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

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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%.\(^5\)\(^,\)\(^6\) 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.\(^7\)^\(^8\)\(^9\)\(^10\) 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.\(^11\)^\(^,\)\(^12\)\(^,\)\(^13\)\(^,\)\(^14\) Recently, dysregulation of miRNA expression has been identified to play an important role in progression and metastasis of CRC.\(^15\) The first report of a role for miRNAs in colon cancer was the downregulation of \(miR-143\) and \(miR-145\),\(^16\) which leads to the development of cancer through EGFP signaling.\(^17\) 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.\(^18\)

\(miR-19a\) is involved in the development of CRC. \(miR-19a\) promotes cell proliferation and migration by targeting \(TIA1\) in CRC.\(^19\) Further, \(miR-19a\) enhances cell proliferation, migration, and invasion by enhancing lymphangiogenesis through \(v\) through thrombospondin-1 in CRC.\(^20\) \(miR-19a\) silencing significantly suppresses the epithelial-mesenchymal transition, invasion, migration, and proliferation of CRC cells.\(^20\)\(^,\)\(^21\)\(^,\)\(^22\) 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 EGFP-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, China). The primer sequences used: human \(CLCA4\) (5'-TTTGGGCTCTTCATCAGG-3' and 5'-GTGTCGTTCATCCAGGCTAT-3'), \(actin\) (F: 5'-TCACCCACACTGTCGCCCATCTCAGA-3' and R: 5'-GGATGCCAAGATTCCATACCCCA-3'), \(miR-19a\) (F: 5'-CTCTG-3' and R: 5'-CACGGC-3').

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CACCATCAGTTTTG-3’) and U6 (F: 5’-CTCGCTTCGGGACGACA-3’ and R: 5’-AACGCTTCAGAAATTGCGT-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 CCK-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 propidium iodide (2.5 mg/ml). Tubes were gently vortexed and incubated for 15 min 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 CaCO2, and in the normal human colon mucosal epithelial cell line NCM460 were subjected to RT-qPCR to measure miR-19a levels. *p<0.05.

![Figure 1](https://example.com/figure1.png)

**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 CaCO2 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 CaCO₂ 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,

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*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 CaCO₂ 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.*  
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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, miRDB, and picTar 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](image_url) 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 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. For example, miR-301a and miR-135b induce CRC cell proliferation, migration, and invasion by regulating the TGF-β signaling pathway. Similarly, miR-638 promotes the proliferation, migration, and invasion of CRC by up-regulating the expression of SOX2 and TSPAN1 proteins. 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. Overexpression of miR-19a is a risk factor for poor prog-

![Figure 6](image_url)
nosis in osteosarcoma, and is linked to the metastatic potential of the lymph nodes. Moreover, overexpression of miR-19a contributes to chemoresistance in ovarian cancer and non-small cell lung cancer. However, the role of miR-19a in CRC is controversial. A prior study suggested that serum miR-19a-3p is upregulated in CRC tissues, and that miR-19a-3p silencing suppresses EMT, invasion, migration, and proliferation of CRC cells. Adenomatous polyposis coli regulates miR-19a through the β-catenin pathway in CRC. 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. 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 promoted, while overexpression of miR-19a inhibited, proliferation, 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. CLCA proteins are activated by Ca²⁺ and play a role in chloride ion conduction in epithelial cells. 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. For example, CLCA1 increases spontaneous differentiation and decreases cell proliferation in CRC, and CLCA2 negatively regulates tumor cell invasion. CLCA4 is primarily expressed in the colon, with a highly similar structure to those of CLCA1 and 2. CLCA4 inhibits the proliferation and invasion of liver cancer cells through PI3K/AKT signaling. 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. Further, inhibition of CLCA4 in breast cancer promotes migration and invasion by regulating EMT. Inhibition of miR153-3p leads to upregulation of CLCA4, which is neuroprotective and reduces the toxicity of CLCA inhibitors in microglia. 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.

