Wang *et al.*
European Journal of Medical Research (2025) 30:352
https://doi.org/10.1186/s40001-025-02625-0

European Journal of Medical Research

RESEARCH

Open Access

miR-182-5p facilitates colorectal cancer progression through manipulating neurocalcin delta mediated Wnt/β-catenin signalling

Pengfei Wang[1,2†], Gang Li[3†], Xianglin Sun[3], Jie Zhang[2], Leijian Shi[2], Xiaoyu Zhou[2], Guohua Wang[3*] and Weichang Chen[1*]

Abstract

Background Colorectal cancer (CRC), a complex and multifactorial disease, has been associated with elevated expression of microRNA miR-182-5p, although its precise regulatory role in CRC progression remains unclear. This study aims to identify potential therapeutic targets to improve clinical outcomes and to decipher the intricate role of miR-182-5p in the pathobiology of CRC.

Methods We conducted comprehensive bioinformatics analyses using GEO databases to investigate differences in miRNA expression between CRC and normal tissues, with a particular focus on miR-182-5p. Its expression levels in CRC cells and tumor tissues were quantified by quantitative real-time PCR (qRT-PCR). The expression of neurocalcin delta (NCALD) and proteins related to Wnt/β-catenin signalling was evaluated by qRT-PCR and Western blotting. Pathological changes in tumor-bearing mice as well as the proliferation, invasion, and migration of CRC cells, were assessed. Tumor cell proliferation and apoptosis were examined using Ki-67 immunohistochemistry and TUNEL staining, respectively. A dual luciferase reporter assay explored the regulatory interaction between miR-182-5p and NCALD.

Results Our findings reveal significantly elevated miR-182-5p levels in CRC tissues and cell lines, positively correlated with tumor invasion depth, differentiation degree, clinical stage, and lymph node metastasis. miR-182-5p appears to accelerate CRC progression in both cell lines and mouse models by downregulating NCALD, thereby enhancing Wnt/β-catenin signalling. This study identifies miR-182-5p as a pivotal enhancer of CRC progression, modulating Wnt/β-catenin signalling via NCALD regulation.

Conclusions The findings position the miR-182-5p/NCALD axis as promising targets for CRC therapy, offering new avenues for treatment strategies.

Trial registration: Retrospectively registered.

Keywords Colorectal cancer, miR-182-5p, Neurocalcin delta, Wnt/β-catenin signalling

†Pengfei Wang and Gang Li contributed equally to this work.

*Correspondence:
Guohua Wang
wgh036@hotmail.com
Weichang Chen
weichangchen@126.com
Full list of author information is available at the end of the article

Introduction

Colorectal cancer (CRC), a predominant malignancy within the gastrointestinal tract, ranks as the third most commonly diagnosed cancer worldwide [1]. In developing countries, the incidence of CRC has been rising annually, attributable to dietary changes linked with economic development [2]. Despite a recent deceleration in its growth rate, the asymptomatic onset and terrible prognosis of CRC exert considerable impact on individual well-being [3, 4], thus posing a significant challenge to public health. Hence, clarifying the potential mechanisms underlying the occurrence, development, and metastasis, as well as devising efficacious prevention and treatment approaches, remain critical in CRC research [5].

The pathogenesis and progression of CRC involve complex biological processes, encompassing multiple stages and the interaction of numerous genes and regulatory factors [6]. MicroRNAs (miRNAs), 21-nucleotide long non-coding RNA molecules, are ubiquitous in organisms and crucially regulate gene expression via transcriptional silencing, degradation, or translational alteration [7, 8]. These molecules are critical in various biological functions, including growth, development, and apoptosis [9]. Mounting evidence underscores the diverse functions of miRNAs in disease regulation, including their significant impact on CRC development [10, 11]. Liu et al. observed heightened levels of exosomal miR-106b-3p in metastatic CRC patients, implicating its role in promoting metastasis by repressing DLC-1 expression [12]. Similarly, Zhang et al. confirmed miR-335-5p as a modulator of CRC cell proliferation, migration, and invasion by inhibiting LDHB expression, suggesting its potential as a therapeutic target [13]. Moreover, a meta-analysis conducted by Gu et al. demonstrated the prognostic value of miRNA-181a/b in CRC [14]. Collectively, these researches spotlight the crucial role of miRNAs in CRC development, offering valuable insights for diagnosis, treatment, and prognosis.

However, only a limited number of miRNAs have been confirmed to play definitive roles in CRC diagnosis, prognosis, or treatment [15, 16]. Su et al. previously identified 44 differentially expressed miRNAs in CRC, comprising 19 downregulated and 25 upregulated ones, through systematic bioinformatics analyses [16]. Among these, miR-182-5p exhibited the most significant upregulation in CRC cells [16]. Despite this, the functional role and underlying mechanisms of miR-182-5p in CRC remain poorly understood. This study aims to elucidate the biological mechanisms through which miR-182-5p contributes to CRC progression, with the expectation that the findings will offer valuable insights for the development of novel diagnostic and therapeutic targets.

Materials and methods

Clinical specimen and cell line acquisition

This study involved the procurement of 30 CRC tissue samples and corresponding adjacent normal tissues, obtained from 2019 to 2020 at The First Affiliated Hospital of Soochow University. All samples were obtained via surgical resection from patients who had not undergone radiotherapy or chemotherapy prior to surgery. Three board-certified pathologists independently confirmed the CRC diagnoses. The First Affiliated Hospital of Soochow University's Ethical Committee approved the research procedure, which was carried out in accordance with ethical guidelines (Approval number: NL09-2019017). Informed consent was obtained from all participants. Furthermore, the normal cell line FHC and the CRC cell lines HT29, SW620, SW480, LOVO, and HCT116 were obtained from the American Type Culture Collection (ATCC, USA).

Cell culture and transfection protocol

These CRC cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco), which was supplemented with 100 U/mL penicillin (Catalog No. 61305, Sigma), 100 μg/mL streptomycin (Catalog No. 85886, Sigma), and 10% fetal bovine serum (FBS, Catalog No. 16140071, Gibco, USA). These cultures were kept in an atmosphere with 5% CO_2 and at a constant temperature of 37 °C. Following the manufacturer's instructions, the NCALD and miR-182-5p transfection procedure was carried out using Lipofectamine 2000 Reagent (Catalog No. 11668019, Invitrogen). When the cell confluence was between 60 and 90%, the transfection was started. In the instance of miR-182-5p transfection, the HCT116 CRC cell line was treated with mimics, inhibitors, and matching controls, using hsa-miR-182-5p mirVanaTM miRNA inhibitor (ID: MH12369) and mimic (ID: MC12369) assays (AMBION, Austin, TX, USA). MirVanaTM miRNA inhibitor as negative control (NC). Simultaneously, logarithmic growth phase LoVo and SW480 CRC cells were plated in 6-well plates and transfected with a miR-182-5p overexpression lentivirus (miR-182-5p-OE) or a control (NC-OE), and a miR-182-5p interference lentivirus (miR-182-5p-shRNA) or a control (NC-shRNA). Regarding NCALD transfections, the constructs NCALD-pcDNA3.1, sh-NCALD, and pCDNA3.1 were transfected into HCT116 CRC cells as required. All plasmids were graciously supported by GenePharma (Shanghai, China). Transfection efficiency was quantitatively evaluated 48-h post-transfection via qRT-PCR. Cells receiving various treatments were then

collected for subsequent analysis, employing qPCR to confirm the efficacy of the transfections.

