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miR-19a targets *CLCA4* to regulate the proliferation, migration, and invasion of colorectal cancer cells

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ABSTRACT

The role of *miR-19a* in colorectal cancer (CRC), a devastating disease with high mortality and morbidity, remains controversial. In the present study, we show that the level of *miR-19a* is significantly higher in clinical CRC tissue samples than in paracancerous tissue samples, and significantly higher in CRC cells lines HT29, SW480, and CaCO2 than in the normal human colon mucosal epithelial cell line NCM460. *miR-19a* mimics and inhibitors were synthesized and validated. Overexpression of *miR-19a* mimics significantly promoted, while *miR-19a* inhibitors inhibited, the proliferation, survival, migration, and invasion of SW480 and CaCO2 CRC cells. Furthermore, mRNA and protein levels of chloride channel accessory 4 (CLCA4) were lower in CRC cells and tissues. Bioinformatics and a luciferase reporter assay confirmed that CLCA4 was a *miR-19a* target. Further, *miR-19a* inhibition increased CLCA4 expression. The inhibitory effect of *miR-19a* on cell growth, survival, migration, and invasion was reversed by knockdown of CLCA4 expression. The data demonstrated that the *miR-19a*/CLCA4 axis modulates phospho-activation of the PI3K/AKT pathway in CRC cells. In conclusion, our results revealed that *miR-19a* overexpression decreases CLCA4 levels to promote CRC oncogenesis, suggesting that *miR-19a* inhibitors have potential applications for future therapeutic of CRC.

Key words: Colorectal cancer; *miR-19a*; CLCA4; proliferation; migration; invasion

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Introduction

Colorectal cancer (CRC) is the third-most common malignant tumor in the world and the fourth leading cause of cancer-related death.[1] The global distribution of CRC varies greatly. In developing countries, especially those in Asia, South America, and Eastern Europe, the morbidity and mortality of CRC have increased rapidly, partly due to aging of the population and unhealthy lifestyles.[2] Early CRC can be effectively treated through surgical resection and adjuvant chemotherapy.[3] However, there are no effective treatments for patients with advanced CRC, especially when metastases are present.[4] The prognosis of patients with CRC metastases is extremely poor, with a 5-year survival rate of 12%.[5-7] In recent years, in-depth understanding of pathways related to the transformation and proliferation of cancer cells has promoted the development of targeted therapies, and a variety of novel therapeutics have been developed for the treatment of CRC.[8] However, CRC is a complex multi-stage process, leaving many disease mechanisms uninvestigated. Further clarifying the detailed molecular mechanisms of the occurrence and development of CRC is essential for identifying novel CRC therapeutics

MicroRNAs (miRNAs) are a class of evolutionarily conserved, non-coding single-stranded small RNAs with a length of approximately 22 nucleotides.[9,10] miRNAs specifically bind to target mRNAs to inhibit their translation, thus repressing target genes at the post-transcriptional level.[11,12] miRNAs affect cell proliferation, differentiation, and apoptosis by regulating activation and expression of critical signaling molecules such as transcription factors, cytokines, growth factors, pro-apoptotic mediators, and anti-apoptotic mediators.[13-15] Recently, dysregulation of miRNA expression has been identified to play an important role in progression and metastasis of CRC.[16] The first report of a role for miRNAs in colon cancer was the downregulation of *miR-143* and *miR-145*,[17] which leads to the development of cancer through EGFP signaling.[18] The AKT-PI3K-Pten pathway downregulates *miR-126*, *miR-497,* and *miR-1* and upregulates *miR-21*, *miR-19,* and *miR-96* to mediate the occurrence and progression of CRC.[19]

miR-19a is involved in the development of CRC. *miR-19a* promotes cell proliferation and migration by targeting *TIA1* in CRC.[20] Further, *miR-19a* enhances cell proliferation, migration, and invasion by enhancing lymphangiogenesis *via* thrombospondin-1 in CRC.[21] *miR-19a* silencing significantly suppresses the epithelial-mesenchymal transition, invasion, migration, and proliferation of CRC cells.[22] However, the role of miR-19a and its specific downstream targets in CRC require further investigation.

Chloride channel accessory 4 (CLCA4) is a member of the calcium-activated chloride channel protein family, which is characterized by multiple symmetrical cysteine motifs in the amino terminus tail.[23] CLCA4 is involved in the occurrence and development of cancer. In bladder cancer, CLCA4 suppresses cellular growth and metastasis through the PI3K/AKT pathway.[24] In breast cancer, *CLCA4* knockdown promotes breast cancer cell migration and invasion.[25] Thus, CLCA4 acts as a tumor suppressor, which is critical for suppression of tumor cell growth, proliferation, migration, and invasion. However, the upstream regulation of CLCA4 in CRC remains incompletely understood and requires further study.

In the present study, we measured the expression levels of *miR-19a* and CLCA4 in CRC tissues and cell lines to elucidate their up- and downstream regulatory relationships. Further, we assessed the effects of *miR-19a* and CLCA4 on growth, migration, and invasion of CRC cells, providing new insights for the diagnosis and treatment of CRC.

Materials and Methods

Samples and cell lines

CRC tissues (16 pairs of cancer and paracancerous tissues) were provided by the Pathology Department of Guangzhou Red Cross Hospital. Written consent was signed by the patients, and the study was approved by the Ethics Committee of the Jinan University. Adjacent non-cancerous tissues were used as controls, and were considered to be paracancerous tissues. The normal human colon mucosal epithelial cell line NCM460 and the CRC cell lines HCT116, HT29, SW480, and CaCO2 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell lines were maintained in DMEM (Hyclone, cat no. #SH30022.01, Logan, UT, USA) containing 10% FBS (Gibco, cat. no. #10270-106, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and 100 U/mL of penicillin and streptomycin (Gibco, cat. no. #15140122) at 37°C in a humid atmosphere containing 5% carbon dioxide.

