Downregulation of microRNA-183-5p inhibits the proliferation and invasion of colorectal cancer cells by inactivating the reticulocalbin-2/Wnt/β-catenin signaling pathway

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Abstract. microRNAs (miRNAs) are frequently aberrantly expressed in colorectal cancer (CRC) and are considered to serve a critical role in the onset and development of CRC by binding to its target transcription factor. The aim of the present study was to examine the role of miRNA (miR)-183-5p in the proliferation, invasion and migration of CRC cells, in addition to its underlying mechanism. Reverse transcription-quantitative polymerase chain reaction analysis was used to detect the expression level of miR-183-5p. MTT and Transwell assays were performed to examine proliferation and invasion in SW620 cells. Western blot analysis was performed to determine the protein expression of reticulocalbin-2 (RCN2), matrix metalloproteinase-2, β-catenin, cyclin D1 and c-Myc. miR-183-5p expression was significantly upregulated in the CRC tissues compared with adjacent normal tissues. In addition, the inhibition of miR-183-5p suppressed proliferation, invasion and migration in SW620 cells. miR-183-5p downregulation or overexpression regulated the CRC cell cycle, invasion and migration by modulating RCN2 expression. Furthermore, the Wnt/β-catenin pathway was observed to be involved in the inhibitory effect of miR-183-5p downregulation in CRC cell proliferation, invasion and migration. These results provided evidence that the downregulation of miR-183-5p inhibits CRC proliferation and invasion by regulating the RCN2/Wnt/β-catenin pathway. miR-183-5p and RCN2 may serve an important role in the molecular etiology of CRC and have potential applications in CRC treatment.

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Introduction

Colorectal cancer (CRC) is the third most common type of cancer worldwide, occurring in millions of people worldwide (1). It may be divided into rectal and colon cancer. The occurrence and development of CRC is closely associated with lifestyle, heredity and colorectal adenoma (2,3). Over the last decade, studies have reported that, in addition to a small number of patients with CRC with a clear familial tendency, the majority of patients have sporadic CRC (4). The cause of the disease is associated with individual genetic mutations, including different types of chromosomal mutations and DNA mismatch repair (5,6). In recent years, the development and application of new treatments and diagnostic technologies have markedly reduced the mortality rate of CRC. However, the pathogenesis of CRC remains only partially understood.

Reticulocalbin-2 (RCN2) is a 55-kDa Ca^{2+}-binding protein, which contains six EF hand motifs and exists in the endoplasmic reticulum (7,8). To date, reports on the effect of RCN2 in cancer are limited. A previous study confirmed that the knockdown of RCN2 significantly inhibited hepatocellular carcinoma cell proliferation by regulating G1/S transition arrest and cyclin D1 expression (9). Wang *et al* (10) reported that RCN2 expression was upregulated in CRC and was correlated with cancer growth and proliferation. These results demonstrated that RCN2 is strongly associated with aggressive cancer behavior and has a potential function in promoting CRC cell proliferation and invasion.

microRNAs (miRNAs) are small non-coding single-stranded RNAs with a length of ~22 nucleotides, which modulate the stability and/or translation of mRNA by regulating the interaction with specific sequences in the coded or untranslated regions (11). miRNAs serve a vital role in cancer tumorigenesis and metastasis (12). Each miRNA has its own specific target gene that regulates multiple genes via a miRNA, and/or multiple miRNAs regulate the same gene, thus forming a complex gene regulatory network involved in the development of various types of cancer (13). Recently, miR-183-5p was reported to be involved in the occurrence and progression of numerous types of cancer, including lung adenocarcinoma and breast cancer (14,15). Furthermore, integrated analysis of miRNA datasets demonstrated that miR-183-5p was

upregulated and directly associated with CRC (16). However, the effect of miR-183-5p on CRC and its underlying mechanism are unclear.

The results of the present study demonstrated that the downregulation of miR-183-5p inhibited proliferation, migration and invasion in SW620 cells. The bioinformatics analysis and luciferase reporter assay results also revealed that RCN2 is a potential target of miR-183-5p. The present evidence collectively suggested that the knockdown of miR-183-5p may serve an antineoplastic role via the RCN2/Wnt/β-catenin axis, which may provide a novel therapeutic target for CRC.

Materials and methods

Tissue samples and cell lines. CRC and adjacent normal tissues were obtained from 45 patients with CRC undergoing resection. There were 22 males and 23 females, aged 30-55 years, with a mean of 38.5±1.4 years. Inclusion criteria were: i) Patients who conformed to the diagnostic criteria of CRC and ii) Patients who had undergone surgical resection. Excluded were patients with other malignant tumors. The specimens were collected between May 2015 and October 2017 in the Department of Gastroenterological Surgery of The Second People's Hospital of Lianyungang (Lianyungang, China). All tissues were confirmed by pathological examination. Informed consent was obtained from all patients and ethical approval was obtained from the Institutional Review Board of The Second People's Hospital of Lianyungang. Samples were fixed before paraffin embedding, usually for 4 to 24 h at room temperature. Fresh tissue was fixed in 4% paraformaldehyde for >24 h. Once the tissue was removed from the fixative, the tissue of the target site was smoothed in a fume hood with a scalpel, and the trimmed tissue and corresponding label placed in the dehydration box. Thereafter, the dehydration box was placed in a hanging basket and dehydrated with a graded series of alcohol. The tissue was embedded in an embedding machine; the melted wax first placed in the embedding frame, and the tissue placed in the embedding frame according and a corresponding label was attached. It was then placed in a -20˚C freezer. When the paraffin was solidified, the wax block was removed from the embedding frame and the wax block trimmed. Finally, the trimmed wax block was placed on a microtome. The thickness of the sections were ~2-3 mm. Normal colorectal cells (CCD-18Co) and CRC cell lines (HT-29, SW116, HCT116, SW480 and SW620) together with 293 cells were purchased from the American Type Culture Collection (Manassas, VA, USA). SW116 and SW620 cells were grown in Leibovitz's L-15 medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.). HCT-116 cells were cultured in Ham's F12K medium (Thermo Fisher Scientific, Inc.) containing 10% FBS. SW480 and HT-29 cells were incubated in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) containing 10% FBS. Cells were cultured at 37˚C in a humidified atmosphere with 5% CO_2.