Cell proliferation analysis via CCK-8 assay

CRC cell lines HCT116, LoVo, and SW480 were seeded at a density of 1.5×10^3 cells per well in 96-well plates. Cells were subsequently treated with miR-182-5p and NCALD, respectively. The cells were then cultivated using DMEM supplemented with 10% FBS for 24, 48, and 72 h at 37 °C in a 5% CO_2 environment. Following the incubation period, each well received 10 μL of the CCK-8 solution (Dojindo, Japan), and an additional hour of incubation was required. Cell viability was then assessed by measuring the optical density (OD) at 450 nm using a microplate reader (BioTek, USA).

Cell migration and invasion assessment

The migratory and invasive capacities of HCT116, LoVo, and SW480 cells were assessed using wound-healing and Transwell assays. For the wound-healing assay, cells (2×105 per well) transfected with either miR-182-5p or NCALD were seeded in 6-well plates and cultivated at 37 °C in a 5% CO_2 atmosphere until they reached approximately 90% confluence. After creating a scratch in the cell monolayer with a 200 μL pipette tip, any unfastened cells were cleaned with PBS. An inverted microscope was used to record the wound closure after a 24-h incubation period in a serum-free solution. The formula (Initial Wound Width–Wound Width at 24 h)/Initial Wound Width was used to calculate the migration rate. For the Transwell invasion assay, the procedure followed the standardized protocol provided by the Cell Invasion Assay Kit (CHEMICON, Millipore, USA), in line with previous reports [17]. Briefly, 4×10^9 cells were resuspended in serum-free media containing 1 μg/mL Mitomycin C to prevent proliferation, and then added to the upper chambers of Millipore Transwell inserts. A medium containing 10% FBS was added to the lower chambers to serve as a chemoattractant. The cells that had passed through the membrane were observed using a Zeiss fluorescence photomicroscope (Carl Zeiss, Germany) after being fixed in methanol and stained with 0.1% crystal violet. After a 24-h incubation period, non-migratory cells on the upper membrane surface were eliminated. One of the steps in the quantification procedure was to count the cells in at least ten random fields.

qRT-PCR

TRIzol reagent (Invitrogen, USA) was used to extract total RNA from CRC cells and tumor tissues. PrimeScript Reverse Transcriptase (Takara) was used to convert 0.5 μg of the isolated RNA to cDNA for reverse transcription, in accordance with the manufacturer's instructions [18]. Thermo Scientific SYBR Green qPCR kit and the ABI7500 real-time PCR system were used to conduct the qRT-PCR analysis. Two minutes of initial denaturation at 95 °C, forty cycles of denaturation at 95 °C for 15 s each, and 30 s of annealing/extension at 60 °C comprised the PCR cycling conditions. The relative expression of mRNAs or miRNAs was measured using the $2^{-\Delta\Delta CT}$ technique with internal controls adjusted to GAPDH and U6. The following primer sequences were used: miR-182-5p: Forward: 5′-ATCACTTTTGGCAATGGTAGAACT-3′, Reverse: 5′-TATGGTTTTGACGACTGTGTGAT-3′; NCALD: Forward: 5′-CAAGTAGTTAGCAAAGGGAGGC-3′, Reverse: 5′-CCTGGAGGTTAGCATGTCTAGT-3′; U6: Forward: 5′-GCTTCGGCAGCACATATACTAA-3′, Reverse: 5′-AACGCTTCACGAATTTGCGT-3′; GAPDH: Forward: 5′-TGTGTCCGTCGTGGATCTGA-3′, Reverse: 5′-CCTGCTTCACCACCTTCTTGA-3′.

Western blot

Protein extraction from cellular and tissue samples was performed using RIPA buffer, with mechanical disruption facilitated by a Qiagen tissue lyser (Qiagen, USA). An assay utilizing BSA was used to determine protein concentrations. We separated 20 μg of protein from each sample on Millipore-provided 10% SDS–PAGE gels to perform electrophoresis. The resolved proteins were then electrotransferred onto PVDF membranes. The membranes were then subjected to blocking using 5% non-fat milk in TPBS. For antibody incubation, we kept the blocked membranes at 4 °C overnight with primary antibodies that targeted NCALD, Wnt3a, β-catenin, GSK-3, and GAPDH. All these primary antibodies were sourced from Abcam and used at a dilution of 1:1000. Post-incubation, we thoroughly washed the membranes three times and then allowed them to incubate with an HRP-conjugated goat anti-mouse IgG secondary antibody for a duration of 10 min at room temperature. Detection of protein bands was achieved using the Amersham Imager 600 and the Thermo Fisher Scientific ECL Chemiluminescent Kit (GE, USA). Protein band intensities were quantified utilizing ImageJ software (USA).

Animal experiment

Six-week-old BALB/c nude mice were purchased from Gem Pharmatech (Nanjing, China), and housed under standard conditions in a barrier facility with 12-h light/dark cycles. HCT116 cells (5×10^6), transfected with agomir-182-5p (miR-182-5p mimics group) or antagomir-182-5p (miR-182-5p inhibitor group) using Lipofectamine 2000 were subcutaneously injected into

the right armpit region of each mouse. Tumor volumes were assessed using the following formula on days 7, 14, and 28 after injection to track the progression of the tumor (mm3) = 1/2 × width2 × length. The tumors were taken out, measured, photographed, and quickly kept in formalin for further analysis when 4 weeks were up. After that, the mice were mercifully put to sleep. Approved by the Animal Protection and Use Committee (Approval No. DW102A.07), this animal study was carried out at The First Affiliated Hospital of Soochow University in strict accordance with established norms for animal welfare.

Fractionation of subcellular materials

The cytoplasmic and nuclear RNA were separated using the Cytoplasmic and Nuclear RNA Purification Kit (Norgen Biotek, Thorold, Canada). The expression levels of NCALD, miR-182-5p, GAPDH, and U6 in the nuclear and cytoplasmic fractions of HCT116 cells were assessed by qRT-PCR.

Histochemical analysis

Tumor tissues were fixed overnight in 4% paraformaldehyde, embedded in paraffin, and sectioned into 4 μm slices. For immunohistochemical analysis, sections were incubated overnight at 4 °C with Ki-67 monoclonal antibodies (1:200, Abcam), followed by incubation with a secondary goat anti-rabbit IgG (H + L) antibody. To prepare sections for the terminal deoxynucleotidyl transferase-mediated UTP end-labelling (TUNEL) assays, 0.1% Triton X-100 in 0.1% sodium citrate was used as a pretreatment. They were then incubated on ice for 2 min and then for 1 h at 37 °C in the dark with 50 μl of the TUNEL reaction mixture. Nuclear counterstaining was executed using 1 mg/ml DAPI. Imaging of the stained sections was performed using either a standard fluorescence or an inverted fluorescence microscope (Leica, Germany; Nikon, Tokyo, Japan).

Dual-luciferase reporter assays

Subcloned complementary DNA (cDNA) fragments encoding the wild-type and mutant forms of NCALD were introduced into the pmirGLO-Basic luciferase reporter vector (Promega) after being cloned downstream of the luciferase gene. After then, miR-182-5p mimics and negative control (NC) mimics were co-transfected into HCT116 cells using these recombinant vectors. The dual-luciferase reporter assay system (Promega) was used to assess luciferase activity 48 h after transfection.

Data interpretation from publicly accessible databases

The miR-182-5p target genes were identified using online prediction tools from RNA22 (https://cm.jefferson.edu/rna22/), Starbase (https://starbase.sysu.edu.cn), and Diana Tools (http://diana.imis.athena-innovation.gr/DianaTools/index.php).

Statistical analysis

All the information gathered for this study was statistically assessed using the SPSS software, version 22.0 (IBM SPSS, USA). The way of displaying the data was mean ± standard deviation (SD). Comparisons between two groups were performed using the Student's *t* test, while one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied for comparisons involving more than two groups. A *P* value of less than 0.05 was considered statistically significant.

