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Original Paper

The CircRNA-ACAP2/Hsa-miR-21-5p/ Tiam1 Regulatory Feedback Circuit Affects the Proliferation, Migration, and Invasion of Colon Cancer SW480 Cells

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Key Words
CircRNA-ACAP2 • Hsa-miR-21-5p • Tiam1 • Colon cancer • SW480 cells • Invasion • Proliferation

Abstract
Background/Aims: Circular RNAs (circRNAs), a type of RNA that is widely expressed in human cells, have essential roles in the development and progression of cancer. CircRNAs contain microRNA (miRNA) binding sites and can function as miRNA sponges to regulate gene expression by removing the inhibitory effect of an miRNA on its target gene. ***Methods:*** We used the bioinformatics software TargetScan and miRanda to predict circRNA-miRNA and miRNAi-Mrna interactions. Rate of inhibiting of proliferation was measured using a WST-8 cell proliferation assay. Clone formation ability was assessed with a clone formation inhibition test. Cell invasion and migration capacity was evaluated by performing a Transwell assay. Relative gene expression was assessed using quantitative real-time polymerase chain reaction and relative protein expression levels were determined with western blotting. circRNA and miRNA interaction was confirmed by dual-luciferase reporter and RNA-pull down assays. ***Results:*** In the present study, the miRNA hsa-miR-21-5p was a target of circRNA-ACAP2, and T lymphoma invasion and metastasis protein 1 (Tiam1) was identified as a target gene of hsa-miR-21-5p. CircRNA-ACAP2 and Tiam1 were shown to be highly expressed in colon cancer tissue and colon cancer SW480 cells, but miR-21-5p was expressed at a low level. SW480 cell proliferation was suppressed when the expression of circRNA-ACAP2 and Tiam1 was decreased and the expression of miR-21-5p was increased *in vivo* and *in vitro*. SW480 cell migration and invasion were also inhibited under the same circumstance. The circRNA-ACAP2 interaction regulated the expression of miR-21-5p, and miR-21-5p regulated the expression

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of Tiam1. Down-regulation of circRNA-ACAP2 promoted miR-21-5p expression, which further suppressed the transcription and translation of Tiam1. ***Conclusion:*** The present study shows that the circRNA-ACAP2/hsa-miR-21-5p/Tiam1 regulatory feedback circuit could affect the proliferation, migration, and invasion of colon cancer SW480 cells. This was probably due to the fact that circRNA-ACAP2 could act as a miRNA sponge to regulate Tiam1 expression by removing the inhibitory effect of miR-21-5p on Tiam1 expression. The results from this study have revealed new insights into the pathogenicity of colon cancer and may provide novel therapeutic targets for the treatment of colon cancer.

Introduction

In China, colon cancer is a malignant tumor with a high incidence that is considered to be related to environmental and genetic factors. Currently, research on the pathogenicity of colon cancer and the underlying molecular mechanism is still in its infancy [1, 2]. The conventional treatment for colon cancer in China consists of surgery, adjuvant therapy, and molecular targeted therapy [3, 4]. However, the prognosis is unsatisfactory and adverse drug effects remain an issue. Therefore, further study on the pathogenicity of colon cancer is required, which will provide empirical evidence for improving the diagnosis, treatment, and prognosis of colon cancer, as well as reducing the economic burden of patients.

Competing endogenous RNA (ceRNA) is a type of RNA that includes mRNA, pseudogenes, long non-coding RNA (lncRNA), and circular RNA (circRNA). circRNAs are a newly discovered type of non-coding RNA that has become a focus of research in cancer-related fields [5]. Unlike linear RNA molecules that contain a 5′ cap and 3′ polyadenylation tail, circRNA forms a covalently closed continuous loop and therefore does not have 5′ and 3′ ends [6, 7] circRNAs can function as a sponge for microRNAs (miRNAs) to regulate gene expression by removing the inhibitory effect of an miRNA on its target gene [8]. A previous study showed that the gene expression of HOTAIR and HER2 was up-regulated in gastric cancer tissue, and high expression levels of HER2 were correlated with the poor prognosis of gastric cancer. HOTAIR could competitively bind to the miRNA miR-331-3p, which positively regulated HER2 expression by functioning as its ceRNA and further promoted the proliferation, migration, and invasion of gastric cancer cells [9]. A recently discovered circRNA that could function as an miRNA sponge for both miR-21 and miR-221 was found to have a significant anti-tumor effect in melanoma, which suggested that circRNAs might be used as potential therapeutic targets [10].

According to our preliminary results, the expression of circRNA-ACAP2 was increased in colon cancer tissue. By using the bioinformatics software TargetScan and miRanda, hsa-miR-21-5p was predicted to be a target of circRNA-ACAP2, and T lymphoma invasion and metastasis protein 1 (Tiam1) was suggested to be a putative target gene of hsa-miR-21-5p. Therefore, we speculated that the circRNA-ACAP2/hsa-miR-21-5p/Tiam1 regulatory feedback circuit could affect the proliferation, migration, and invasion of colon cancer SW480 cells. By exploring the hypothesis, we may gain insights into the pathogenicity of colon cancer and provide new therapeutic targets for the treatment of colon cancer.