Transfection of miRNA mimic and inhibitor. SW620 cells were transfected with 50 nM hsa-miR-183-5p mimics or inhibitor (Wuhan GeneCreate Biological Engineering Co., Ltd., Wuhan, China), or with adenovirus RCN2 or RCN2 small interfering (si)RNA at the same time (50 nM; Shanghai GeneChem Co., Ltd., Shanghai, China) using Lipofectamine® 2000 transfection reagent (Thermo Fisher Scientific, Inc.) for 48 h, according to the manufacturer's protocol. The sequences of hsa-miR-183-5p used in this study was: 5'UAUGGCACUGGUAGAAUUCAC U3'; The sequences of small-interfering RNA for RCN2 is: Forward 5'-GCGTGAGATGGTACGAACT-3', reverse 5'-AGGCTTACACCCTCATACAT-3'. All experimental control samples were treated with an equal concentration of a non-targeting control mimic sequence (negative controls).

MTT analysis. The proliferation of SW620 cells was measured using MTT assay kits (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Cells were seeded at $2x10^3$ cells/well in 96-well plates and were cultured for 24-96 h. Next, MTT assay was performed at 0, 24, 48, 72 and 96 h, after which the optical density values were recorded. The formazan was dissolved by DMSO in the MTT experiment and its absorbance was measured at the wavelength of 490 nm.

Cell cycle assays. The SW620 cells were harvested following transfection with miRNA mimic or inhibitor, and adenovirus RCN2 or RCN2 siRNA. The cell cycle assay was performed using a propidium iodide (PI) cell cycle detection kit (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China), according to the manufacturer's protocol, and detection was performed with a FACScan flow cytometer and FACSCanto II version 4.1 (BD Biosciences, San Jose, CA, USA).

Transwell assay. SW620 cells were resuspended in 100 μl serum-free medium and plated in the top chamber of each insert (8-μm pore size; Corning Inc., Corning, NY, USA) with a Matrigel-coated membrane (BD Biosciences) for the Transwell assay. SW620 cells were incubated in serum-free medium for 24 h, and subsequently trypsinized and suspended with culture medium containing 0.1% FBS (Thermo Fisher Scientific, Inc.) albumin at a concentration of $4x10^4$ cells/ml. Subsequently, 500 μl cell suspension was added to each well for 36 h. The lower chambers of the inserts were filled with Dulbecco's modified Eagle's medium (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) with 10% FBS. After 24 h, the invasive cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet at room temperature. The number of invasive cells was counted in 5 randomly selected fields of view and images acquired. After 24 h, the invasive cells were fixed with 4% paraformaldehyde and stained with 0.5% crystal violet at 37˚C for 20 min. The number of invasive cells was counted in 5 randomly selected fields of view and images acquired under a light microscope (magnification, x400).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from CRC and adjacent normal tissues, and CRC cell lines, were extracted using TRIzol® (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The purity of the RNA was determined by measuring the absorbance at 260-280 nm using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc.). RT was performed using a PrimeScript RT kit (cat. no. RR036A; Takara Bio, Inc., Otsu, Japan), and RT-qPCR was performed

using a SYBR Premix kit (cat. no. RR420A; Takara Bio, Inc.). Total RNA was reverse transcribed into complementary DNA (cDNA) in a reaction system (25 μl) composed of 5 μl 5X M-MLV RT buffer, 2 μl dNTP mix (2.5 mM), 1 μl RNase inhibitor (30 U/μl), 1 μl M-MLV Reverse Transcriptase and 16 μl double distilled H_2O. The RNA was reverse transcribed into cDNA according to the instructions of the reverse transcription kit: Pre-denatured at 95˚C for 5 min, denatured at 95˚C for 15 sec, annealed at 60˚C for 30 sec, extended at 72˚C for 30 sec, 40 cycles, and maintained at 72˚C for 10 min. The data were normalized to the levels of GAPDH and were further analyzed using the $2^{-\Delta\Delta Cq}$ method (17).

The primers used in the study were as follows: miR-183-5p forward 5'TCACTTAAGATGGTCACGGTAU3', and reverse 5'ATAGACCAACAGGTGTACTGA3'; RCN2 forward 5'CCC GACCTCTTCAGCGGGCA3' and reverse, 5'CTTGGGGCA GGGGCTCTTGAC-3'; and GAPDH forward 5'TGGATT CGACTTAGACTTGACCT-3', and reverse 5'GGTGGGTTA TGGTCTTCAAAAGG3'.