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 regulates 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.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Center MM, Jemal A, Smith RA, Ward E. Worldwide variations in colorectal cancer. CA Cancer J Clin 2009;59:366-78.
3. Chen X, Qiu H, Chen Y, Wang M, Zhu P, Pan S, et al. A comparison of bevacizumab Plus TAS-102 and TAS-102 monotherapy for metastatic colorectal cancer: A systematic review and meta-analysis. Front Oncol 2021;11:690515.
4. Pratt M, Forbes JD, Knox NC, Bernstein CN, Van Domselaar G. Microbiome-mediated immune signaling in inflammatory bowel disease and colorectal cancer: support from meta-omics data. Front Cell Dev Biol 2021;9:716604.
5. Messersmith WA. NCCN guidelines updates: Management of metastatic colorectal cancer. J Natl Compr Canc Netw 2019;17:599-601.
6. Spartalis C, Schmidt EM, Elmasry M, Schulz GB, Kirchner T, Horst D. In vivo effects of chemotherapy on oncogenic pathways in colorectal cancer. Cancer Sci 2019;110:2529-39.
7. Torring ML, Falborg AZ, Jensen H, Neil RD, Weller D, Reguilon I, et al. Advanced-stage cancer and time to diagnosis: An International Cancer Benchmarking Partnership (ICBP) cross-sectional study. Eur J Cancer Care 2019;28:11.
8. Piawah S, Venook AP. Targeted therapy for colorectal cancer metastases: A review of current methods of molecularly targeted therapy and the use of tumor biomarkers in the treatment of metastatic colorectal cancer. Cancer 2019;125:4139-47.
9. Jiang M, Jin S, Han J, Li T, Shi J, Zhong Q, et al. Detection and clinical significance of circulating tumor cells in colorectal cancer. Biomark Res 2021:9:85.
10. Grillo TG, Quaglio AEV, Beraldo RF, Lima TB, Baima JP, Di Stasi LC, et al. MicroRNA expression in inflammatory bowel disease-associated colorectal cancer. World J Gastrointest Oncol 2013;11:995-1016.
11. Macias S, Michlewski G, Caceres JF. Hormonal regulation of microRNA biogenesis. Mol Cell 2009;36:172-3.
12. Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol 2014;15:509-24.
13. Shadbad MA, Asadzadeh Z, Derakhshani A, Hosseinkhani N, Mohktarzadeh A, Baghbannazadeh A, et al. A scoping review on the potentiality of PD-L1-inhibiting microRNAs in treating colorectal cancer: Toward single-cell sequencing-guided biocompatible-based delivery. Biomed Pharmacother 2021;143:112213.
14. Pidikova P, Herichova I. miRNA clusters with up-regulated expression in colorectal cancer. Cancers (Basel) 2021;13:2979.
15. Ghafoori-Fard S, Hussen BM, Badrloiu E, Abak A, Taheri M. MicroRNAs as important contributors in the pathogenesis of colorectal cancer. Biomed Pharmacother 2021;140:111759.
16. He J, Wu F, Han Z, Hu M, Lin W, Li Y, et al. Biomarkers (mRNAs and non-coding RNAs) for the diagnosis and prognosis of colorectal cancer: From the body fluid to tissue level. Front Oncol 2021;11:632834.
17. Michael MZ, SM OC, van Holst Pellekaan NG, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882-91.
18. Pagliuca A, Valvo C, Fabrizi E, di Martino S, Biffoni M, Runci D, et al. Analysis of the combined action of miR-143 and miR-155 on oncogenic pathways in colorectal cancer cells reveals a coordinate program of gene repression. Oncogene 2013;32:4806-13.
19. Danielsen SA, Eide PW, Neshbakken A, Guren T, Leithe E, Lothe RA. Portrait of the PI3K/AKT pathway in colorectal cancer. Biochim Biophys Acta 2015;1855:104-21.
20. Liu Y, Liu R, Yang F, Cheng R, Chen X, Cui S, et al. miR-19a promotes colorectal cancer proliferation and migration by targeting TIA1. Mol Cancer 2017;16:51.
21. Yin Q, Wang PP, Peng R, Zhou H. MiR-19a enhances cell proliferation, migration, and invasiveness through enhancing lymphangiogenesis by targeting thrombospodin-1 in colorectal cancer. Biochem Cell Biol 2019;97:731-9.
22. Yu FB, Sheng J, Yu JM, Liu JH, Qin XX, Mou B. MiR-19a-3p regulates the Forkhead box F2-mediated Wnt/beta-catenin signaling pathway and affects the biological functions of colorectal cancer cells. World J Gastroenterol 2020;26:627-44.