Results

miR-182-5p is significantly promoted in CRC tissues and cell lines

To elucidate the role of miRNAs in CRC, an initial investigation was conducted on miRNA expression profiles using microarray data sets from two independent CRC cohorts. This analysis identified a group of miRNAs with altered expression in CRC, with miR-182-5p showing significant upregulation in both cohorts. The dysregulated expression of miR-182-5p in CRC was further verified by further bioinformatics analysis, as shown in Fig. 1A–D. A comparative analysis revealed that CRC tissues had significantly higher levels of miR-182-5p than normal tissues (Fig. 1E). Furthermore, in comparison with the normal cell line FHC, the expression levels of miR-182-5p were considerably greater in the CRC cell lines HT29, SW620, SW480, LOVO, and HCT116 (Fig. 1F). HCT116, LoVo, and SW480 cells were chosen for further investigations due to their

(See figure on next page.)

Fig. 1 Elevated expression of miR-182-5p in colorectal cancer (CRC) tissues and cell lines. **A, C** Hierarchical clustering heatmaps display the differential expression of miRNAs in CRC vs. normal tissues, derived from RNA sequencing data in the GEO database. Upregulated miRNAs are shown in red, downregulated ones in blue, with miR-182-5p highlighted by a red line. **B, D** Bioinformatics analysis revealing the expression pattern of miR-182-5p in CRC. **E** Comparative analysis of miR-182-5p expression levels between CRC tissues and normal tissues (control). **F** qRT-PCR analysis of miR-182-5p expression in various CRC cell lines (HT29, SW620, SW480, LOVO, and HCT116) vs. the normal cell line FHC. Data are presented as mean ± SD; $^{**}P < 0.01$, comparing CRC groups to the FHC group

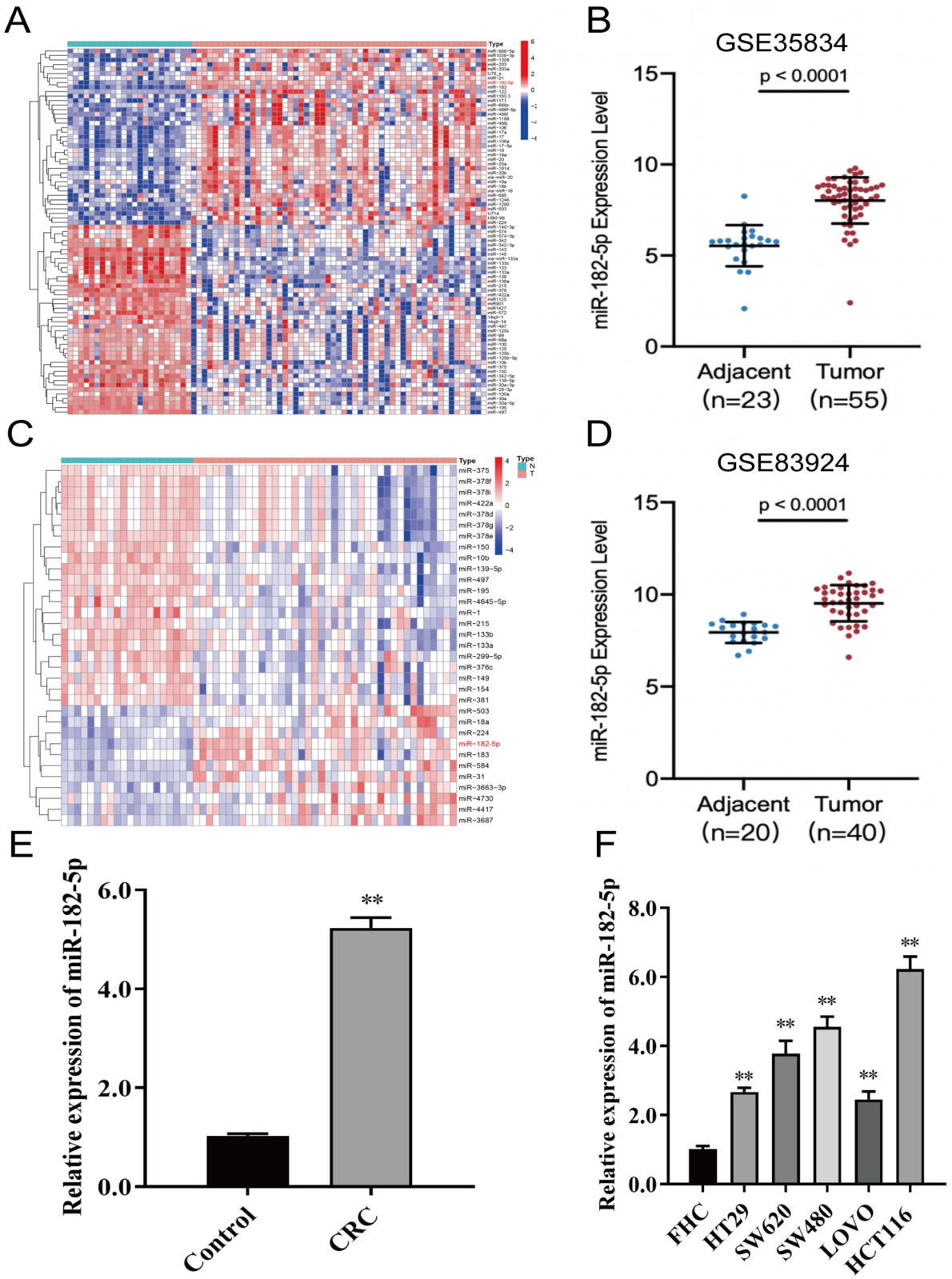

Fig. 1 (See legend on previous page.)

prevalent use in colorectal cancer research and distinct molecular characteristics, including a strong responsiveness to miRNA regulation and widespread application in the investigation of the Wnt/β-catenin signalling system. In addition, as shown in Table 1, a significant association was found between the expression of miR-182-5p and several clinical indicators, such as tumor invasion depth, degree of differentiation, clinical stage, and lymph node metastasis.

Table 1 Clinical features of 30 CRC patients

Clinical features	Number of cases	miR-182-5p Expression χ^2	*P* value
Sex			> 0.05
Male	18	8.21 ± 8.58	
Female	12	7.30 ± 7.25	
Age			> 0.05
< 60	12	7.10 ± 6.02	
≥ 60	18	7.26 ± 8.16	
Tumor site			> 0.05
Colon	10	6.25 ± 5.42	
Rectum	20	7.41 ± 3.69	
Invasion depth			< 0.05
T2	11	4.15 ± 5.02	
T3	19	7.58 ± 8.23	
Differentiation			< 0.05
High + Middle differentiation	9	4.26 ± 1.36	
Low + none differentiation	21	10.41 ± 8.45	
Lymph node metastasis			< 0.05
Negative	10	4.24 ± 3.34	
Positive	20	8.61 ± 4.65	
TNM stage			< 0.05
I + II	13	5.32 ± 7.36	
III + IV	17	9.11 ± 8.14	

CRC cells invasion, migration, and proliferation are all aided by miR-182-5p

Using lentiviral transfection techniques, LoVo and SW480 cell lines were genetically altered to either overexpress or inhibit miR-182-5p to study the impact of this gene on CRC cell behaviors. Post-transfection analysis demonstrated a significant rise in miR-182-5p levels in the overexpression group and a noteworthy drop in the knockdown group in comparison with their respective controls, confirming the successful manipulation of miR-182-5p (Fig. 2A). To examine the role of miR-182-5p in the development of CRC, miR-182-5p mimics and inhibitors were utilized to alter the expression of the protein in HCT116 cells. We next evaluated the changes in cell invasion, migration, and proliferation. Treatment with miR-182-5p mimics significantly enhanced its expression, whereas inhibitors decreased it (Fig. 2B). The CCK-8 experiment demonstrated that miR-182-5p mimics increased HCT116 cell proliferation, whereas the inhibitor reduced it. Consequently, overexpression of miR-182-5p boosted LoVo and SW480 cell proliferation, whereas its inhibition lowered it (Fig. 2C). In addition, miR-182-5p mimics greatly improved HCT116 cells' capacity for invasion and migration, while the inhibitor dramatically decreased these activities, according to Transwell and wound-healing experiment results. Similarly, overexpression of miR-182-5p increased, whereas its inhibition decreased the ability of LoVo and SW480 cells to invade and migrate (Fig. 2D–O).