Western blot analysis. Proteins from the cells were extracted using radioimmunoprecipitation assay lysis buffer (Sigma-Aldrich; Merck KGaA). The extracted proteins were determined by a bicinchoninic acid kit (Sigma-Aldrich; Merck KGaA). Subsequently protein (45 μg per lane) was subjected to 10% SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% non-fat milk at the room temperature for 1 h, and incubated with antibodies against RCN2 (cat. no. ab231912), matrix metalloproteinase (MMP)-2 (cat. no. ab37150), β-catenin (cat. no. ab6302), cyclin D1 (cat. no. ab1663), C-myc (cat. no. ab32072), GAPDH (cat. no. ab181602) and β-actin (cat. no. ab8226) at a dilution of 1:1,000 (all Abcam, Cambridge, MA, USA) overnight at 4˚C. Following washing five times (5 min each), the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (cat. no. ab6940; Abcam) at a dilution of 1:2,000 with secondary antibody dilution buffer (cat. no. P0023D; Beyotime Institute of Biotechnology, Shanghai, China) for 2 h at room temperature. The bands were visualized using a chemiluminescence detection kit (Nanjing KeyGen Biotech Co., Ltd.) and analyzed using Image J version 1.8.0 (National Institutes of Health, Bethesda, MD, USA).

Luciferase reporter assay. Human RCN2 cDNA containing wild-type (wt) and mutant (mut) target sites for miR-183-5p was chemically synthesized and inserted into a pMIR-REPORT™ vector (Shanghai GeneChem Co., Ltd.). The pMIR-REPORT™ β-galactosidase control vector (Shanghai GeneChem Co., Ltd.) was used as a reference. 293 cells were seeded and cultured in 6-well plates at a density of $1.2x10^6$ cells per well. Then they were co-transfected with miR-183-5p mimics or inhibitor and wt or mut reporter plasmid using the Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A Dual Luciferase Reporter Gene Assay System D0010-100T, (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was used to analyze the luciferase activity at 48 h post-transfection according to the manufacturer's protocol. The relative luciferase activity was calculated as the ratio of firefly luciferase activity vs. *Renilla* luciferase activity.

Bioinformatics analysis. In the present study, CoGeMiR (release v1.2b, June 2008) was used to calculate the conservation levels of miR-183-5p (18). miRanda (http://www.miranda-im.org/, release IM vo.10.78, April 2018), miRDB (release 5.0 August 2014) and TargetScan (http://www.targetscan.org/, release 7.2, March 2018) were used to identify the proteins that may potentially interact with miR-183-5p, the specific methods being based on previous studies (19,20).

Statistical analysis. SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA) was used to analyze the data. Data were presented as the mean ± standard deviation. All the experiments were performed in triplicate and repeated three times. The significant differences between different groups were analyzed using an independent-samples t-test (comparison between two groups). Comparisons among more than two groups were performed with one-way analysis of variance and multiple comparisons between the groups were performed using the Student-Newman-Keuls method. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-183-5p is upregulated in CRC tissues and cell lines. To study the role of miR-183-5p in CRC, bioinformatics analysis was performed to identify its biological features and potential function. RT-qPCR was performed to determine the expression of miR-183-5p in 45 CRC tissues and their adjacent normal colorectal tissues, and miR-183-5p was significantly upregulated in the CRC tissues compared with the adjacent normal tissues (Fig. 1A). The expression of miR-183-5p was also examined in normal colorectal cells (CCD-18Co) and CRC cell lines (HT-29, SW116, HCT116, SW480 and SW620), and the expression of SW620 in CRC cells was observed to be the most significantly increased compared with CCD-18Co cells (Fig. 1B).

Inhibition of miR-183-5p suppresses the proliferation, invasion and migration of SW620 cells. To further investigate the function of miR-183-5p in CRC, a variety of *in vitro* assays were performed, including MTT proliferation and Transwell invasion/migration assays. The knockdown of miR-183-5p was verified through RT-qPCR, and the expression of miR-183-5p was significantly downregulated (Fig. 2A). The present results demonstrated that the downregulation of miR-183-5p inhibited SW620 cell proliferation, which was consistent with the corresponding MTT assay results (Fig. 2B), and it contributed to the suppression of the number of invasive and migratory cells (Fig. 2C). The findings indicated that the inhibition of miR-183-5p suppressed CRC cell proliferation, invasion and migration, and may be a potential therapeutic target for CRC.

RCN2 is a target of miR-183-5p. To further investigate the possible mechanism of action of miR-183-5p in CRC (Fig. 3), a combination of TargetScan, miRanda and miRDB was used to predict the genes that interacted with miR-183-5p. Among the potential targets of miR-183-5p, the present study focused on RCN2, which exhibited the capability to