Constructs and transfection

The *CLCA4* gene (NM_012128.4) sequence was downloaded for comparison. *CLCA4* cDNA was amplified *via* PCR to obtain full-length human *CLCA4*, which was validated by gene sequencing and subsequently inserted into a pcDNA3.1 or pEGFP-C1 vector according to the manufacturer's instructions (Realgene, Nanjing, China). Hairpin RNAs (shRNAs) encoding a knockdown sequence against *CLCA4* were inserted into the GV248 vector (GeneChem, Shanghai, China). Synthetic *miR-19a* mimics, *miR-19a* inhibitors, and scrambled negative control RNAs (control mimics and inhibitors) were purchased from GenePharma (Shanghai, China). Lipofectamine 2000 (Invitrogen, cat. no. #11668027 was used for transfection with the indicated constructs or fragments according to the manufacturer's instructions. At 6 h after transfection, medium was changed to DMEM (Gibco, #11965092) supplemented with 2% FBS, and cells were harvested 48 h after transfection for total RNA or protein isolation.

Immunohistochemistry

Tissue samples were baked at 55°C for 1.5 h, deparaffinized with xylene and rehydrated using an alcohol gradient. Tissue slides were then treated with 3% hydrogen peroxide in methanol for 30 min, and antigens were retrieved in 0.01 M sodium citrate buffer (pH 6.0) using a microwave oven. Subsequently, the samples were blocked with 10% normal goat serum, and then were incubated with a primary antibody against CLCA4 (cat.no #ab197347, 1:500, Abcam, Cambridge, UK) at 4°C overnight. The slides were then incubated with a non-biotin horseradish-peroxidase detection system (Gene Tech). Immunohistochemical images were captured and analyzed by Image J software (NIH). Slides with no primary antibody were used as negative controls.

RT-qPCR

TRIzol reagent (Life Technologies, Carlsbad, CA, USA) was used to extract total cellular RNAs from cell lines or tissue samples according to the manufacturer's instructions. For miRNA reverse transcription, an EasyScript cDNA Synthesis SuperMix (cat. no. # AE301-02, TransGen Biotech, China) was used. Real-time PCR was performed using TransStart Top Green quantitative PCR (qPCR) SuperMix (cat. no. #AQ131-01, TransGen Biotech,. The primer sequences used: human CLCA4 (5'-TTTGGGGCTCTTACATCAGG-3' and 5'-GTGTCGTTCATCCAGGCATT-3'), *actin* (F: 5'- TCACCCACACTGTGCCCATCTACGA -3' and R: 5'-GGATGCCACAGGATTCCATACCCA -3'), miR-19a (F: 5'-CCTCTG-TTAGTTTTGCATAGTTGC-3' and R: 5'-CAGGC-

CACCATCAGTTTTG-3') and U6 (F: 5'- CTCGCTTCG-GCAGCACA -3' and R: 5'- AACGCTTCACGAATTTGCGT -3'). *miR-19a* and *CLCA4* mRNA expression levels were normalized to those of *U6* and *beta-Actin*, respectively. All PCR reactions were repeated at least three times.

Western blotting

Proteins from cells or tissue samples were isolated in RIPA lysis buffer (cat. no. # P0013C, Beyotime, Shanghai, China) with freshly added PMSF (cat. no. #ST506, Beyotime). Proteins were then separated by 8% SDS-PAGE and transferred to PVDF membranes (cat. no. # IPVH00010, Millipore Corp, Billerica, MA, USA). After blocking with a 5% skim milk solution, membranes were incubated with primary antibodies (CLCA4, cat. no. #ab197347, 1:1000 or GAPDH, cat. no. #ab8245, 1:2000, both from Abcam) overnight at 4°C. After washing, membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 h, and blots were then visualized using an ECL kit (cat. no. #P0018S, Beyotime).

Cell counting Kit-8 (CCK-8) assay

Cell growth was detected using the CKK-8 assay. Cells were cultured in 96-well plates and incubated overnight in DMEM medium with 10% FBS. After transfection, CCK-8 solution (cat. no. #CK04-500, Dojindo, Japan) was added 24, 48, 72, or 96 h post-transfection, according to the manufacturer's instructions. To quantify cell proliferation, the absorbance was measured at 450 nm and data were analyzed.

Flow cytometry

To detect apoptosis, cells stained with an Annexin V-FITC/PI Dual Staining Kit (cat. no. #640914, Biolegend, San Diego, CA, USA) were subjected to flow cytometric analysis. Briefly, cells were trypsinized and harvested. Next, 100 µL of the cell suspension was transferred to a tube and mixed with 5 µL Annexin V-FITC (1 mg/ml) and 5 µL propodium iodide (2.5 mg/mL). Tubes were gently vortexed and incubated for 15 m at room temperature in the dark. Binding buffer (400 µL) was added, and the samples were analyzed using a FACS machine (BD Biosciences, San Jose, CA, USA). Each experiment was conducted in triplicate.

Transwell assay

To determine cellular migration and invasion ability, cells were assessed using a Transwell system (Corning, Lowell, MA, USA). After transfection, cells were suspended in RPMI medium and added to the upper chamber. After a 24 h incubation, cells on the surface of upper chamber were removed by scraping with a cotton swab. The membrane was stained with 0.1% crystal violet for 10 min and migrated/invaded cells were counted under an optical microscope (IX81, Olympus, Tokyo, Japan). For the invasion assay, 10% Matrigel glue (Corning BioCoat, Corning, NY, USA) was pre-laid in the upper chamber for 6 h.

Luciferase reporter assay

Wildtype (WT) and mutant (Mut) *CLCA4* 3' untranslated regions (UTRs) with the *miR-19a* binding site were cloned into the pGL3-control vector (cat. no. # E1751, Promega, Madison, WI, USA) to generate the luciferase reporter construct (the sequences that matched are shown in Figure 4F). Luciferase activity assays were performed using a Dual Luciferase Assay System (Promega) according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (SPSS Inc, Chicago, IL, USA). Data are expressed as means ± SD. Student's *t*-tests were used for comparison between two groups. A one-way analysis of variance (ANOVA) was applied for multiple group comparisons; $p<0.05$ was considered statistically significant.