Materials and Methods

Sample collection

We collected tumor samples and distant normal mucous tissues (>5 cm from the margin of the tumor) from 21 patients with colon cancer who received surgical resection at our hospital from January 2015 to December 2016. No patient had received neoadjuvant chemotherapy or chemotherapy prior to surgery, and all tissue samples were pathologically confirmed. Tumor-node-metastasis staging of the patients was according to the 2015 National Comprehensive Cancer Network clinical practice guidelines. Informed consent

was obtained from each patient from the archives of the Central Hospital of Panyu District Hospital and with approval from the institutional ethics committee (Guangzhou, China).

Cell culture

FIHC, HCT-116, HT-29, and SW480 cell lines were purchased from the Shanghai Institute of Cell Biology (Shanghai, China). The cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/Nutrient Mixture F-12 Ham containing 10% fetal bovine serum, and maintained in a humidified incubator at 37°C with 5% CO_2. The cells in logarithmic growth with 95% viability were subjected to further experiment.

Quantitative real-time PCR

Total RNA samples were extracted using TRIzol according to the manufacturer's instructions. M-MLV reverse transcriptase was used to generate cDNA, which was later used as a PCR template. Quantitative real-time PCR (qRT-PCR) was performed in triplicate. The expression of U6 and β-actin was used as an internal control. The primers used in qRT-PCR analysis are shown in Table 1.

Table 1. Primers for qRT-PCR

Gene	Sequence (5′–3′)	Length (bp)
β-actin (H)	F: GTGGCCGAGGACTTTGATTG R: CCTGTAACAACGCATCTCATATT	73
circRNA-ACAP2	F: GAATGGGATTCGAGACCTG R: TTCTTCCAAAGCTGCCTGT	122
miR-21-5p	F: ACACTCCAGCTGGGTAGCTTATCAGACTGA R: TGGTGTCGTGGAGTCG	89
Tiam1	F: GATCCACAGGAACTCCGAAGT R: GCTCCCGAAGTCTTCTAGGGT	278
RT (miRNAs)	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTCCCAT	89
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT	89

Table 2. siRNAs used for vector construction

Gene	siRNA (5′–3′)
circRNA-ACAP2	Sense: GGCAGCATACAGGAAGATGAA Antisense: UUCAUCUUCCUGUAUGCUGCC
Negative control	Sense: UUCUCCGAACGUGUCACGUUUC Antisense: GAAACGUGACACGUUCGGAGAA
Tiam1	Sense: UUGACAAGCAUUUACAACGU Antisense: CGUUGUAAAUGCUUGUCAAAC

Cell transfection

The design and synthesis of small interfering RNAs (siRNAs) for circRNA-ACAP2 and Tiam1, the construction of a lentiviral vector over-expressing miR-21-5p, and the construction of a lentiviral vector expressing siRNAs for circRNA-ACAP2 and Tiam1 were conducted by Sangon Biotech (Shanghai) Co., Ltd. The inhibitor for miR-21-5p was purchased from Guangzhou RiboBio Co., Ltd. Transfection was performed using Lipofectamine 2000 according to the manufacturer's instructions (Table 2).

WST-8 assay

Cells at a density of 5.0×10^4 cells/mL were plated in a 96-well plate and divided into five groups: miR-21-5p group (miR-2-5p over-expression), NC group (infected with negative control lentiviral vector), control group (no infection), sh-Tiam1 group (infected with lentiviral vector expressing short hairpin (sh)-Tiam1), and sh-circRNA-ACAP2 group (infected with lentiviral vector expressing sh-circRNA-ACAP2). The cells were cultivated in serum-free DMEM for 6 h, then further cultured for 24, 48, and 72 h before the addition of 10 μL Cell Counting Kit-8 (5 mg/mL) to the culture medium in each well. After incubation for 2 h at 37°C, absorbance at 450 nm was measured using a multifunctional microplate reader (excitation wavelength at 450 nm, reference wavelength at 655 nm; Bio-Rad Laboratories, Hercules, CA). The inhibitory rate of cell proliferation was calculated according to the following formula: Inhibitory rate of cell proliferation (%) = (control group absorbance − experimental group absorbance)/(control group absorbance − blank group absorbance) × 100.

Colony formation assay

SW480 cells in logarithmic growth were plated at a density of 1200 cells/well in a 6-well plate and incubated at 37°C with 5% CO_2 for 7 days. The supernatant in each well was discarded and the cells were washed twice with a 1× phosphate-buffered saline (PBS) solution. The cells were then fixed for 15 min in 4% paraformaldehyde and stained for 15 min with crystal violet in methanol. Upon discarding the staining solution, the plates were allowed to air-dry, and the colonies were observed under a microscope.

Scratch wound assay

Cells in logarithmic growth were plated and allowed to grow until a confluent monolayer had formed. The cell monolayer was scraped in a straight line with a pipette tip to create a gap. The cell debris was removed by washing 3 times with 1× PBS. Culture medium was added again and the cells were allowed to grow for 24 and 48 h. Microscopic images were taken at 0, 24, and 48 h. For each image, the distance between one side of the scratch and the other was measured using ImageJ software (National Institutes of Health, Bethesda, MD).