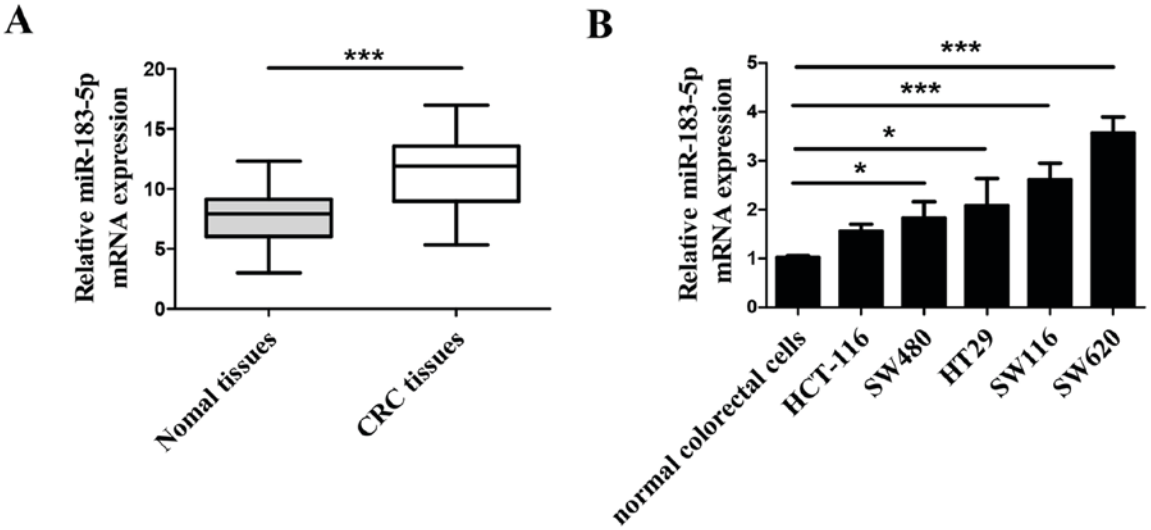

Figure 1. miR-183-5p is upregulated in CRC tissues and cell lines. (A) RT-qPCR was used to determine the expression of miR-183-5p in 45 adjacent normal colorectal and CRC tissues. (B) RT-qPCR was performed to examine the expression of miR-183-5p in normal colorectal cells (CCD-18Co) and CRC cell lines (HT29, SW116, HCT116, SW480 and SW620 cells). All data are expressed as the mean ± standard deviation (n=3). *P<0.05, ***P<0.001. CRC, colorectal cancer; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; miR, microRNA.

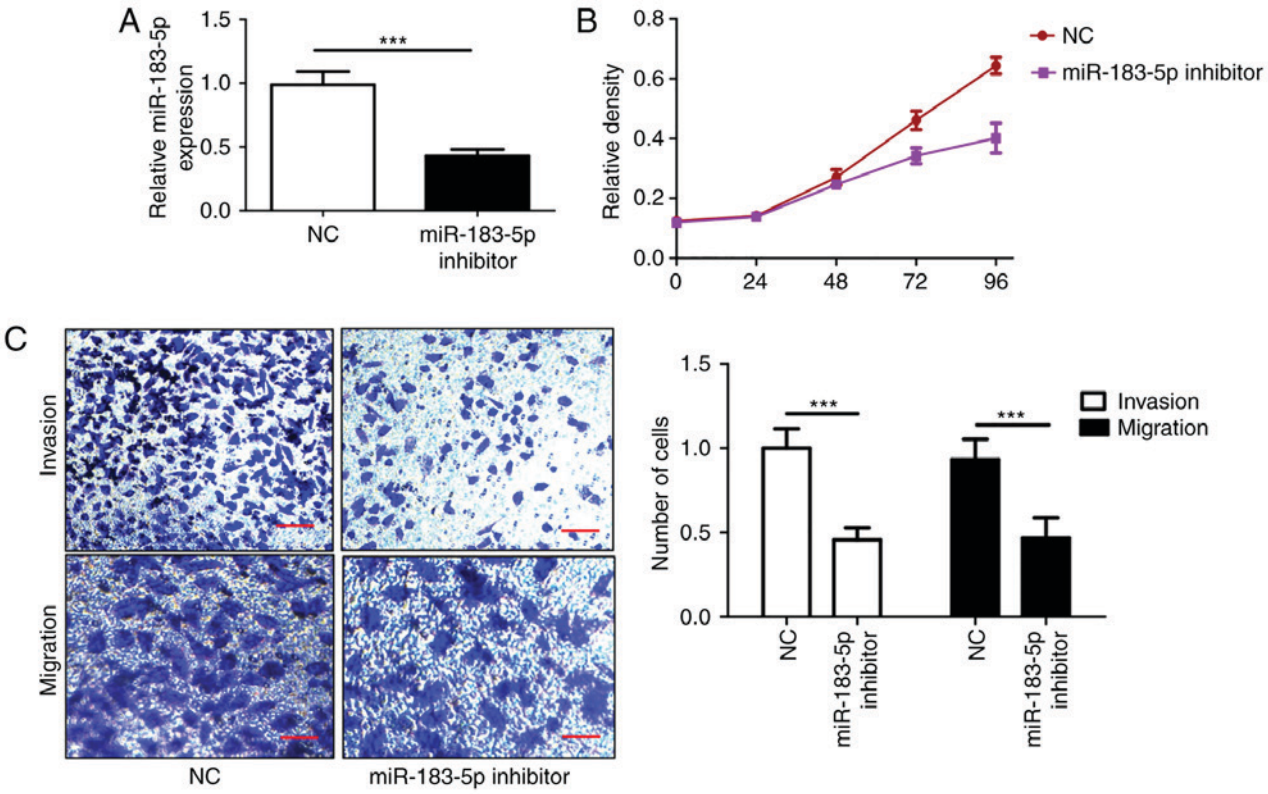

Figure 2. Inhibition of miR-183-5p suppresses the proliferation, invasion and migration of SW620 cells. (A) Reverse transcription-quantitative polymerase chain reaction analysis was used to determine the mRNA expression of miR-183-5p in SW620 cells. (B) An MTT assay was used to investigate the role of miR-183-5p expression in the proliferation of SW620 cells. (C) A Transwell assay was used to determine the role of miR-183-5p expression in the invasion of SW620 cells. Scale bars, 100 μm. All data are expressed as the mean ± standard deviation (n=3). ***P<0.001. miR, microRNA; NC, negative control.