NCALD is identified as a direct target of miR-182-5p

TargetScan was used to anticipate probable targets in the search for miR-182-5p downstream effectors. This process resulted in the putative target NCALD being identified, as shown in Fig. 3A. Positions 79–86 on the 3'UTR of NCALD were found to contain a particular binding site for miR-182-5p, suggesting that miR-182-5p and NCALD may interact in CRC cells. Figure 3B, C illustrates the significant decline in NCALD expression in CRC tissues as compared to control samples, as determined by comparative analysis. In co-transfection studies utilizing miR-182-5p mimics or NC mimics and wild-type NCALD

(See figure on next page.)

Fig. 2 miR-182-5p enhances migration, invasion, and proliferation of CRC Cells. **A** qPCR data demonstrating miR-182-5p overexpression and knockdown in LoVo and SW480 cells. $^{**}P < 0.01$ between the miR-182-5p-OE and NC–OE groups; $^{\#\#}P < 0.01$ between the miR-182-5p-shRNA and NC-shRNA groups. **B** After treatment with mimics or inhibitors, changes in the miR-182-5p expression, using U6 as a reference. $^{**}P < 0.01$ for mimics vs. mimics NC group; $^{\#\#}P < 0.01$ for inhibitor vs. inhibitor NC group. **C** CCK-8 assay measuring HCT116, LoVo, and SW480 cell proliferation at 24, 48, and 72 h after treatment. $^{**}P < 0.01$ vs. reference group. **D–I** Transwell experiments measuring the impact of miR-182-5p on the invasion of HCT116, LoVo, and SW480 cells. $^{**}P < 0.01$ for the mimics NC group vs. the mimics group or the miR-182-5p-OE group vs. the NC-OE group; $^{\#\#}P < 0.01$ for the inhibitor NC group vs. the inhibitor NC group or the miR-182-5p-shRNA group vs. the NC-shRNA group. **J–O** Wound healing tests investigating the miR-182-5p-affected migration of HCT116, LoVo, and SW480 cells. $^{**}P < 0.01$ for the mimics NC group vs. the mimics group or the miR-182-5p-OE group vs. the NC–OE group; $^{\#\#}P < 0.01$ for the inhibitor NC group vs. the inhibitor NC group or the miR-182-5p-shRNA group vs. the NC-shRNA group. Scale bar = 100 μm. Data are presented as mean ± SD

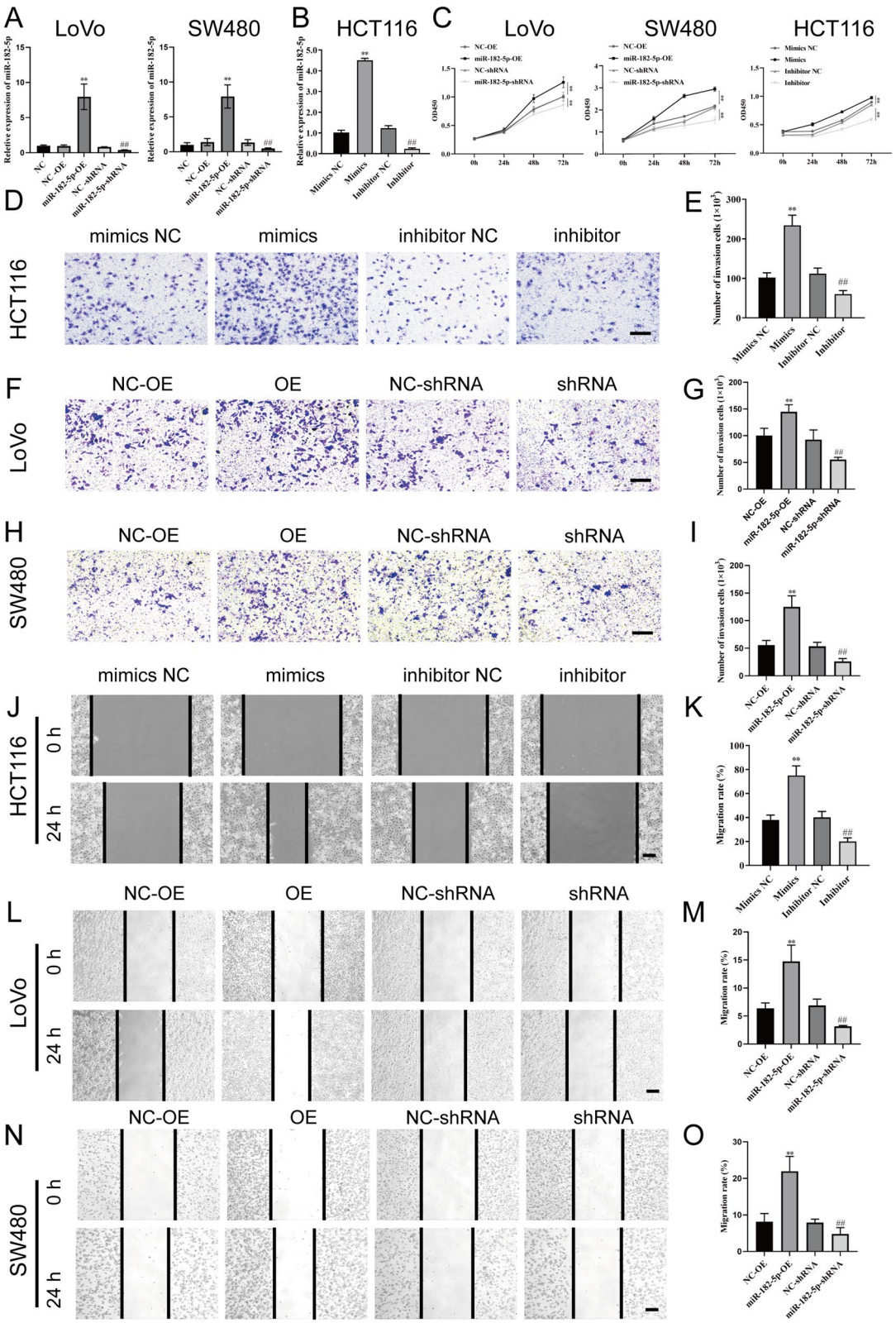

Fig. 2 (See legend on previous page.)