Results

Upregulation of *miR-19a* in CRC

We determined the levels of *miR-19a* in clinical CRC tissue samples and paracancerous margin tissue samples as controls using RT-qPCR, which revealed that *miR-19a* levels were higher in CRC tissue samples than in paracancerous tissue samples (Figure 1A). Furthermore, the expression levels of *miR-19a* in cultured CRC cell lines HT29, SW480, and CaO_2, and in the normal human

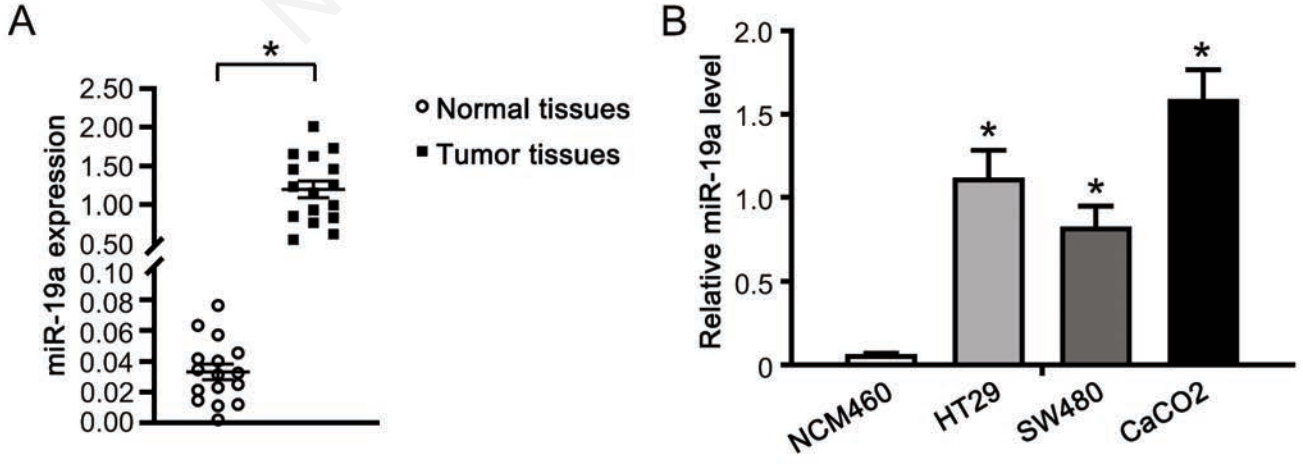

Figure 1. Expression levels of *miR-19a* are upregulated in CRC tissues and cell lines. A) RT-qPCR was used to measure expression levels of *miR-19a* in 19 pairs of CRC tumor tissues and adjacent paracancerous control tissues. B) The normal human colon mucosal epithelial cell line NCM460 and the CRC cell lines HT29, SW480, and $CaCO_2$ were subjected to RT-qPCR to measure *miR-19a* levels. *p<0.05.

colon mucosal epithelial cell line NCM460 as a control, were also measured using RT-qPCR, which revealed that *miR-19a* levels were higher in CRC cell lines than in the NCM460 cell line (Figure 1B). These results indicated that *miR-19a* is overexpressed in CRC.

miR-19a promoted proliferation, migration and invasion functions of CRC cells

Next, we determined the role of *miR-19a* in CRC development. Fragments of *miR-19a* mimics and inhibitors and control fragments (*miR-NC* and *inhibitor-NC*) were purchased. These fragments were first overexpressed in the CRC cell lines $CaCO_2$ and SW480 to determine their effect on *miR-19a* expression. Overexpression of *miR-19a* mimics significantly upregulated *miR-19a* levels in both cell lines (Figure 2A). Conversely, overexpression of *miR-19a* inhibitors markedly decreased *miR-19a* levels (Figure 2B). Further, cells with *miR-19a* overexpression or inhibition were subjected to analyses to assess proliferation,