bind to miR-183-5p (Fig. 3A). To further confirm the interaction between RCN2 and miR-183-5p, mimics were used to overexpress miR-183-5p (Fig. 3B). The wt RCN2 and mut RCN2 reporter vectors were constructed to perform the luciferase reporter assay. The luciferase activity of wt RCN2 was increased in the miR-183-5p mimics group, while the transfection with miR-183-5p inhibitors decreased the luciferase activity of wt miR-183-5p. In addition, miR-183-5p mimics or miR-183-5p inhibitors had no significant effect on the luciferase activity of mut RCN2 (Fig. 3C). Furthermore, the overexpression of miR-183-5p in SW620 cells increased the expression of RNC2 compared with the control group;

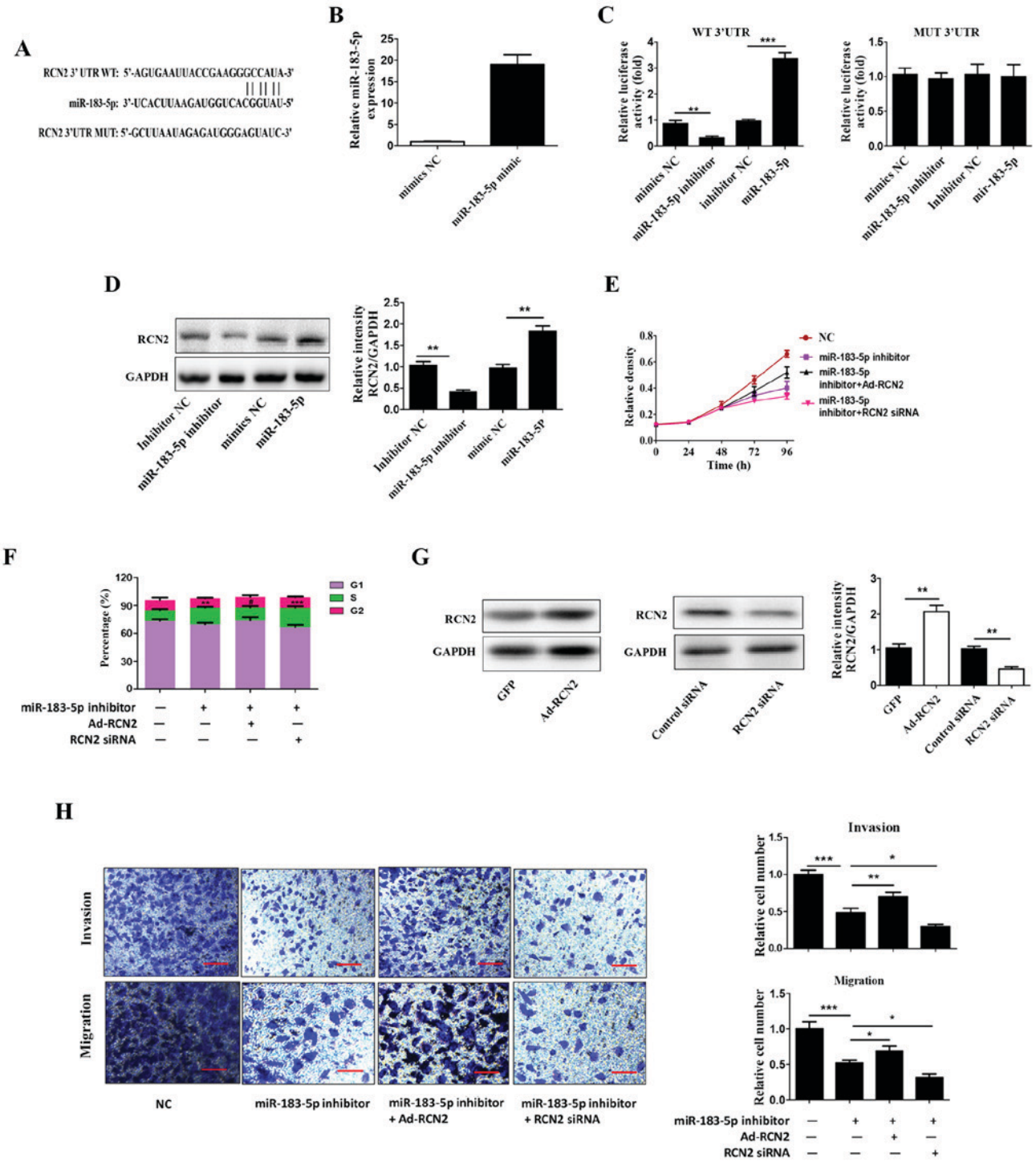

Figure 3. RCN2 is a target of miR-183-5p. (A) RCN2 can bind to miR-183-5p. (B) Reverse transcription-quantitative polymerase chain reaction analysis was used to examine the transfection efficiency of miR-183-5p mimic. (C) A luciferase reporter assay was performed to confirm the interaction between RCN2 and miR-183-5p. (D) Western blot analysis was used to determine the protein expression of RCN2. (E) An MTT assay demonstrated that miR-183-5p inhibitor and RCN2 siRNA further enhanced cell proliferation in SW620 cells. (F) Cell cycle analysis indicated that the inhibition of miR-183-5p significantly increased the percentage of CRC cells in the S phase via RCN2. (G) Western blot analysis was used to determine the protein expression of RCN2. (H) A Transwell assay was used to determine whether the miR-183-5p inhibitor and RCN2 siRNA further enhanced cell invasion in SW620 cells. Scale bars, 100 μm. All data are expressed as the mean ± standard deviation (n=3). *P<0.05, **P<0.01, ***P<0.001. RCN2, reticulocalbin-2; CRC, colorectal cancer; miR, microRNA; siRNA, small interfering RNA; UTR, untranslated region; WT, wild-type; MUT, mutant; NC, negative control; Ad, adenovirus.