Transwell assay

Matrigel was diluted in a pre-chilled serum-free medium at a volume ratio of 1:3, and then 40 μL of pre-chilled serum-free medium was added to a pre-chilled Transwell chamber. Matrigel was then solidified by incubating at 37°C for 2 h. Excess liquid was removed from the chamber, and 100 μL and 600 μL serum-free medium was added to the upper and lower chamber, respectively. The plate containing Transwell chambers was then incubated overnight at 37°C. On the next day of cell transfection, 1.0×10^5 cells were re-suspended in 100 μL serum-free DMEM/F12 medium. The cells were added to the upper chamber, and 600 μL complete medium was added to the lower chamber at the same time. After incubation at 37°C in 5% CO_2 for 24 and 48 h, surface cells and Matrigel were removed using a cotton swab in the upper chamber, and the cells were observed under an inverted microscope.

Western blot analysis

When the cells reached 80% confluence, the medium was discarded and the cells were washed with pre-chilled 1× PBS. Then, 320 μL cell lysis buffer (RIPA with 3.2 μL PMSF) was added to the cells in order to extract cellular protein. After a 30-min incubation on ice, the cells were scraped into a 1.5-mL centrifuge tube and subjected to centrifugation at 4°C for 15 min at 12, 000 rpm. Protein quantification was performed using a Nano Drop ND-1000. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransferred to a polyvinylidene fluoride membrane. The membrane was incubated with a primary antibody against Tiam1 at 4°C overnight, washed extensively with 0.1% Tween-20 in PBS, and incubated with a secondary antibody conjugated to horseradish peroxidase (1:1000) at room temperature for 3 h. Immunolabeling was visualized using an electrochemiluminescence system.

In vivo treatment

A total of 25 androgen BALB/c nude mice (weight, 18–20 g) were purchased from Guangdong Medical Laboratory Animal Center (Foshan, China; animal production license, no: 440035458020). Five cell lines (SW480, SW480-NC, sh-circRNA-ACAP2-SW480, sh-Tiam1-SW480, and miR-21-5p-SW480) were digested with 0.25% trypsin, washed with PBS, counted by trypan blue staining, and adjusted to a concentration of 1.0×10^6 cells/mL, and 0.1-mL aliquots were used each time. After mixing with Matrigel (Beijing Xia Si Biotechnology Co., Ltd., Beijing, China), the cells were injected subcutaneously between the abdominal ribs of specific pathogen free-grade male nude mice aged up to 4 weeks. The tumor weight of the mice was observed.

Biotin-coupled circRNA and miRNA capture

Biotin-coupled miRNA and circRNA pull-down assays were performed as described previously [11, 12]. Briefly, 3′ end biotinylated circRNA-ACAP2 (digoxin-5′-TCACACAGGCAGCTTTGGAAGAAG-3′-Digoxin; RiboBio, Guangzhou, China) was transfected into SW480 cells at a final concentration of 20 nM for 1 day. Biotin-coupled RNA complexes were pulled down by incubating the cell lysates with 4 MyOne streptavidin C1 Dynabeads (Invitrogen, Carlsbad, CA). The abundance of circRNA-ACAP2 or miR-21-5p in the bound fractions was evaluated by qRT-PCR analysis.

Luciferase reporter assay

Genomic DNA was extracted from SW480 cells and used as a template for the linear sequence of circRNA, 3′ untranslated region (3′UTR) sequence of mRNA, and *Xho*I and *Not*I restriction sites were introduced. The PCR amplification product was double-digested with respective enzymes and cloned into the psiCHECK-2 vector. The ligated products were then transformed into *Escherichia coli* DH5a cells. A blue/white screening assay and PCR were performed to identify positive clones. A dual-luciferase assay (Promega, Madison, WI) was then conducted according to the manufacturer's instructions.

Statistical analysis

Data are expressed as the mean ± standard deviation and processed using the statistical software SPSS 20.0 (SPSS Inc., Chicago, IL). Statistical comparisons were performed using one-way analysis of variance.

Results

CircRNA-ACAP2 and Tiam1 are expressed at a high level in colon cancer tissue and colon cancer SW480 cells, whereas miR-21-5p is expressed at a low level

In order to examine the relative expression of circRNA-ACAP2, Tiam1, and miR-21-5p in colon cancer tissue and colon cancer SW480 cells, pathologically confirmed colon cancer tissues and distant normal mucous tissues from newly diagnosed patients were collected, and normal colonic FIHC cells and colon cancer HCT-116, HT-29, and SW480 cell lines were cultured. The relative expression of circRNA-ACAP2, Tiam1, and miR-21-5p was determined using qRT-PCR. The result showed that circRNA-ACAP2 and Tiam1 were expressed at a high level, whereas miR-21-5p was expressed at a low level in colon cancer tissue and colon cancer SW480 cells (Fig. 1). These observations suggested that circRNA-ACAP2 and Tiam1 might contribute to the progression of colon cancer, whereas miR-21-5p might function as a tumor suppressor.