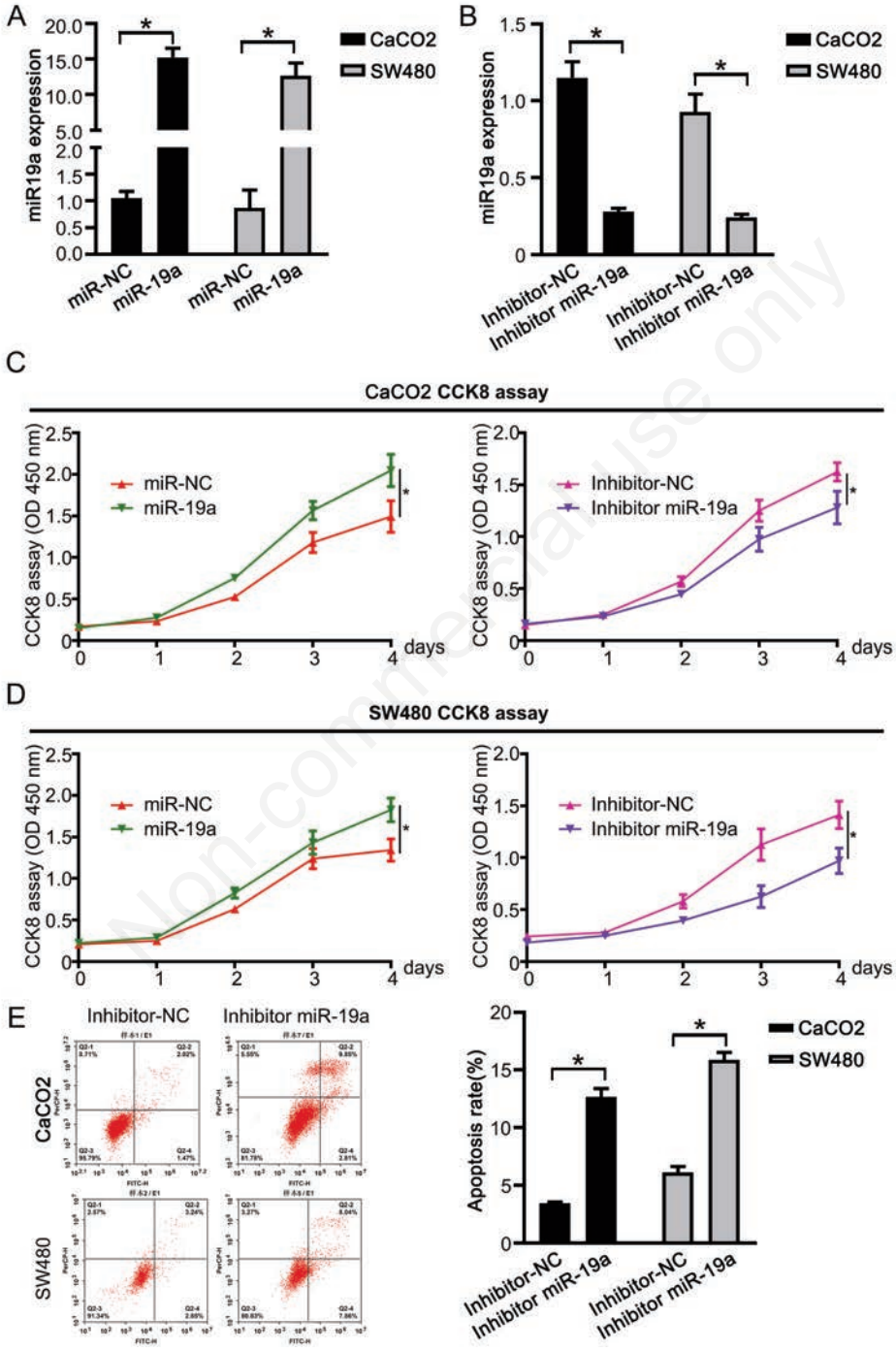

Figure 2. Regulatory role of miR-19a in growth and apoptosis of CRC cells. A,B) Synthesized *miR-19a* mimics or inhibitors or controls (miR-NC or inhibitor-NC, respectively) were transfected into SW480 and $CaCO_2$ cells, and *miR-19a* was measured by RT-qPCR. C,D) CRC cells transfected with *miR-19a* mimics or inhibitors were subjected to a CCK-8 assay. The growth rate, indicated by the OD at 450 nm, is shown. E) Cells were treated as in (C) and were subjected to flow cytometry to detect apoptosis. *p<0.05.; n=3.

migration and invasion functions. CCK-8 proliferation assays revealed that *miR-19a* overexpression significantly promoted, while *miR-19a* inhibition significantly suppressed, cell viability in both CaCO2 (Figure 2C) and SW480 (Figure 2D) cells. Flow cytometry assays revealed that inhibition of *miR-19a* expression induced apoptosis in both cell lines (Figure 2E), whereas overexpression of *miR-19a* did not (*data not shown*). A Transwell assay revealed that transfection of *miR-19a* mimics significantly promoted, while transfection of *miR-19a* inhibitors suppressed, the migration and invasion ability of both CaCO2 (Figure 3A-B) and SW480 (Figure 3C-D) cells. Taken together, these results suggested that *miR-19a* functions as an oncogene to promote the growth, migration, and invasion of CRC cells.

CLCA4 was a direct *miR-19a* target in CRC

We next determined the downstream effector(s) of *miR-19a* in CRC. Bioinformatics analysis by TargetScan,[26] miRDB,[27] and picTar[28] online software revealed a variety of potential *miR-19a* targets, and among them, CLCA4 was identified as a target gene of interest. We thus measured CLCA4 expression in CRC. The mRNA level (Figure 4A) measured by RT-qPCR and the protein level (Figure 4 B,C) measured by Western blotting were both significantly lower in CRC cell lines than in NCM460 cells. Clinical CRC tissues were subjected to immunohistochemistry, revealing that protein levels of CLCA4 were significantly lower in CRC tissues than in paracancerous controls (Figure 4D). The quantified data from each pair of samples showed the same trend (Figure 4E). Informatics analysis tools identified a *miR-19a* target sequence in the 3'UTR of *CLCA4* (Figure 4F). We thus constructed a luciferase reporter assay to determine the direct regulatory relationship between *miR-19a* and CLCA4. Wildtype (WT) and mutant (Mut) 3'UTR constructs of CLCA4 containing the indicated sequences (Figure 4F) were co-transfected with *miR-19a* mimics in both CaCO2 and SW480 cells. Overexpression of *miR-19a* significantly suppressed the luciferase signal of the WT *CLCA4* 3'UTR reporter, but not that of the Mut *CLCA4* 3'UTR reporter (Figure 4F). These data suggested that CLCA4 is a direct target of *miR-19a* in CRC.

The *miR-19a*/CLCA4 axis regulated growth, apoptosis, migration, and invasion of CRC cells

Next, we determined if *miR-19a* targeted CLCA4 to regulate CRC development. CaCO2 and SW480 CRC cells were transfected with *miR-19a* inhibitors, with or without an shRNA vector targeting *CLCA4*. Western blotting revealed that *miR-19a* inhibitors significantly increased CLCA4 protein levels in both cell lines, which was abolished by co-transfection of *shCLCA4*-encoding plasmids (Figure 5 A,B). Overexpression of *miR-19a* inhibitors suppressed the growth of CaCO2 (Figure 5C) and