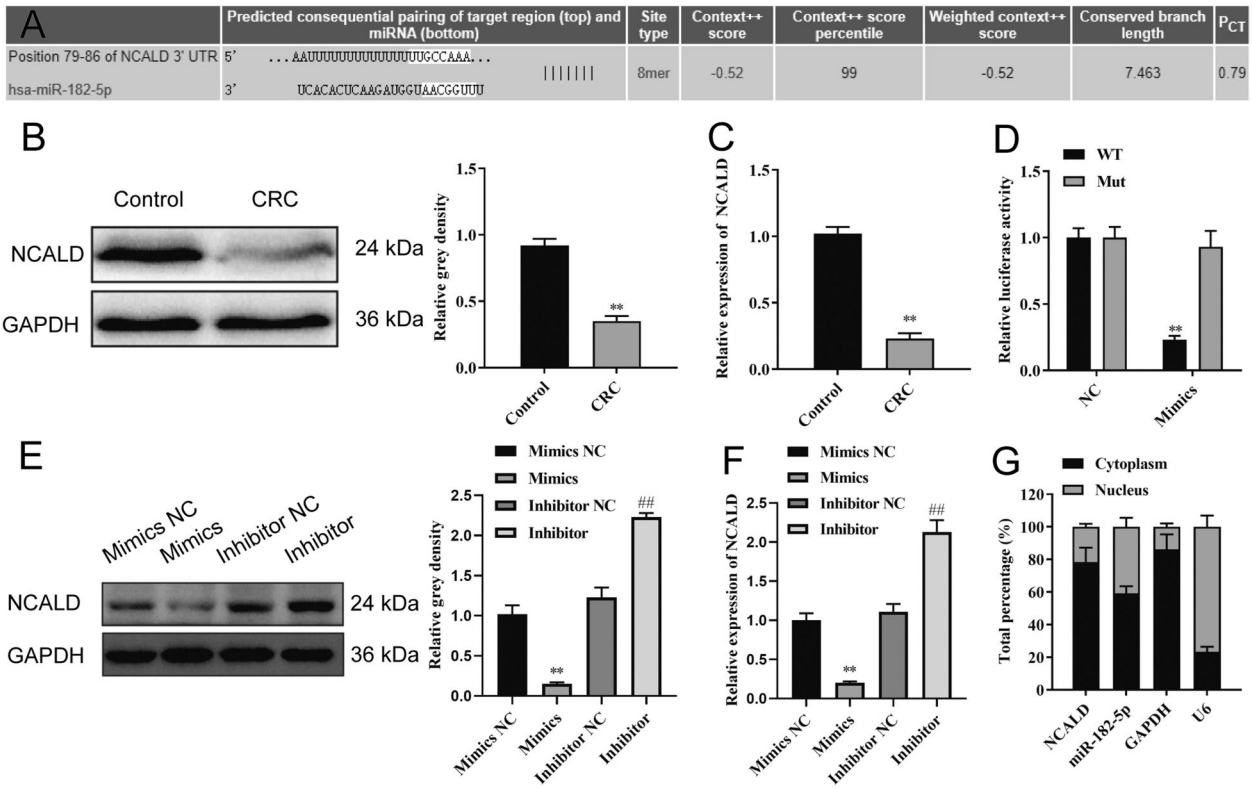

Fig. 3 Identification of NCALD as a direct target of miR-182-5p. **A** TargetScan prediction reveals a potential binding site for miR-182-5p at positions 79–86 on the 3'UTR of NCALD. The context + + score indicates the overall score, with a lower value signifying a higher likelihood of the goal. PCT is the score derived from evolutionary conservation among species, with elevated values signifying greater conservation. **B, C** Analysis of NCALD expression in CRC tissues by Western blot and qRT-PCR; $^{**}P < 0.01$ between the CRC group and the control group. **D** Dual luciferase reporter assays were employed to investigate the association between miR-182-5p and NCALD. A significant difference is noted between the negative control (NC) group and the miR-182-5p mimic group, with $^{**}P < 0.01$. **E, F** Assessment of the impact of miR-182-5p on NCALD expression using Western blot and qRT-PCR. $^{**}P < 0.01$ between the miR-182-5p mimics and the NC groups; $^{\#\#}P < 0.01$ between the miR-182-5p inhibitor and the NC groups. **G** In HCT116 cells, subcellular fractionation tests were performed to determine the distribution of NCALD and miR-182-5p. Data are presented as mean ± SD

pmirGLO vectors, the miR-182-5p mimics group demonstrated a substantial reduction in luciferase activity associated with NCALD–WT, in contrast to the NC group. However, this effect was not observed with mutant NCALD vectors (Fig. 3D). Following transfection with miR-182-5p mimics, NCALD levels were considerably lower compared to control groups, and following transfection with miR-182-5p inhibitors, NCALD levels were significantly greater (Fig. 3E, F). NCALD and miR-182-5p were mostly detected in the cytoplasm of HCT116 cells, according to subcellular fractionation studies (Fig. 3G).

CRC cells proliferative, invasive, and miR-182-5p-mediated migration are facilitated by NCALD downregulation

To elucidate NCALD's function in the pathogenesis of CRC, we examined the impact of NCALD overexpression and knockdown on the migration, invasion, and proliferation of LoVo cells. The confirmation of the effective modulation of NCALD expression may be shown in Fig. 4A, B. The CCK-8 test findings demonstrated a substantial reduction in cell proliferation in the over-NCALD group and increased cell proliferation in the sh-NCALD group relative to the control group (Fig. 4C). Furthermore, NCALD overexpression dramatically reduced HCT116 cell invasion and migration, while NCALD suppression increased them, according to data from Transwell and wound-healing experiments (Fig. 4D–G). Moreover, our findings imply that NCALD's effects on HCT116 cell invasion, migration, and proliferation may be somewhat offset. Specifically, the application of miR-182-5p mimics appeared to negate the effects of NCALD overexpression, while miR-182-5p inhibitors seemed to alleviate the impact of NCALD knockdown, as demonstrated in Fig. 4C–G.