the expression of RCN2 was decreased when miR-183-5p was downregulated in SW620 cells, compared with the control group (Fig. 3D). To investigate whether the effects of miR-183-5p were dependent on RCN2, co-transfection with miR-183-5p inhibitor and RCN2 overexpression adenovirus or siRNA was performed. The overexpression and knockdown efficiency of RCN2 are presented in Fig. 3G. As demonstrated in Fig. 3F, miR-183-5p knockdown significantly increased the percentage of CRC cells in the S phase, which was reversed by the over-expression of RCN2,

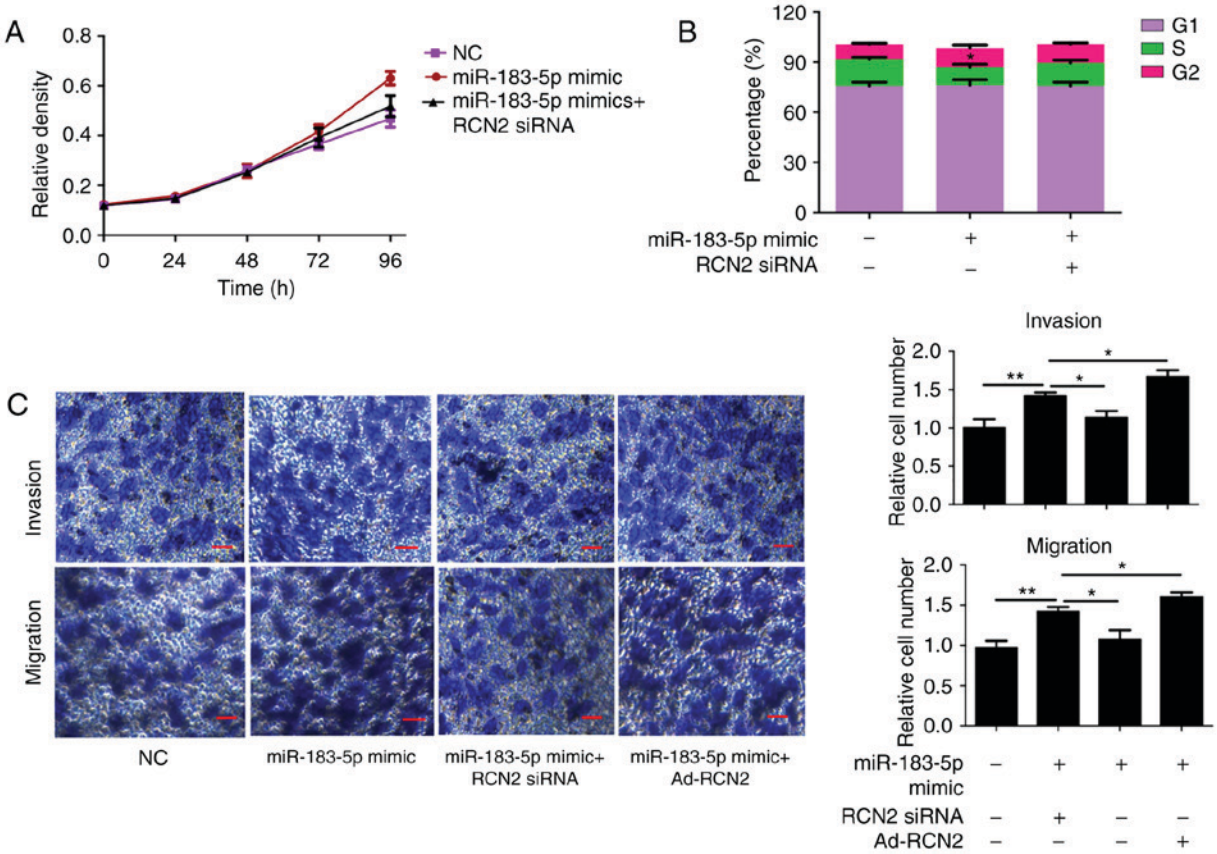

Figure 4. Overexpression of miR-183-5p promotes CRC cell proliferation, invasion and migration via RCN2. (A) An MTT assay revealed that miR-183-5p overexpression promoted CRC cell proliferation, and RCN2 knockdown led to proliferation retardation in CRC cells in which miR-183-5p was overexpressed. (B) Cell cycle analysis revealed that miR-183-5p upregulation significantly inhibited the percentage of CRC cells in the S phase via RCN2. (C) Transwell results demonstrated that the increased migratory and invasive potential of miR-183-5p-overexpressing cells was rescued by RCN2 silencing in SW480 cells. Scale bars, 100 μm. All data are expressed as the mean ± standard deviation (n=3). *P<0.05, **P<0.01. RCN2, Reticulocalbin-2; CRC, colorectal cancer; miR, microRNA; NC, negative control; siRNA, small interfering RNA; Ad, adenovirus.

and the percentage of CRC cells in the S phase was also increased to a greater extent following RCN2 silencing. It was also observed that the overexpression of RCN2 reversed the anti-proliferative and anti-invasive effect of miR-183-5p knockdown in SW620 cells. The results also demonstrated that co-transfection with miR-183-5p inhibitor and RCN2 siRNA further inhibited cell proliferation, invasion and migration (Fig. 3E and H).