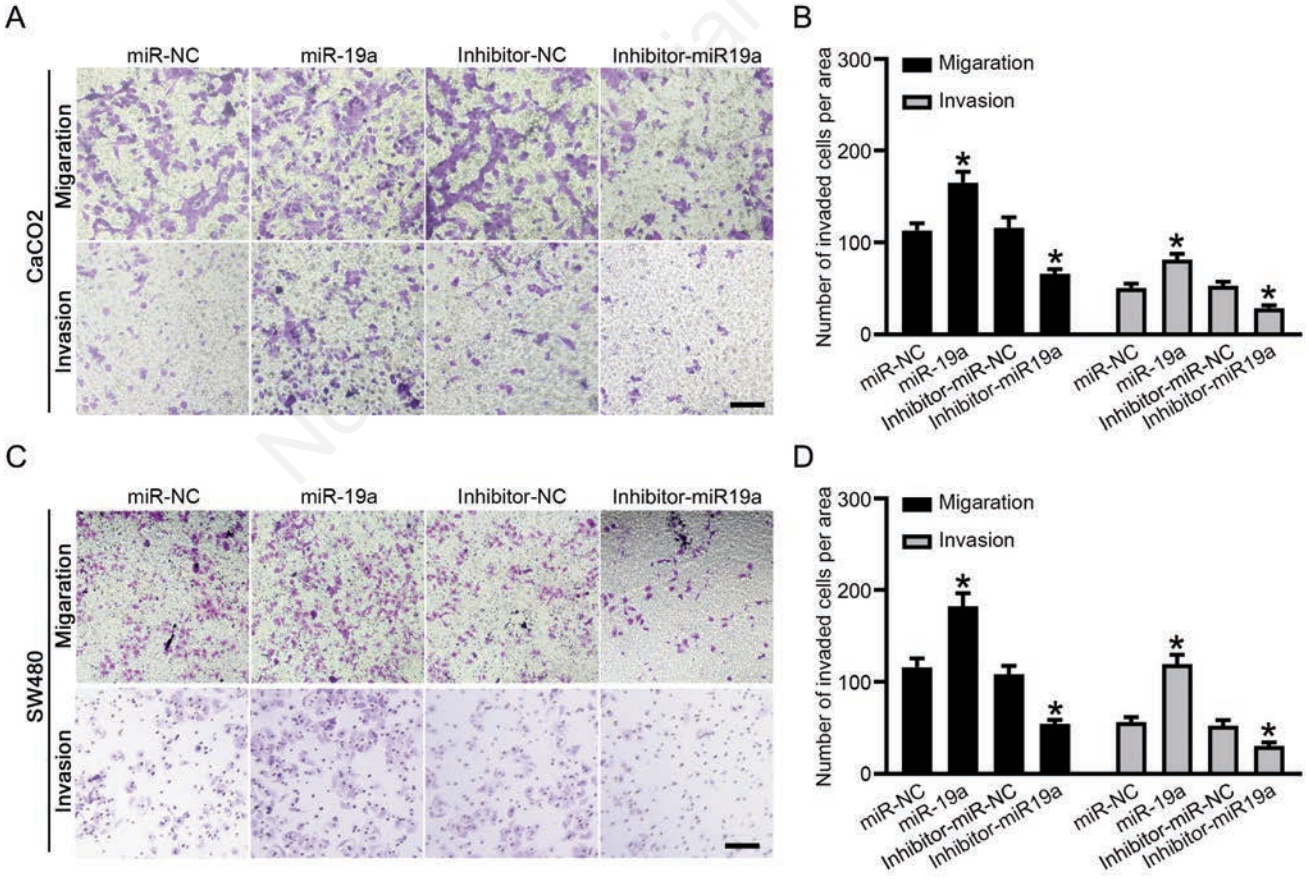

Figure 3. Effect of miR-19a on CRC cell migration and invasion. Cells were treated as in Figure 2, and were then subjected to migration and invasion assays. Representative images of migrated and invaded cells are shown (A,C), and cells were counted for quantitative analyses as shown in (B,D). **p<0.01; ***p<0.001; n=3; scale bars: 20 μm.

SW480 (Figure 5D) cells, but this inhibitory effect could be reversed by co-transfection of *shCLCA4* (Figure 5 C,D). Furthermore, *miR-19a* inhibition increased apoptosis, which was also abolished by *CLCA4* knockdown (Figure 5 E,F). The same trend was observed in Transwell assays in both CaCO2 (Figure 5 G,H) and SW480 (Figure 5 I,J) cells. Taken together, these results indicated that *miR-19a* regulates CRC cell growth, apoptosis, migration, and invasion by modulating CLCA4 protein levels.

miR-19a/CLCA4 regulated the PI3K/AKT pathway in CRC cells

CLCA4 is related to the PI3K/AKT pathway in other cancers such as hepatocellular and breast cancers.[25,29] Thus, we determined if *miR-19a*/CLCA4 could also regulate this pathway in CRC. CaCO2 and SW480 (Figure 6A) cells were transfected with *miR-19a* mimics with or without *CLCA4*-encoding plasmids. *miR-19a* overexpression significantly decreased CLCA4 protein levels, and increased phosphorylated PI3K levels and phosphorylated AKT