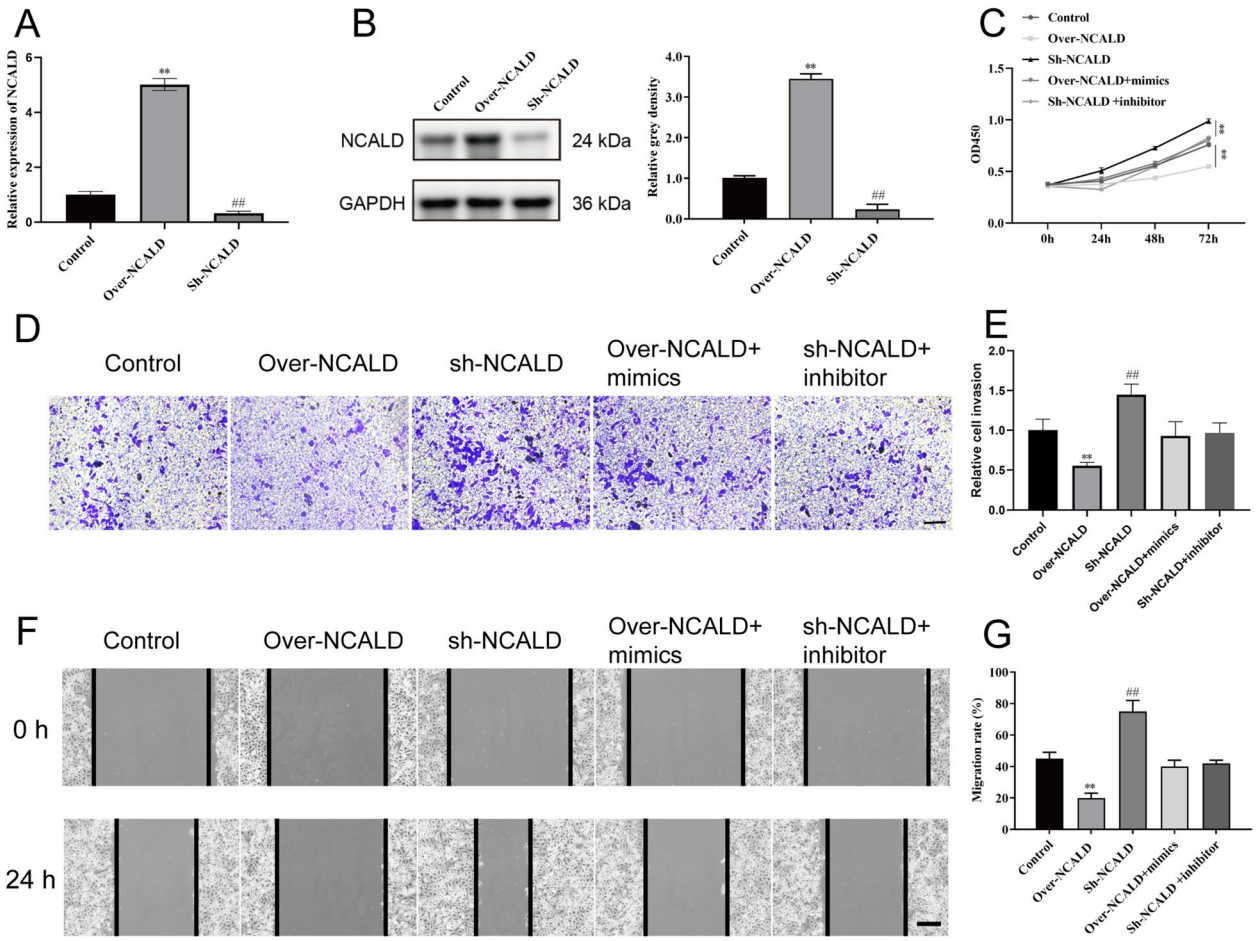

Fig. 4 Impact of NCALD modulation on miR-182-5p-regulated cellular dynamics in LoVo Cells. **A, B** Validation of NCALD overexpression and knockdown efficiency in LoVo cells using qRT-PCR and Western blot analyses, with GAPDH serving as an internal control. **C** CCK-8 assay evaluating the proliferation of LoVo cells under NCALD modulation and its reversal by miR-182-5p at 24-, 48-, and 72-h post-treatment. **D, E** Transwell assays assessing the invasive capacity of LoVo cells in response to NCALD modulation and its reversal by miR-182-5p. **F, G** Wound healing assays analyzing the migration ability affected by NCALD and its reversal by miR-182-5p. $^{**}P < 0.01$ for over-NCALD group vs. control group; $^{\#\#}P < 0.01$ for sh-NCALD group vs. control group. Scale bar = 100 μm. Data are presented as mean ± SD

NCALD downregulation activates Wnt/β-catenin signalling

The formation and progression of CRC are significantly influenced by the Wnt/β-catenin signalling pathway. To investigate the possible regulatory impact of NCALD on this pathway, we looked at the expression of three important proteins: Wnt3a, GSK-3β, and β-catenin. When comparing CRC tissues to controls, analysis at the mRNA and protein levels revealed large increases in Wnt3a and β-catenin expression, combined with a dramatic decrease in GSK-3β expression (Fig. 5A, B). In HCT116 cells, overexpression of NCALD (over-NCALD group) led to a notable reduction in Wnt3a and β-catenin levels, while GSK-3β expression was increased relative to the NC group. As opposed to the over-NCALD group, the NCALD knockdown (sh-NCALD group) showed an inverted expression pattern with increased Wnt3a and β-catenin and decreased GSK-3β expression (Fig. 5C, D). These findings suggest a regulatory connection between the stimulation of the Wnt/β-catenin signalling pathway and the downregulation of NCALD.

Role of miR-182-5p in CRC progression through NCALD modulation

In vivo investigations were carried out to confirm the involvement of miR-182-5p in the advancement of CRC. Mice overexpressing miR-182-5p (mimic group) exhibited significantly increased Ki-67 staining, indicating higher tumor cell proliferation, whereas inhibition of miR-182-5p led to reduced proliferation (Fig. 6A). Western blot analysis supported these observations, showing decreased NCALD expression in the miR-182-5p mimic group and increased expression in the inhibitor group (Fig. 6B, C). Moreover, due to the very low transfection efficiency of the miR-182-5p mimic and inhibitor groups,

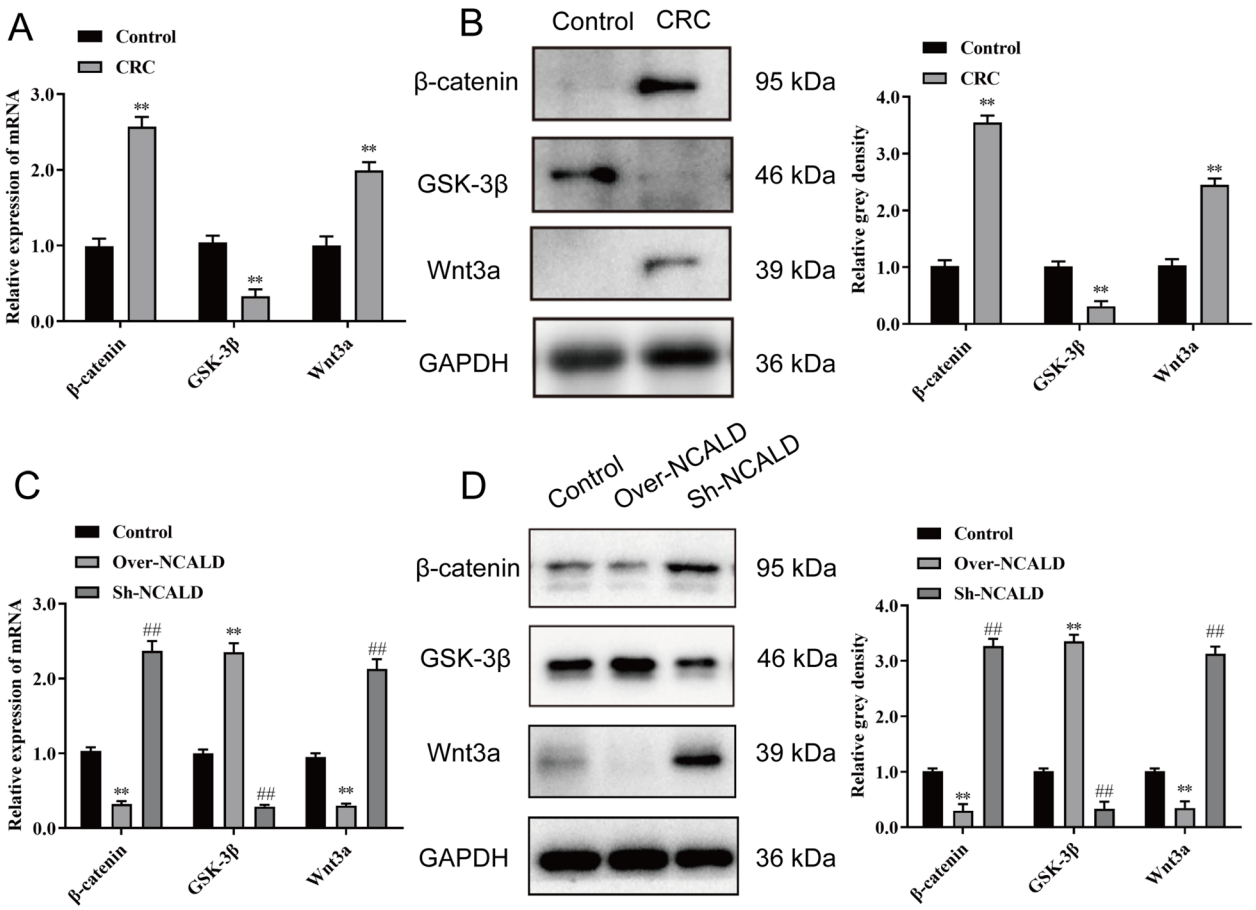

Fig. 5 NCALD downregulation triggers the activation of Wnt/β-catenin signalling. **A, B** Expression levels of Wnt/β-catenin signalling-related proteins (Wnt3a, GSK-3β, and β-catenin) in CRC tissues were analyzed using qRT-PCR and Western blot. $^{**}P < 0.01$ vs. control group. **C, D** Effects of NCALD overexpression and knockdown on the expression of Wnt3a, GSK-3β, and β-catenin at both mRNA and protein levels were analyzed in CRC tissues, with GAPDH used as an internal control. $^{**}P < 0.01$ for the over-NCALD group vs. control group; $^{\#\#}P < 0.01$ for the sh-NCALD group vs. control group. Data are presented as mean ± SD

no differences were detected in the results of the TUNEL assay for apoptosis (Fig. 6D). The subcutaneous transplantation of tumor cells into mice further demonstrated that tumors in the miR-182-5p mimic group were significantly larger than those in the inhibitor group (Fig. 6E, F). Collectively, these findings robustly support that miR-182-5p enhances CRC progression by modulating NCALD expression.

