (A) The miRNA-145-5p combination locations on CBR3-AS1 WT and Mut. (B) The luciferase reporter activity of co-transfected CBR3-AS1 WT and Mut with miRNA-145-5p. (C) RT-qPCR was utilized to measure the expression level of miRNA-145-5p. Data are presented as average ± SD of triple experiments.

Figure 3. MiRNA-145-5p was disrupted in UC. (A) The expression of MiRNA-145-5p in control and LPS-treated Caco-2 cells. (B) MiRNA-145-5p’s effectiveness mirrors transfection. (C) CCK-8 was implemented to calculate cell viability. (D) Tunel was applied to determine the Caco-2 cells’ apoptosis ratio. (E) TNF-α, IFN-γ, IL-6, as well as IL-17, were assessed using an ELISA kit. Data are presented as average ± SD of triple experiments.

Figure 4. FN1 is miRNA-145-5p downstream mRNA. (A) Targetscan 7.0 predicted the binding sites between miRNA-145-5p and FN1. (B) The correlation between miRNA-145-5p and FN1 was confirmed using the dual relative luciferase assay. (C) FN1 expression levels were measured by RT-qPCR. Data are presented as average ± SD of triple experiments.
downstream mRNA of the miRNA (Figure 4A). To address this issue, we carried out a dual relative luciferase approach. We showed that miRNA-145-5p overexpression decreased the luciferase output of the FN1-WT reporter gene (Figure 4B). In Caco-2 cells transfected with miRNA-145-5p mimic, the FN1 expression level was affected, according to RT-qPCR results (Figure 4C). As a consequence, our findings suggest that miRNA-145-5p controls the FN1 in Caco-2 cells in an opposing manner.

**miRNA-145-5p/FN1 axis participated in the impact of CBR3-AS1 silenced on UC cells**

We co-cultured Caco-2 with sh-CBR3-AS1, sh-CBR3-AS1 with miRNA-145-5p inhibitor, or combination with sh-FN1 to ensure the connection of miRNA-145-5p, FN1, and CBR3-AS1 in UC. In Caco-2 cells treated with LPS, FN1 was highly expressed (Figure 5A). By transfecting sh-FN1 and miRNA-145-5p inhibitors, respectively, FN1 and miRNA-145-5p were reduced (Figure 5B). The viability of LPS-treated Caco-2 cells that were transfected with sh-CBR3-AS1+miRNA-145-5p inhibitor+sh-FN1 was higher than that treated with sh-CBR3-AS1+miRNA-145-5p inhibitor (Figure 5C). However, inhibition of CBR3-AS1 restored the viability of LPS-stimulated Caco-2 cells, whereas co-transfection of miRNA-145-5p inhibitor was reversed completely. Apoptosis was inhibited by the transfection of sh-CBR3-AS1 into LPS-treated Caco-2 cells, whereas the co-transfection of the miRNA-145-5p inhibitor increased the percentage of apoptotic cells. The co-transfection of sh-FN1 reversed the above finding (Figure 5D). Tunel demonstrated that LPS enhanced cell apoptosis rate. TNF-α, IFN-γ, IL-6, as well as IL-17 levels, were all noticeably increased by LPS and decreased by the sh-CBR3-AS1 transfection. The above-mentioned effects were subsequently reversed by down-regulating FN1 (Figure 5E).

**Discussion**

The intestinal mucosa is affected by inflammation and ulcerative alterations in UC, a chronic non-specific IBD. The mucosa, colonic submucosa, and rectum have all experienced the majority of the stress from UC. Uncertainty surrounds the pathophysiology of UC, though. According to some arguments, one key cause of UC is an immune system malfunction. Immunosuppressants, aminosalicylates, and corticosteroids are often used in clinical therapy to treating ulcerative colitis.

In this study, we outlined the molecular mechanism by which sh-lncRNA CBR3-AS1 alleviated UC that was induced by LPS. This analysis demonstrated that lncRNA CBR3-AS1 contributed to the development of UC via miRNA-145-5p, indicating that lncRNA CBR3-AS1 acted as a new UC biomarker.

More proof was found to support the idea that lncRNAs were important in UC. Different lncRNAs have been classified as biomarkers in UC. LncRNA MALAT1 and ANRIL both take part in the UC process concurrently (27). Yang W discovered that the PI3K pathway was used by lncRNA H19 to control UC (28). The lncRNA NORAD was also up-regulated in UC and used NF-κB to induce inflammation (29). However, just a little amount of study has been done to analyze the function of the lncRNA CBR3-AS1 in UC. Our research showed that the lncRNA CBR3-AS1 was promoted in UC. These discoveries improved our understanding of the role of the lncRNA CBR3-AS1 in the regulation of UC, which may help us find the pathogenic mechanism of UC more effectively.

Numerous studies have shown how miRNAs and circRNAs controlled the development of illnesses (30, 31). Forerunners discovered many miRNAs to be implicated in UC (32). Pierdomenico M discovered that miR-320 was connected to UC (33). By triggering an immunological response, Singh U. et al. demonstrated that miR-155 protected UC (34). On the other hand, miR-133a was shown to suppress UC (35). Since they have verified link sites, experimental evidence here established miRNA-145-5p as the lncRNA CBR3-AS1’s downstream gene. We explained that the level of lncRNA CBR3-AS1 in UC was contrast-controlled with the level of miRNA-145-5p, which was down-regulated.

FN1 has previously been linked to human disorders such as renal fibrosis (38), spondylometaphyseal dysplasia (37), and oral squamous cell carcinoma (36). Additionally, FN1 was shown to be strongly expressed in UC and to be regulated by miRNA-145-5p in this study.

In conclusion, our research confirms that miRNA-145-5p/FN1 is modulated by lncRNA CBR3-AS1 to aid in the UC process. Our findings show that the lncRNA CBR3-AS1/miRNA-145-5p/FN1 offers a fresh approach to treating ulcerative colitis.

**Declaration**

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

**Availability of data and material**
All data generated or analyzed during this study are included in this published article.
Author Contributions Statement
All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Jingmei Cao, Qing Zhao, and Qing Jia. The first draft of the manuscript was written by Jingmei Cao and Yiming Li, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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