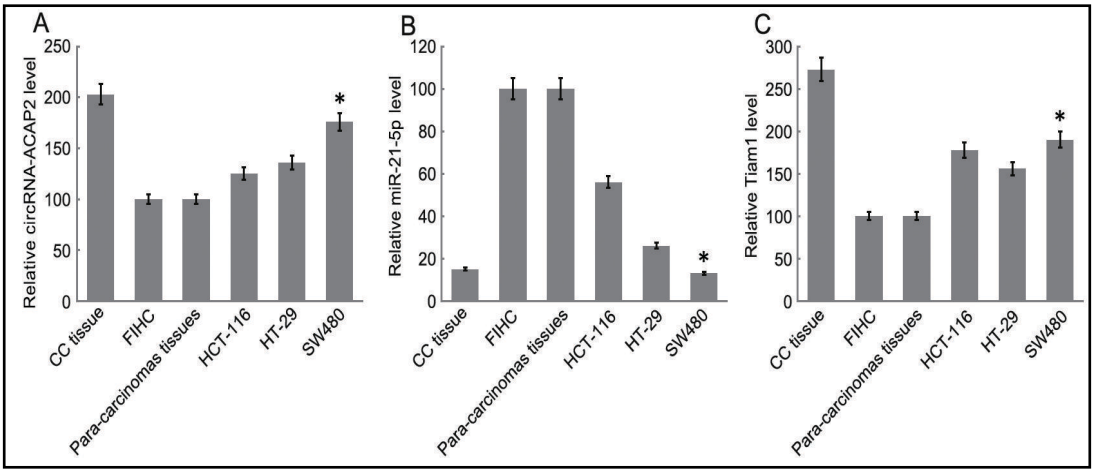

Fig. 1. Relative expression of circRNA-ACAP2, Tiam1, and miR-21-5p determined by qRT-PCR. (A) Relative expression of circRNA-ACAP2. Each bar represents the mean of three independent experiments. *P<0.05 compared with FIHC, HCT-116, and HT-29 cell lines. (B) Relative expression of miR-21-5p. Each bar represents the mean of three independent experiments. *P<0.01 compared with FIHC, HCT-116, and HT-29 cell lines. (C) Relative expression of Tiam1. Each bar represents the mean of three independent experiments. *P<0.05 compared with FIHC, HCT-116, and HT-29 cell lines.

CircRNA-ACAP2, Tiam1, and miR-21-5p affect the proliferation and clonogenicity of SW480 cells

WST-8 and colony formation assays were employed to examine the proliferation and clonogenicity of colon cancer SW480 cells after altering the expression levels of circRNA-ACAP2, Tiam1, and miR-21-5p. The results suggested that the proliferation and clonogenicity of SW480 cells were significantly suppressed following the down-regulation of circRNA-ACAP2 and Tiam1 expression as well as the up-regulation of miR-21-5p expression (Fig. 2).

CircRNA-ACAP2, Tiam1, and miR-21-5p affect the migration and invasion of SW480 cells

In order to determine the effect of circRNA-ACAP2, Tiam1, and miR-21-5p expression on the invasion of SW480 cells, scratch wound and Transwell assays were performed using SW480 cells transfected with lentiviral vectors expressing sh-circRNA-ACAP2, sh-Tiam1, or miR-21-5p. The results showed that the cell migration rate was decreased in the sh-circRNA-ACAP2, sh-Tiam1, and miR-21-5p groups compared with the control and NC groups (Fig. 3). The number of cells invading the other side of the chamber was significantly less in the sh-circRNA-ACAP2, sh-Tiam1, and miR-21-5p groups than in the control and NC groups (Fig. 4). These results suggested that the migration and invasion of SW480 cells were significantly suppressed following the down-regulation of circRNA-ACAP2 and Tiam1 expression as well as the up-regulation of miR-21-5p expression.

CircRNA-ACAP2, Tiam1, and miR-21-5p together could affect tumor growth

SW480 tumor xenografts were established in Athymic nude mice to evaluate the effects of circRNA-ACAP2, Tiam1, and miR-21-5p on clone cancer growth *in vivo*. Compared with the untreated animals, application of sh-circRNA-ACAP2, sh-Tiam1, and miR-21-5p significantly decreased the tumor mass, whereas the negative control group showed no effect (Fig. 5). No body weight loss or diarrhea was observed and all animals (treated as well as untreated) survived. These results showed that reduced expression of circRNA-ACAP2 and Tiam1 or over expression of miR-21-5p can effectively inhibit colon cancer growth *in vivo*.