Discussion

CRC, a common malignancy of the gastrointestinal tract, is characterized by a complex and not fully understood pathogenesis [19]. This complexity underscores the urgent need for in-depth research into CRC's pathophysiology and the development of diagnostic markers to improve patient survival rates [20]. Aligning with previous studies that reported an upregulation of miR-182-5p in CRC cells [16], our investigation also identified a significant elevation in miR-182-5p levels in CRC tissues and cell lines. This increase was found to be positively associated with factors, such as tumor invasion depth, cellular differentiation, clinical stage, and lymph node metastasis. This study further reveals that miR-182-5p tends to the progression of CRC by modulating NCALD, which in turn affects the Wnt/β-catenin signalling pathway. These findings suggest that miR-182-5p is a viable option for CRC treatment and diagnostic approaches.

MicroRNAs (miRNAs) are increasingly recognized as crucial regulators in the progression of CRC, affecting various biological processes [21, 22]. For instance, miR-17-5p has been shown to regulate epithelial–mesenchymal transition (EMT) in CRC cells; miR-31 enhances the radiosensitivity of CRC cells; and aberrant expression of miR-9 has been associated with poor prognosis in CRC patients [23–25]. A thorough investigation

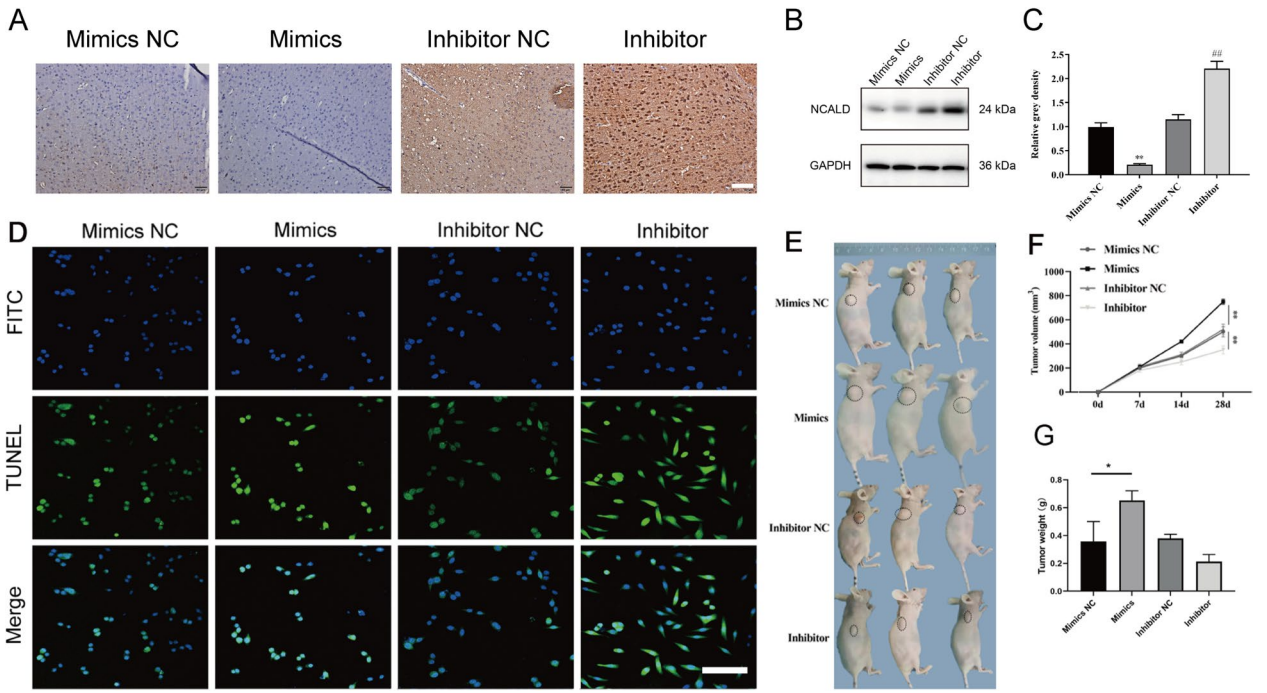

Fig. 6 miR-182-5p facilitates CRC progression through NCALD regulation. **A** Ki-67 immunohistochemical staining was performed to assess tumor cell proliferation following miR-182-5p modulation. Scale bar = 50 μm. **B, C** Western blot analysis showing the expression of NCALD in response to miR-182-5p overexpression or inhibition. $^{**}P < 0.01$ between the miR-182-5p mimics and NC groups; $^{\#\#}P < 0.01$ between the miR-182-5p inhibitor and NC groups. **D** TUNEL assay was used to evaluate apoptosis in CRC cells influenced by miR-182-5p. Scale bar: 200 μm. **E** Representative images of subcutaneous tumorigenesis of CRC in mice and excised tumors after the experiment. **F** Measurement of tumor volume at 7, 14, and 28 day post-induction in mice, showing the influence of miR-182-5p. **G** Measurement of tumor weight post-induction in mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the indicated group. Data are mean ± SD

using bioinformatics by Su et al. confirmed that CRC tissues exhibit a substantial overexpression of miR-182-5p. Numerous investigations have linked miR-182-5p to the pathophysiology of CRC. For instance, the suppression of miR-182 has been reported to inhibit CRC development by promoting apoptosis [26].

Moreover, Cheng et al. found that the long noncoding RNA GAS5, which sponging miR-182-5p reduces CRC cell growth, influences the expression of FOXO3a [27]. In the present study, we observed significantly elevated expression levels of miR-182-5p in CRC tissues and cell lines. Furthermore, miR-182-5p expression was positively correlated with key clinicopathological features, including tumor invasion depth, degree of differentiation, clinical stage, and lymph node metastasis. These findings support the oncogenic potential of miR-182-5p in CRC. Our in vitro experiments further demonstrated that miR-182-5p promotes CRC cell proliferation, invasion, and migration, while in vivo data confirmed its role in accelerating tumor development. Collectively, these results underscore the critical role of miR-182-5p in CRC pathogenesis and highlight its potential as a therapeutic target.

To alter the expression of specific genes, miRNA, a naturally occurring single-stranded non-coding RNA molecule, targets the 3' untranslated region (3'UTR) of those genes. In particular, miR-182-5p has been connected to several cancers through its effects on radioresistance, cell proliferation, and the inhibition of cell invasion and multiplication [28–30]. For instance, miR-182-5p has been shown by Jin et al. to downregulate MTDH in CRC cells, which reduces metastasis and cell proliferation [31]. Similar findings were made by Yan et al., who discovered that miR-182-5p inhibits VEGF-C expression to reduce tumorigenesis, angiogenesis, and lymphangiogenesis in colon cancer [32]. NCALD was shown to be the main target of miR-182-5p in our investigation. Low NCALD expression was associated in earlier studies by Feng et al. with treatment resistance and a poor prognosis for epithelial ovarian cancer [33]. Shi et al. reported that the overexpression of lncRNA00673, which suppresses NCALD, led to increased tumor proliferation in non-small cell lung cancer [34].