23. Elble RC, Pauli BU. Tumor suppression by a proapoptotic calcium-activated chloride channel in mammary epithelium. J Biol Chem 2001;276:4050-7.

24. Hou T, Zhou L, Wang L, Kazobinka G, Zhang X, Chen Z. CLCA4 inhibits bladder cancer cell proliferation, migration, and invasion by suppressing the PI3K/AKT pathway. Oncotarget 2017;8:93001-13.

25. Yu Y, Walia V, Elble RC. Loss of CLCA4 promotes epithelial-to-mesenchymal transition in breast cancer cells. PLoS One 2013;8:e83943.

26. Agarwal V, Bell GW, Nam JW, Bartel DP. Predicting effective microRNA target sites in mammalian mRNAs. Elife 2015;4:e05005.

27. Wang X. Improving microRNA target prediction by modeling with unambiguously identified microRNA-target pairs from CLIP-igation studies. Bioinformatics 2016;32:1316-22.

28. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nat Genet 2005;37:495-500.

29. Lui Z, Chen M, Xie LK, Liu T, Zou ZW, Li Y, et al. CLCA4 inhibits cell proliferation and invasion of hepatocellular carcinoma by suppressing epithelial-mesenchymal transition via PI3K/AKT signaling. Aging (Albany NY) 2018;10:2570-84.

30. Zhang Y, Wang J. MicroRNAs are important regulators of drug resistance in colorectal cancer. Biol Chem 2017;398:929-38.

31. Falzone L, Lupo G, La Rosa GRM, Crimi S, Anfuso CD, Yu Y, Walia V, et al. Adenomatous polyposis coli (APC) regulates miR17-92 cluster through beta-catenin pathway in colorectal cancer. Oncogene 2016;35:4558-68.

32. Chen M, Lin M, Wang X. Overexpression of miR-19a inhibits colorectal cancer angiogenesis by suppressing KRAS expression. Oncol Rep 2018;39:619-26.

33. Shen P, Qu L, Wang J, Ding Q, Zhou C, Xie R, et al. LincRNA LINC00342 contributes to the growth and metastasis of colorectal cancer via targeting miR-19a-3p/NPEPL1 axis. Cancer Cell Int 2021;21:105.

34. Patil KS, Basak I, Pal R, Ho HP, Alves G, Chang EJ, et al. A novel role of microRNA-410 in regulating the Forkhead box F2-mediated Wnt/beta-catenin signaling pathway in colorectal cancer. Oncol Rep 2018;39:133-40.

35. Ma K, Pan X, Fan P, He Y, Gu J, Wang W, et al. Loss of miR-638 in vitro promotes cell invasion and a mesenchymal-like transition by influencing SOX2 expression in colorectal carcinoma cells. Mol Cancer 2014;13:118.

36. Zhang J, Fei B, Wang Q, Song M, Yin Y, Zhang B, et al. MicroRNA-638 inhibits cell proliferation, invasion and regulates cell cycle by targeting tetraspanin 1 in human colorectal carcinoma. Oncotarget 2014;5:12083-96.

37. Li Z, Li Y, Wang Y. MiR-19a promotes invasion and epithelial to mesenchymal transition of bladder cancer cells by targeting RhoB. J BUON 2019;24:797-804.

38. Peng Y, Huang D, Ma K, Deng X, Shao Z. MiR-19a as a prognostic indicator for cancer patients: a meta-analysis. Biosci Rep 2019;39:BSR20182370.

39. Li Y, Lv S, Ning H, Li K, Zhou X, Xv H, et al. Down-regulation of CASC2 contributes to cisplatin resistance in gastric cancer by sponging miR-19a. Biomed Pharmacother 2018;108:1775-82.

40. Cao X, Lai S, Hu F, Li G, Wang G, Luo X, et al. miR-19a contributes to gefitinib resistance and epithelial-mesenchymal transition in non-small cell lung cancer cells by targeting c-Met. Sci Rep 2017;7:2939.

41. Li Y, Lauriola M, Kim D, Francesconi M, D’Uva G, Shibata D, et al. Adenomatous polyposis coli (APC) regulates miR17-92 cluster through beta-catenin pathway in colorectal cancer. Oncogene 2016;35:4558-68.