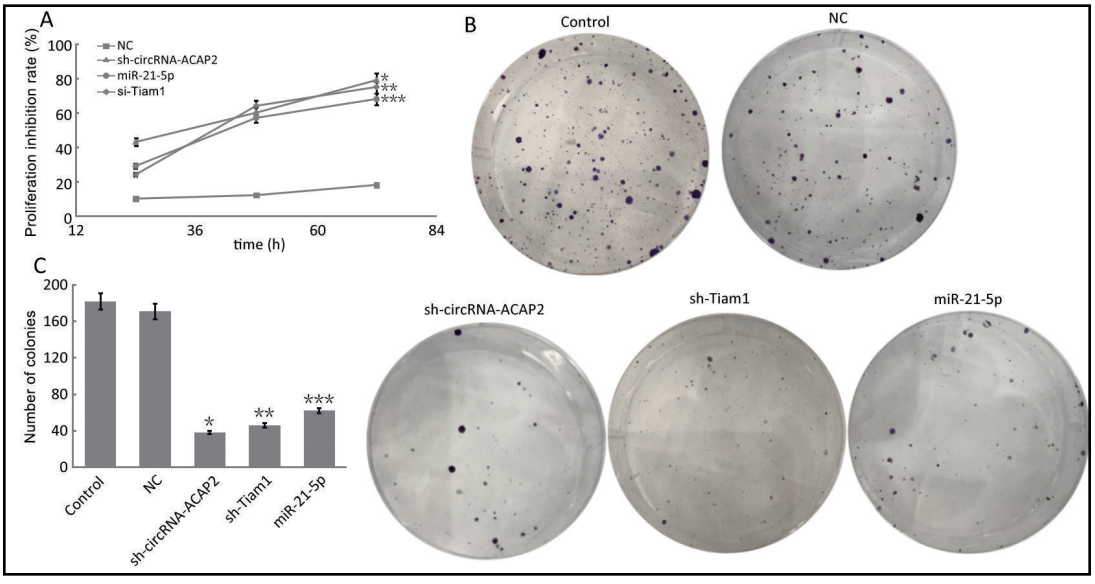

Fig. 2. CircRNA-ACAP2, Tiam1, and miR-21-5p affect the proliferation and clonogenicity of SW480 cells. (A) SW480 cell proliferation determined by a WST-8 cell proliferation assay. Each bar represents the mean of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with the NC group. (B) Image of colony-forming SW480 cells. (C) Counting of colony-forming SW480 cells. Each bar represents the mean of three independent experiments. *P<0.05, **P<0.05, ***P<0.01 when compared with the control and NC groups.

CircA-ACAP2 can bind to miR-21-5p

In order to investigate whether miR-21-5p could bind to circRNA-ACAP2, we used a circRNA-ACAP2-specific probe to pull down its associated miRNAs. qRT-PCR results showed that miR-21-5p was greatly enriched in the circRNA-ACAP2 precipitation complex (Fig. 6). These results indicated that circRNA-ACAP2 could bind to miR-21-5p.

CircRNA-ACAP2 inhibits miR-21-5p expression, thus relieving the inhibition of Tiam1 translation by miR-21-5p

Using the bioinformatics software TargetScan and miRanda, hsa-miR-21-5p was predicted to be a target of circRNA-ACAP2, and Tiam1 was suggested to be a target gene of hsa-miR-21-5p (Fig. 7A, C). CircRNA-ACAP2 wild-type (circRNA-ACAP2-WT), circRNA-ACAP2 mutant (circRNA-ACAP2-Mut), Tiam1-3′UTR wild-type (Tiam1-3′UTR-WT), and Tiam1-3′UTR mutant (Tiam1-3′UTR-Mut) expression vectors were constructed and co-transfected with miR-21-5P mimics (miR-21-5p) into 293T cells, and luciferase activity was examined. Luciferase activity was significantly decreased in 293T cells co-transfected with circRNA-ACAP2-WT, Tiam1-3′UTR-WT, and miR-21-5P mimics, suggesting that circRNA-ACAP2 might interact with miR-21-5p, and miR-21-5p might interact with Tiam1. However, luciferase activity was not affected in 293T cells co-transfected with circRNA-ACAP2-Mut, Tiam1-3′UTR-Mut, and miR-21-5P

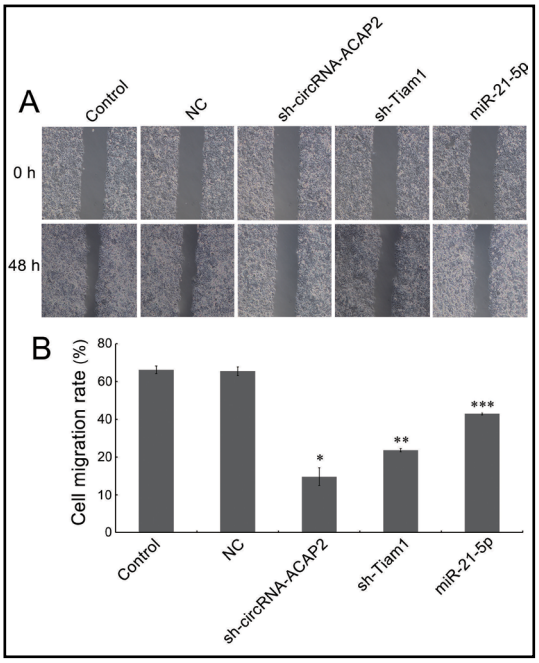

Fig. 3. The migration rate of SW480 cells determined by a scratch wound assay. (A) Microscopic images of migrating cells in different groups. (B) Bar graph representing the migration rate in each group. Each bar represents the mean of three independent experiments. *P<0.05, **P<0.01, ***P<0.05 when compared with the control and NC groups.