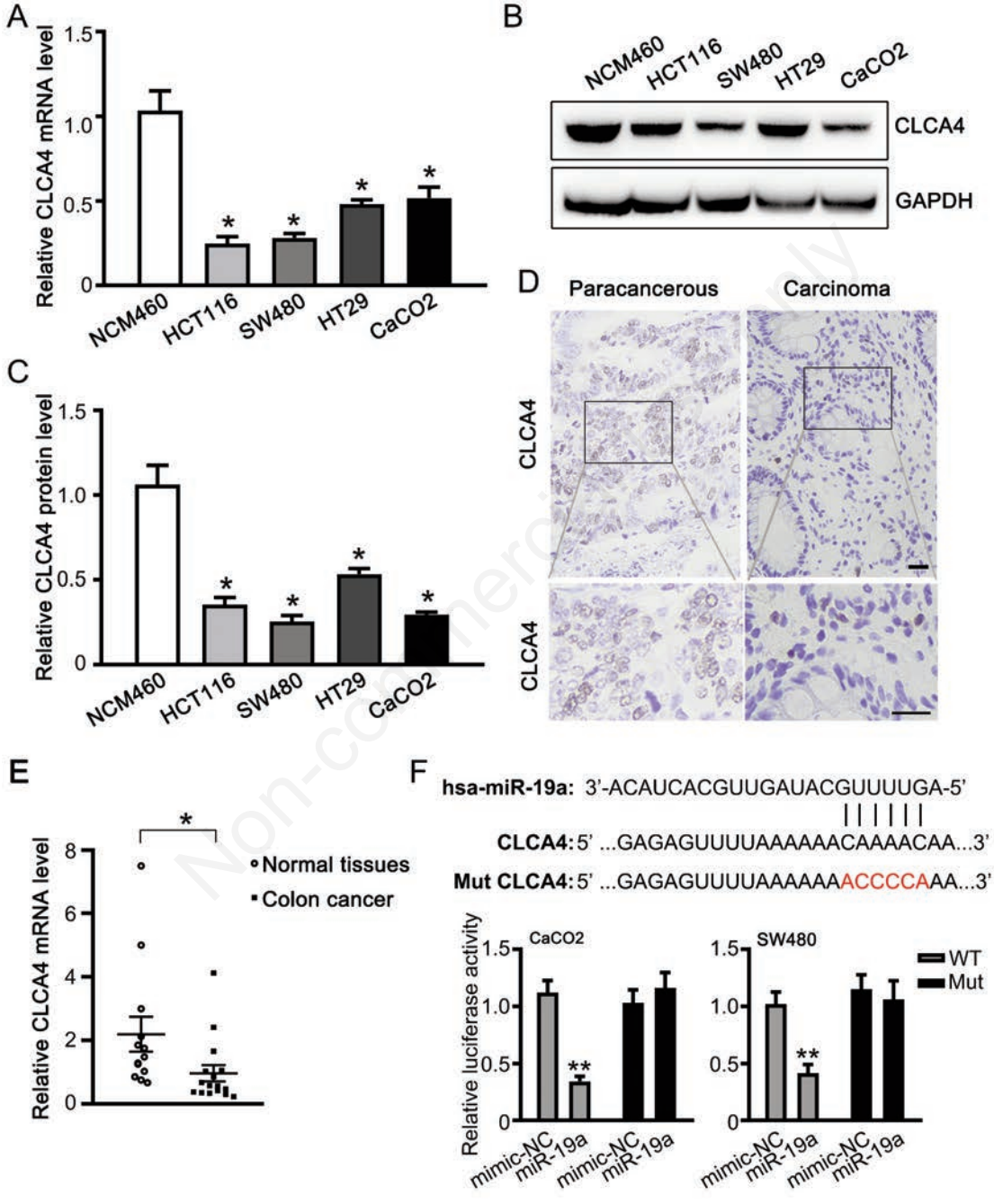

Figure 4. Expression of CLCA4 in CRC. A) *CLCA4* mRNA levels were detected in CRC and control cell lines. B) Protein levels of CLCA4 were detected in CRC and control cell lines, with quantified data shown in (C). D) The distribution of CLCA4 in CRC tissues was assessed by immunohistochemistry; scale bar: 50 μm. The relative expression level of CLCA4 in IHC images was measured as shown in (E). (F) Sequence of the *CLCA4* 3'UTR with the miR-19a target sequence is shown, and the mutant (Mut) sequence miR-19a target sequence is shown in red. Cells were transfected with luciferase reporter genes expressing WT or Mut *CLCA4* 3' UTRs, with or without *miR-19a* mimics. Relative luciferase activity is shown. *p<0.05; **p<0.01.