Overexpression of miR-183-5p promotes proliferation, invasion and migration via RCN2. In the present study, miR-183-5p was overexpressed to verify the role of miR-183-5p in CRC. MTT assays showed that miR-183-5p overexpression promoted CRC cell proliferation, and RCN2 knockdown led to a decrease in proliferation in CRC cells in which miR-183-5p was overexpressed (Fig. 4A). The upregulation of miR-183-5p significantly inhibited the percentage of CRC cells in the S phase, an effect which was reversed by the downregulation of RCN2 (Fig. 4B). The Transwell assay results illustrated that the increased migratory and invasive potential of miR-183-5p-overexpressed cells was rescued by RCN2 silencing in SW480 cells (Fig. 4C). Notably, the overexpression of RCN2 did not further increase the proliferation, invasion and migration of miR-183-5p-treated cells (data not shown).

Downregulation of miR-183-5p or RCN2 inhibits Wnt/β-catenin signaling pathway. The aforementioned results demonstrated that miR-183-5p was positively associated with RCN2 levels, and that the downregulation of miR-183-5p inhibited the proliferation and invasion of CRC cells. However, its potential molecular mechanisms remain unknown. The Wnt/β-catenin pathway serves an important role in the development of tumors. A recent study reported that miR-183 may have an inhibitory effect in osteosarcoma by regulating Wnt/β-catenin (9). Therefore, the present study aimed to investigate the effects of miR-183-5p on Wnt/β-catenin pathway downstream genes, including cyclin D1, c-Myc and MMP-2, in the SW620 cell line. β-Catenin protein and its downstream targets, cyclin D1, c-Myc and MMP-2, were all demonstrated to be reduced in CRC cells in which miR-183-5p or RCN2 were downregulated (Fig. 5). These data suggested that miR-183-5p downregulation inhibits CRC cell proliferation, invasion and migration by blocking the Wnt/β-catenin pathway.

Discussion

An increasing body of evidence has suggested that the pathogenesis and progression of CRC is a complex biological process attributed to the dysregulation of numerous oncogenes and tumor-suppressive genes (21). Although diagnostic and