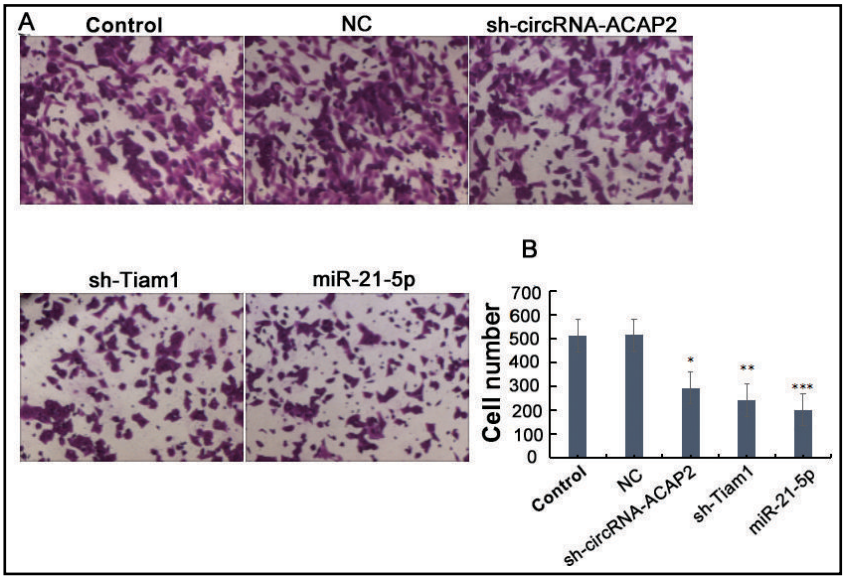

Fig. 4. Invasion of SW480 cells determined by a Transwell assay. (A) Microscopic images of different groups of cells invading the bottom of the Transwell chamber. (B) Bar graph representing the number of invading cells in each group. Each bar represents the mean of three independent experiments. *P<0.05, **P<0.05, ***P<0.01 when compared with the control and NC groups.

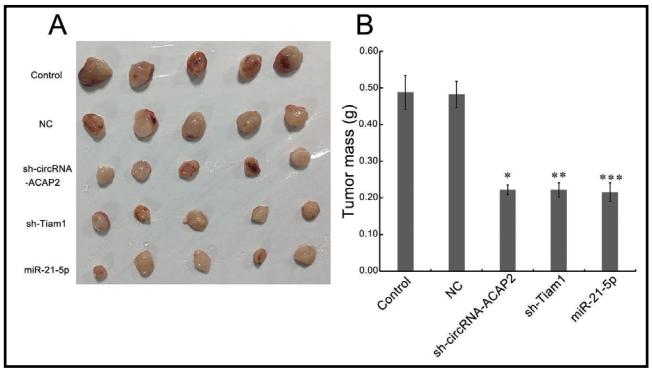

Fig. 5. Effect of circRNA-ACAP2, Tiam1, and miR-21-5p on colon cancer xenografts. (A) Tumor photograph. (B) Body weight of the nude mice in each group; *P<0.05, **P<0.01, ***P<0.01 compared with the NC and control groups.

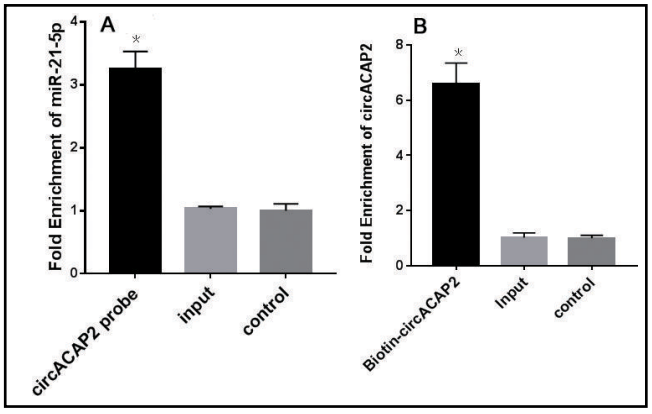

Fig. 6. CircRNA-ACAP2 binding to miR-21-5p in SW480 cells. (A) CircACAP2 in SW480 cell lysis was pulled down and enriched with a circACAP2-specific probe and then detected using qRT-PCR. *P<0.01 when compared with the input and control groups. (B) miR-21-5p was pulled down and enriched with a circACAP2-specific probe and then detected using qRT-PCR. *P<0.05 when compared with the input and control groups.