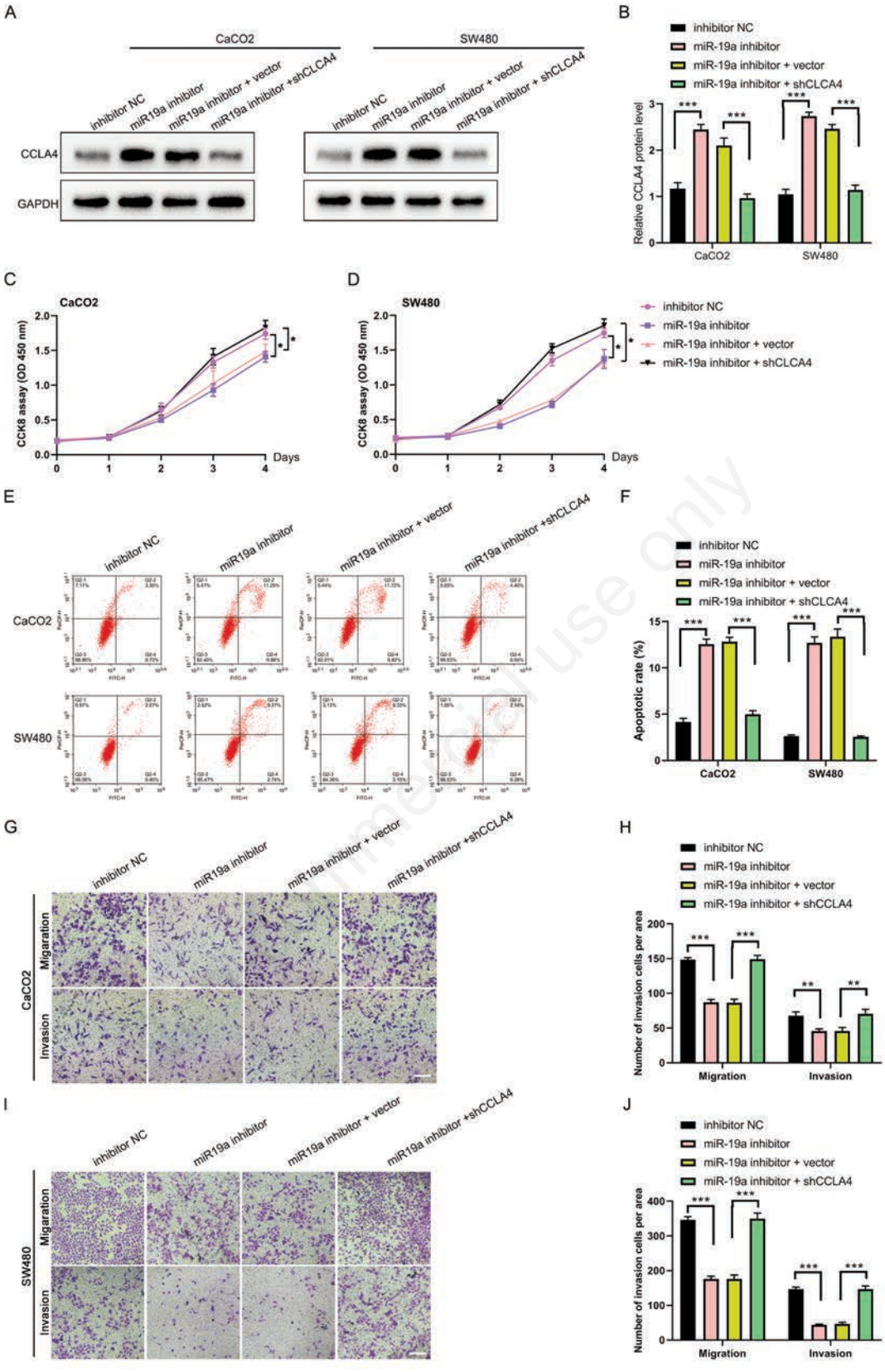

Figure 5. The *miR-19a*/CLCA4 axis regulates cell development of CRC cells. A,B) Cells were transfected with *miR-19a* inhibitor or NC, with or without an *shCLCA4*-encoding plasmid; cells were then subjected to Western blotting with CLCA4 antibody. The images and relative expression levels of CLCA4 are shown. C,D) CCK-8 assay to detect the growth of CRC cells treated as in (A). E,F) Flow cytometry assay to detect cell apoptosis in CRC cells treated as in (A). Transwell assay to detect cell migration (G-H) and invasion (I-J) in CRC cells treated as in (A). **p<0.01; ***p<0.001; n=3; scale bars: 20 μm.

levels, but did not affect total levels of PI3K and AKT (Figure 6B). Quantified data revealed that *miR-19a* overexpression significantly decreased PI3K (Figure 6C) and AKT (Figure 6D) phosphorylation, which was abolished by *CLCA4* overexpression. These data indicated that the *miR-19a*/CLCA4 axis could function via activation of the PI3K/AKT pathway in CRC cells.

Discussion

In the present study, we demonstrated that *miR-19a* expression was upregulated in CRC tissue and cell lines. Further, overexpression of *miR-19a* promoted, while inhibition of *miR-19a* suppressed, proliferation, migration, and invasion of both CaCO2 and SW480 CRC cells. Inhibition of *miR-19a* also significantly increased apoptosis in CRC cells. CLCA4 levels were downregulated in CRC tissues and in multiple CRC cell lines. We identified CLCA4 as a direct target of *miR-19a* and further demonstrated that *miR-19a* regulated the growth, apoptosis, migration, and invasion of CRC cells by modulating the CLCA4 expression level. Furthermore, the *miR-19a*/CLCA4 axis activated the PI3K/AKT pathway in CRC cells. This study thus identifies a role for the *miR-19a*/CLCA4 axis in CRC.

Prior studies have indicated that miRNA dysregulation is a predictor of poor prognosis of various digestive cancers, including CRC, oral cancer, and gastric cancer.[30-32] For example, *miR-301a* and *miR-135b* induce CRC cell proliferation, migration, and invasion by regulating the TGF-β signaling pathway.[33,34] Similarly, *miR-638* promotes the proliferation, migration, and invasion of CRC by up-regulating the expression of SOX2 and TSPAN1 proteins.[35,36] *miR-19a* expression contributes to the development of multiple cancers. For example, in bladder cancer cells, *miR-19a* is upregulated, promoting cell invasion and EMT *via* targeting RhoB.[37] Overexpression of *miR-19a* is a risk factor for poor prog-