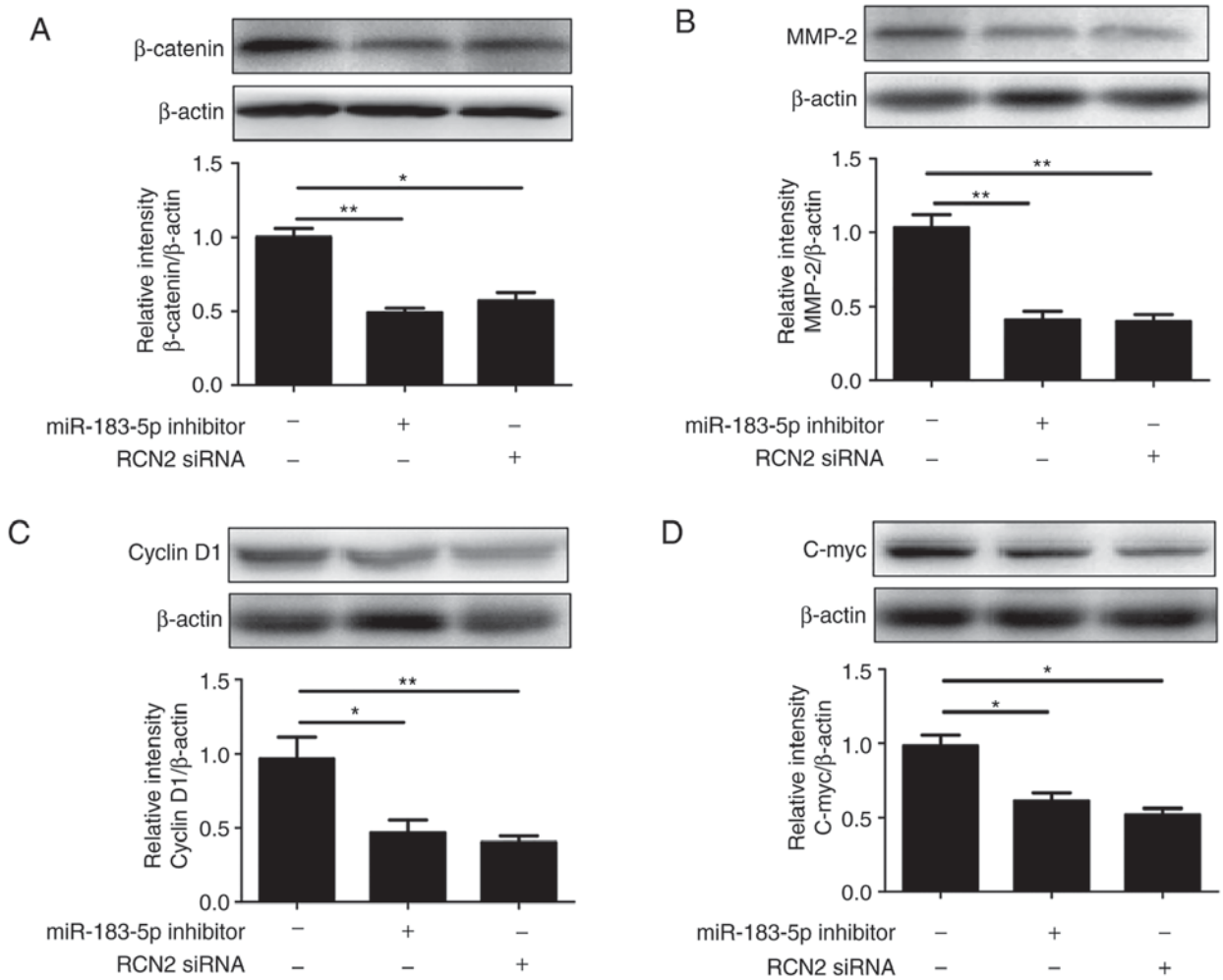

Figure 5. Downregulation of miR-183-5p or RCN2 inhibits the Wnt/β-catenin signaling pathway. Western blot analysis was used to determine the protein expression of (A) β-catenin, (B) MMP-2, (C) cyclin D1 and (D) c-Myc and in the SW620 cell line. All data are expressed as the mean ± standard deviation (n=3). *P<0.05, **P<0.01. RCN2, reticulocalbin-2; miR, microRNA; MMP-2, matrix metalloproteinase-2; siRNA, small interfering RNA.

therapeutic techniques have improved, the clinical outcomes and prognosis of numerous patients with CRC remains very poor. Moreover, there are few reliable markers available to accurately predict early-stage CRC, making the diagnosis and treatment particularly challenging.

In recent years, many scholars have confirmed that miR-183-5p is upregulated and involved in the occurrence and development of various types of malignant tumors. Cheng *et al* (15) reported that the expression level of miR-183-5p was markedly upregulated in breast cancer tissues compared with the adjacent normal tissues. The overexpression of miR-183-5p significantly promoted cell proliferation and inhibited cell apoptosis in breast cancer cells. Miao *et al* (22) demonstrated that miR-183-5p is overexpressed in pancreatic cancer and oncogenic miR-183-5p may serve as a pancreatic cancer biomarker. In addition, integrated analysis of miRNA datasets revealed that miR-183-5p was upregulated in CRC tissues and may serve a key role in the occurrence and development of CRC (16). However, the biological role of miR-183-5p in CRC remains to be elucidated. In the present study, it was demonstrated that miR-183-5p was significantly upregulated in CRC tissues compared with adjacent normal tissues. The knockdown of miR-183-5p impeded the migration and invasion of SW620 CRC cells. These results suggested that miR-183-5p may serve an important role in CRC progression.

At present, there have been no reports on the effect and mechanism of miR-183-5p on inhibiting the invasion and proliferation of CRC tumors, to the best of our knowledge. As a member of the RCN family, the transcription factor RCN2 became a focus of the present study. A number of previous studies have demonstrated that RCN1 serves an important role in a variety of solid tumors, including renal, breast, lung and liver cancer, and may promote tumor invasion and migration in these malignancies (23-26). In addition, RCN2, a homolog of RCN1, has been reported to be aberrantly upregulated in CRC tissues, and is associated with the overall survival of patients with CRC (10). However, it is not clear whether specific factors or genes are involved in the regulation of RCN2 expression in CRC. The majority of studies have demonstrated that miRNAs serve biological roles in different diseases by negatively regulating downstream genes. However, certain studies have suggested that miRNAs serve a role by positively regulating downstream genes, including the tumor suppressor p53, which may be positively regulated by miR-542-3p in cancer (27). Notably, in the present study, it was observed that miR-183-5p was

able to positively regulate the expression of RCN2 to promote invasion and migration in SW620 cells. The present data suggested that the reduced expression of miR-183-5p alleviated CRC cell invasion and migration by decreasing RCN2 expression, and may have an anti-proliferative effect in CRC.

Wnt/β-catenin signaling serves a critical role in regulating cell proliferation, invasion and differentiation by regulating downstream target genes, including cyclin D1, c-Myc and MMP-2 (28,29). The western blot analysis results demonstrated that the downregulation of miR-183-5p or RCN2 expression inhibited the protein expression of β-catenin, cyclin D1, c-Myc and MMP-2. These data demonstrated that the inhibition of miR-183-5p suppressed CRC cell growth and invasion by blocking the Wnt/β-catenin pathway.

In conclusion, the present results indicated that miR-183-5p functions as an oncogenic transcriptional gene that promotes migration, invasion and metastasis in CRC by specifically regulating the RCN2 expression. These findings suggested that miR-183-5p and RCN2 serve an important role in the molecular etiology of CRC and indicate its potential applications in CRC treatment. However, further studies are required to clarify the potential mechanisms of miR-183-5p in CRC.

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Availability of data and materials

The datasets generated during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YM, GW, LX and JZ designed the study and performed the experiments. FL, LQ and XY performed the statistical analyses and wrote the manuscript. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate

Informed consent was obtained from all patients and ethical approval was obtained from the Institutional Review Board of The Second People's Hospital of Lianyungang.

Patient consent for publication

Written informed consent was obtained from all patients.

Competing interests

The authors declare that they have no competing interests.

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