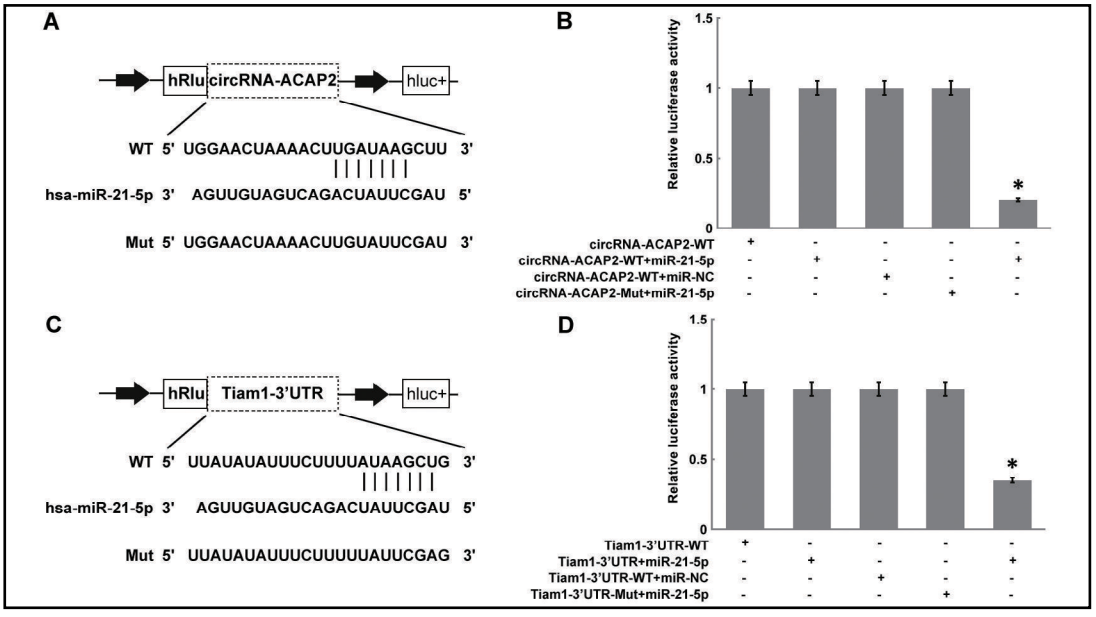

Fig. 7. Construction of double fluorescent reporter gene vectors to verify the interaction of circRNAs/miRNAs and miRNAs/mRNAs. (A) Diagram for the construction of double fluorescent reporter vectors. (B) Effects of the inhibition of miR-21-5p (miR-NC), miR-21-5P mimics (miR-21-5p), circRNA-ACAP2 wild-type (circRNA-ACAP2-WT), and circRNA-ACAP2 mutant (circRNA-ACAP2-Mut) on the luciferase activity of miR-21-5p in 293T cells by luciferase reporter assays. Each bar represents the mean of three independent experiments. *P<0.05. (C) Diagram for the construction of double fluorescent reporter vectors. (D) Effects of the inhibition of miR-21-5p (miR-NC), miR-21-5P mimics (miR-21-5p), Tiam1-3′UTR wild type (Tiam1-3′UTR-WT), and Tiam1-3′UTR mutant (Tiam1-3′UTR-Mut) on the luciferase activity of miR-21-5p in 293T cells by luciferase reporter assays. Each bar represents the mean of three independent experiments. *P<0.01.