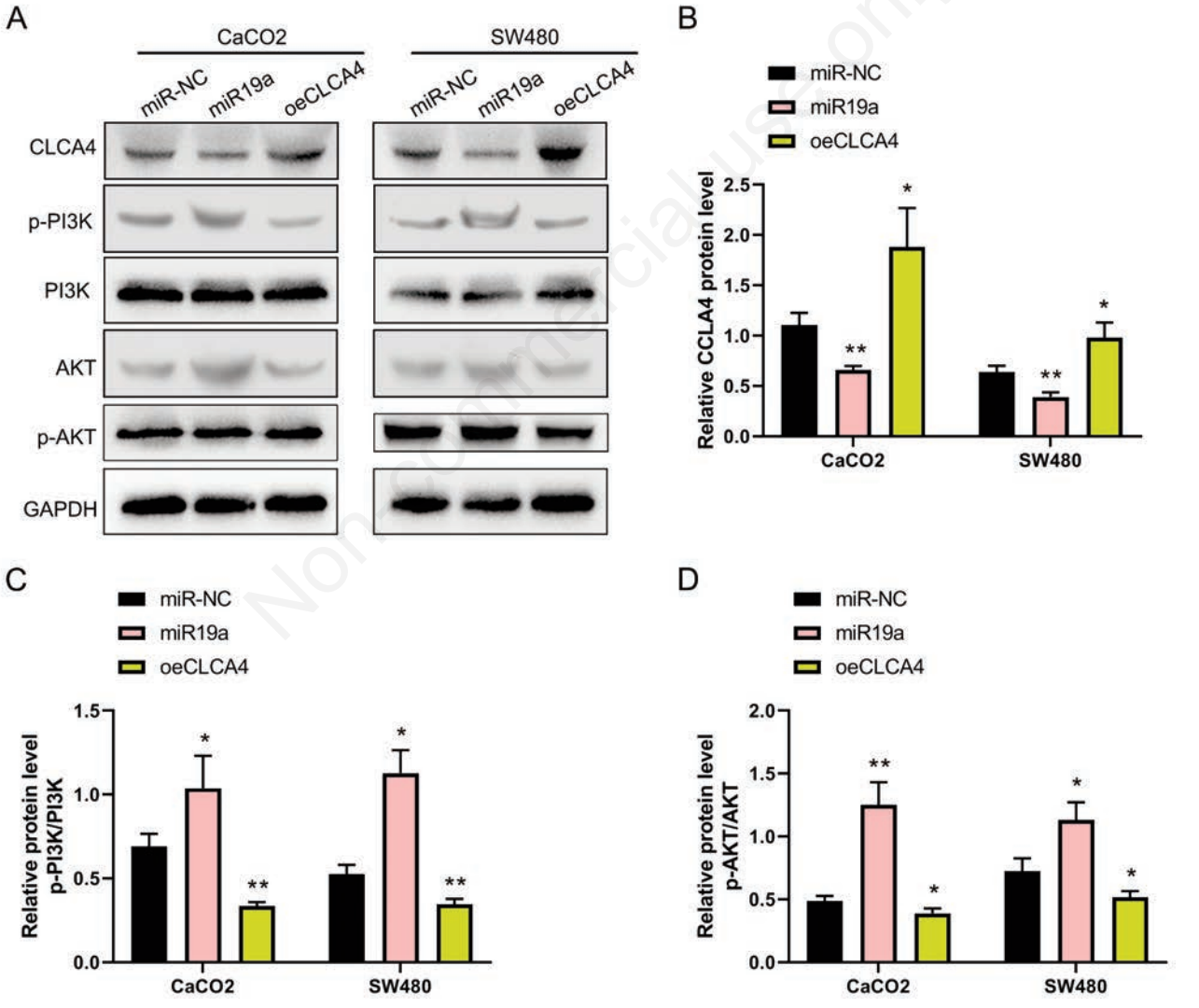

Figure 6. The *miR-19a*/CLCA4 axis regulates activation of the PI3K/AKT pathway. A) $CaCO_2$ and SW480 CRC cells were transfected with *miR-19a* mimics fragments. NC, negative control; 48 h later, cells were subjected to Western blotting with anti-CLCA4, anti-PI3K, anti-phosphorylated PI3K (p-PI3K), anti-AKT, anti-phosphorylated AKT (p-AKT), and anti-GAPDH (loading control) antibodies. B) Quantified data of relative levels of CLCA4, p-PI3K/PI3K and p-AKT/AKT are shown. n=3; *p<0.05.

nosis in osteosarcoma, and is linked to the metastatic potential of the lymph nodes.[38] Moreover, overexpression of *miR-19a* contributes to chemoresistance in ovarian cancer and non-small cell lung cancers.[39,40]

However, the role of *miR-19a* in CRC is controversial. A prior study suggested that serum *miR-19a-3p* is upregulated in CRC patients, and that *miR-19a-3p* silencing suppresses EMT, invasion, migration, and proliferation of CRC cells.[22] Adenomatous polyposis coli regulates *miR-19a* through the β-catenin pathway in CRC.[41] Prior studies have also demonstrated that *miR-19a-3p* is downregulated in CRC and that overexpression of *miR-19a* inhibits CRC angiogenesis by suppressing KRAS expression.[42,43] In the present study, we found that *miR-19a* was significantly upregulated in tumor samples and in CRC cell lines, suggesting an oncogenic function for *miR-19a* in CRC. Further, inhibition of *miR-19a* suppressed, while overexpression of *miR-19a* promoted, growth, migration, and invasion of CRC cells.

There are four CLCA family members (CLCA1, CLCA2, CLCA3, CLCA4), all of which are located on chromosome 1p31-1p22 in the human *CLCA* gene.[44] CLCA proteins are activated by Ca^{2+} and play a role in chloride ion conduction in epithelial cells.[45] In addition, CLCA family members are not only involved in a variety of biological processes, including cell differentiation, adhesion, apoptosis, and inflammatory processes, but also in multiple tumors.[46] For example, CLCA1 increases spontaneous differentiation and decreases cell proliferation in CRC,[47,48] and CLCA2 negatively regulates tumor cell invasion.[49,50]

CLCA4 is primarily expressed in the colon, with a highly similar structure to those of CLCA1 and 2.[46] CLCA4 inhibits the proliferation and invasion of liver cancer cells through PI3K/AKT signaling.[29] Our findings demonstrated that CLCA4 was downregulated in both clinical CRC tissues and CRC cell lines and was a target of *miR-19a*, which contributed to the development of CRC. Consistently, overexpression of CLCA4 countered the oncogenic functions of *miR-19a*.

In bladder and liver cancers, CLCA4 inhibits proliferation and invasion of cancer cells through PI3K/AKT signaling.[24,29] Further, inhibition of CLCA4 in breast cancer promotes migration and invasion by regulating EMT.[25] Inhibition of miR153-3p leads to upregulation of CLCA4, which is neuroprotective[51] and reduces the toxicity of CLCA inhibitors in microglia.[52] Thus, CLCA4 could function by regulating the PI3K/AKT signaling pathway. In the present study, overexpression of *miR-19a* resulted in activation of PI3K/AKT pathway, and overexpression of *CLCA4* significantly suppressed the phosphorylation level of this pathway, which is consistent with previous reports.[24,29]

In the present study, we identified upregulation of *miR-19a* and downregulation of CLCA4 in CRC. Further, we demonstrated that CLCA4 was a direct target of *miR-19a*, and that miR-19a regulated the proliferation, migration, and invasion of CRC cells by targeting CLCA4. In summary, the study identified a novel role for the *miR-19a*/CLCA4 axis in regulating CRC cell growth, migration, and invasion, which could provide new insights into the clinical management of CRC.

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