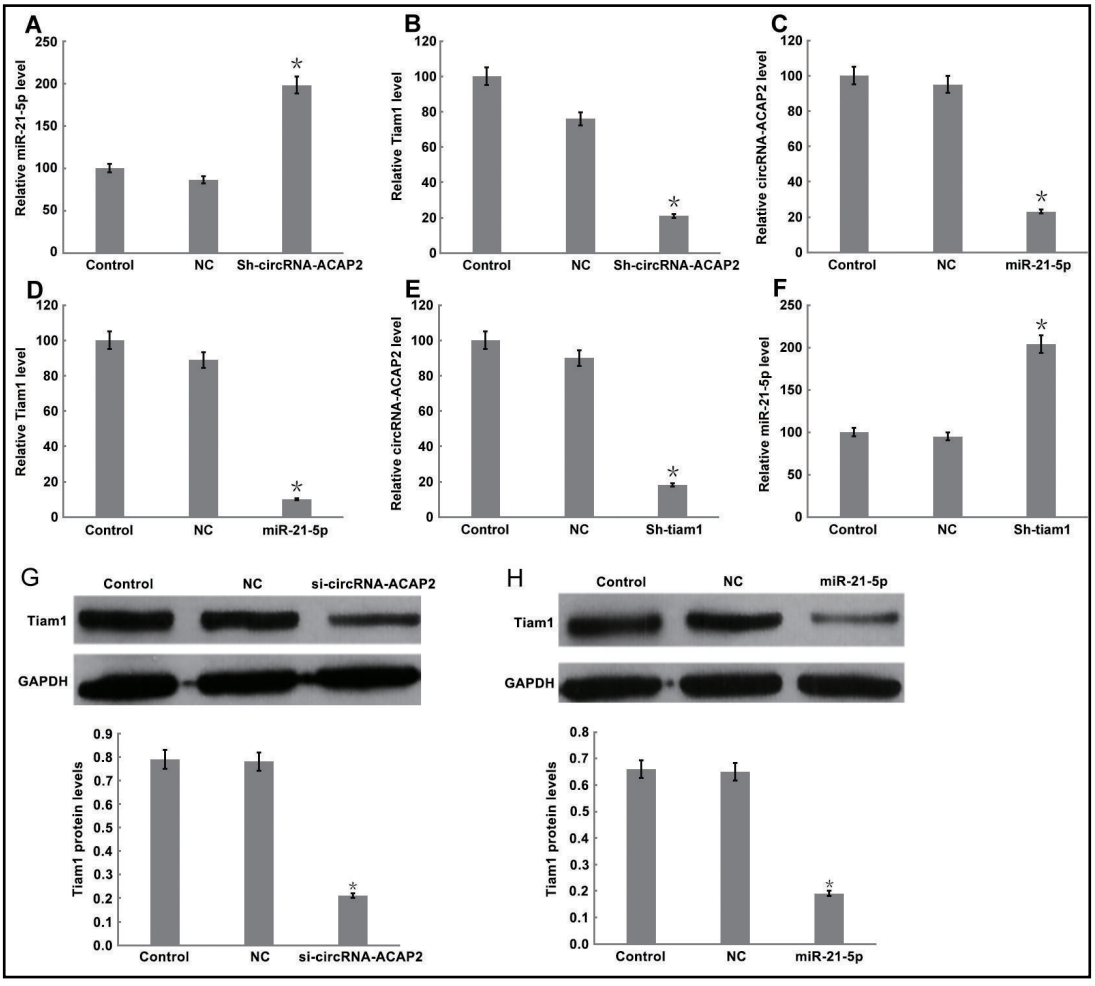

Fig. 8. Relative expression of circRNA-ACAP2, Tiam1, and miR-21-5p detected by qRT-PCR and western blotting. (A) Relative expression of miR-21-5p upon the down-regulation of circRNA-ACAP2 expression. Each bar represents the mean of three independent experiments. *P<0.01. (B) Relative expression of Tiam1 upon the down-regulation of circRNA-ACAP2 expression. Each bar represents the mean of three independent experiments. *P<0.05. (C) Relative expression of circRNA-ACAP2 upon the over-expression of miR-21-5P. Each bar represents the mean of three independent experiments. *P<0.05. (D) Relative expression of Tiam1 upon the over-expression of miR-21-5P. Each bar represents the mean of three independent experiments. *P<0.01. (E) Relative expression of circRNA-ACAP2 upon the down-regulation of Tiam1 expression. Each bar represents the mean of three independent experiments. *P<0.05. (F) Relative expression of miR-21-5P upon the down-regulation of Tiam1 expression. Each bar represents the mean of three independent experiments. *P<0.01. (G) Relative protein expression of Tiam1 when circRNA-ACAP2 expression was knocked down. Each bar represents the mean of three independent experiments. *P<0.05. (H) Relative protein expression of Tiam1 when circRNA-ACAP2 was over-expressed. Each bar represents the mean of three independent experiments. *P<0.01.

mimics, suggesting that no interaction existed (Fig. 7B, D). Moreover, we found that upon down-regulation of circRNA-ACAP2 expression, the expression of miR-21-5p was increased and that of Tiam1 was decreased (Fig. 8A, B, G). Following miR-21-5p over-expression, the expression of circRNA-ACAP2 and Tiam1 was decreased (Fig. 8C, D, H). Down-regulating Tiam1 expression resulted in the down-regulation of circRNA-ACAP2 expression and up-regulation of miR-21-5P expression (Fig. 8E, F). These results suggested that circRNA-ACAP2 inhibits miR-21-5p expression, thus relieving the inhibition of Tiam1 translation by miR-21-5p.

Discussion

CircRNAs are a type of endogenous RNA that are largely expressed in the eukaryotic transcriptome and participate in the regulation of gene expression [13]. They play an essential role in tumorigenesis and cancer progression [14]. Studying their regulatory mechanisms will provide insights into the future prevention and treatment of tumors. By using microarray analysis, a previous study showed that hsa_circ_0007331 was highly expressed in colon cancer tissue. hsa_circ_0007331 is encoded by the ACAP2 gene, a homolog of *Caenorhabditis elegans* CNT-1, which has a pro-apoptotic function and an identical phosphoinositide-binding pattern to that of CNT-1. Its inactivation or down-regulation in human cells may contribute to the development of cancer [15, 16].

Tiam1 is up-regulated in a variety of cancers and its expression level is related to the metastatic potential of each cancer type [17]. miR-21-5p is involved in the regulation of epithelial-mesenchymal transition and stem-like cells in keratinocytes, as well as the regulation of PTEN [18]. The results from the present study also show that circRNA-ACAP2 and Tiam1 were expressed at a high level, whereas miR-21-5p was expressed at a low level in colon cancer tissue and colon cancer SW480 cells (Fig. 1). This observation suggested that circRNA-ACAP2 and Tiam1 might contribute to the progression of colon cancer, whereas miR-21-5p might function as a tumor suppressor. The down-regulation of circRNA-ACAP2 and Tiam1 expression as well as the up-regulation of miR-21-5p expression significantly suppressed the proliferation and invasion of SW480 cells.

An increasing number of studies have shown that some circRNAs have specific binding site for miRNAs, and miRNAs can suppress the degradation of the corresponding mRNA by competitively binding to exonic circRNAs [19, 20]. In this study, we first demonstrated that hsa-miR-21-5p was predicted as a target of circRNA-ACAP2 using the bioinformatics software TargetScan and miRanda, and Tiam1 was a putative target gene of hsa-miR-21-5p. The down-regulation of circRNA-ACAP2 promoted miR-21-5p expression, which further suppressed the transcription and translation of Tiam1. Therefore, we speculated that circRNA-ACAP2 could act as an miRNA sponge to regulate Tiam1 expression by removing the inhibitory effect of miR-21-5p on Tiam1 expression, and eventually affect the proliferation and invasion of SW480 cells. Some studies have shown that circRNAs can affect the expression of mRNAs by competitively binding to miRNAs. For instance, circWDR77 affected the expression of FGF-2 by competitively binding to miR-124, which further regulated the proliferation and migration of vascular smooth muscle cells [21]. CircHIPK2 increased SIGMAR1 expression by suppressing miR-124-2HG activity [22]. In bladder cancer, circHIPK3 affected the expression of heparanase by competitively binding to miR-558 [23]; circRNA MYLK and lncRNA H19 increase the expression of DNMT3, VEGFA, and ITGB1 by suppressing miR-29a-3P activity [24].

Conclusion

Our study is the first to demonstrate that down-regulating the expression of circRNA-ACAP2 and Tiam1 as well as up-regulating miR-21-5p expression could significantly suppress the proliferation and invasion of SW480 cells. This was probably due to the fact that circRNA-ACAP2 could act as an miRNA sponge to regulate Tiam1 expression by removing the inhibitory effect of miR-21-5p on Tiam1 expression. Our findings suggest that the circRNA-ACAP2/hsa-miR-21-5p/Tiam1 feedback circuit plays a critical role in the progression and development of colon cancer. The ceRNA network and pathway might be novel clinical markers and therapeutic targets for patients with colon cancer.

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Disclosure Statement

The authors declare that they have no competing interests.

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