Moreover, individuals with high expression of NCALD who have cytogenetically normal acute myeloid leukemia have a poor prognosis [35], suggesting that NCALD may

act as a tumor suppressor in a range of cancers. The widespread use of HCT-116 cells in colorectal cancer research, along with their significant responsiveness to miRNA regulation, especially with Wnt/β-catenin signalling. Simultaneously, LoVo cells have distinct genetic traits that make them especially appropriate for studying the regulation of NCALD expression. Our findings demonstrate that NCALD silencing promotes HCT116 CRC cell motility, invasion, and proliferation. Interestingly, we discovered that miR-182-5p might inhibit NCALD's effects on HCT116 cell migration, invasion, and proliferation. This suggests that NCALD downregulation may play a major role in the miR-182-5p-mediated proliferation, invasion, and migration of HCT116 cells. To the best of our knowledge, by elucidating the role and significance of the protein in the context of CRC, our work provides new insights into the therapeutic potential of NCALD.

Numerous animal species include the highly expressed and vital Wnt/β-catenin signalling pathway [36]. It is essential for controlling several physiological and pathological cellular processes, including differentiation, apoptosis, proliferation, and others [37]. A new study has demonstrated the important significance of the Wnt/β-catenin pathway in the advancement of colorectal cancer [38]. For example, the compound Rg3 has been shown to inhibit β-catenin's nuclear translocation in colon cancer cells, thereby hindering colorectal tumor growth [39]. The lncRNA HOTAIR, implicated in CRC progression and chemotherapy resistance, may exert its effects through the activation of the Wnt/β-catenin pathway and modulation of miR-203a-3p [40]. Furthermore, the inhibition of ZFP36 in CRC disrupts the EMT by interfering with the Wnt/β-catenin pathway [41]. In CRC tissues, we found that the Wnt/β-catenin signalling pathway was promoted in our investigation. Subsequent research showed that this mechanism is activated by NCALD downregulation, and inhibited by NCALD overexpression. Notably, this study shows that miR-182-5p regulates Wnt/β-catenin signalling mediated by NCALD, which in turn affects the development of CRC. The Wnt/β-catenin signalling pathway was shown to be active in CRC tissues in this investigation. Our research also showed that NCALD overexpression inhibits this crucial pathway, whereas downregulation of the protein promotes the signalling pathway. Most importantly, our results demonstrate that miR-182-5p modifies Wnt/β-catenin signalling, which is mediated by NCALD, and is implicated in the development of CRC.

Nevertheless, this study possesses specific limitations. The luciferase test lacks data pertaining to inhibitors. The demonstration experiment of the Wnt signalling pathway did not include miR-182-5p. In subsequent research, we intend to perform a more thorough examination of the synergistic effects of miR-182-5p and NCALD on Wnt/β-catenin signalling. The TUNEL assay indicated that the transfection effectiveness of both the miR-182-5p mimic and inhibitor groups was comparatively low, potentially altering the apoptotic pathway of colorectal cancer cells and thereby impacting the outcomes of the TUNEL assay. Furthermore, due to its sensitivity, early apoptosis or minimal levels of apoptosis may not be identifiable by TUNEL. No difference in apoptosis was seen as indicated by the TUNEL assay.

Conclusion

In this work, we found that CRC tissues and cell lines had a considerable elevation of miR-182-5p, which was positively linked with important clinical criteria, such as tumor invasion depth, differentiation, stage, and metastasis. Importantly, it was discovered that miR-182-5p accelerated the progression of CRC by controlling NCALD, suggesting that the miR-182-5p/NCALD axis may be a viable target for treatment.

Abbreviations

CRC	Colorectal cancer
miR-182-5p	MicroRNA-182-5p
NCALD	Neurocalcin delta
Wnt/β-catenin	Wnt/β-catenin signaling pathway
GEO	Gene expression omnibus
qRT-PCR	Quantitative real-time polymerase chain reaction
CCK-8	Cell counting kit-8
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
NC	Negative control
CCK-8	Cell counting kit-8 assay
UTR	Untranslated region
GSK-3β	Glycogen synthase kinase-3 beta
FBS	Fetal bovine serum
DMEM	Dulbecco's modified eagle medium
PVDF	Polyvinylidene fluoride
DAPI	4′,6-Diamidino-2-Phenylindole
EMT	Epithelial–mesenchymal transition
FOXO3a	Forkhead Box O3a
SPSS	Statistical Package for the Social Sciences

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-025-02625-0.

Supplementary material 1.

Acknowledgements

Not applicable.

Author contributions

WPF, LG, and SXL conducted the experiments and were responsible for data collection. ZJ, SLJ, and ZXY were involved in data analysis and interpretation. WPF, LG, and WGH contributed to writing and reviewing the manuscript. WGH and CWC were responsible for conceptualizing and overseeing the project.

WGH and CWC also revised the manuscript and provided essential materials. All authors have read and approved the final manuscript for publication.

Funding
This research was funded by the Key Medical Research Projects of Jiangsu Provincial Health Commission (No. ZD2022040), the Nantong City Basic Research and People's Livelihood Science and Technology Plan (No. JCZ21115), and the Nantong Science and Technology Project (MS22016066). The funding bodies was not involved in the study design, data collection, analysis, interpretation, or manuscript preparation.

Availability of data and materials
Original data presented in this study are available in online repositories (https://www.jianguoyun.com/p/DQgWoKYQlvWCChjE3asFIAA).

Declarations

Ethics approval and consent to participate
The Ministry of Health's Chinese Animal Management Rules were strictly followed during every animal experiment carried out for this study. The First Affiliated Hospital of Soochow University Animal Protection and Use Committee approved the study (Approval No. DW102A.07). We have made steps to reduce animal suffering and keep the number of animals we use to a minimum. The First Affiliated Hospital of Soochow University Ethical Committee's ethical standards were followed in every research involving human subjects (approval number: NL09-2019017).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Gastroenterology, The First Affiliated Hospital of Soochow University, Suzhou 215006, Jiangsu, China. [2]Department of Gastroenterology, Affiliated Qidong Hospital of Nantong University, Qidong 226200, Jiangsu, China. [3]Institute of Special Environmental Medicine, Nantong University, Chongchuan District, 9 Seyuan Road, Nantong 226019, Jiangsu, China.

Received: 8 November 2024 Accepted: 22 April 2025
Published online: 02 May 2025

References

1. Chan DKH, Buczacki SJA. Tumour heterogeneity and evolutionary dynamics in colorectal cancer. Oncogenesis. 2021;10(7):53.
2. Pasdar Y, Shadmani FK, Fateh HL, Soleimani D, Hamzeh B, Ghalandari M, et al. The burden of colorectal cancer attributable to dietary risk in Middle East and North African from 1990 to 2019. Sci Rep. 2023;13(1):20244.
3. Pallan A, Dedelaite M, Mirajkar N, Newman PA, Plowright J, Ashraf S. Postoperative complications of colorectal cancer. Clin Radiol. 2021;76(12):896–907.
4. Galli F, Scotto L, Ravenda S, Zampino MG, Pravettoni G, Mazzocco K. Personality factors in colorectal cancer: a systematic review. Front Psychol. 2021;12: 590320.
5. Qi C, Chen Y, Zhou Y, Huang X, Li G, Zeng J, et al. Delineating the underlying molecular mechanisms and key genes involved in metastasis of colorectal cancer via bioinformatics analysis. Oncol Rep. 2018;39(5):2297–305.
6. Kasi A, Handa S, Bhatti S, Umar S, Bansal A, Sun W. Molecular pathogenesis and classification of colorectal carcinoma. Current Colorectal Cancer Rep. 2020;16(5):97–106.
7. Jorge AL, Pereira ER, Oliveira CS, Ferreira EDS, Menon ETN, Diniz SN, et al. MicroRNAs: understanding their role in gene expression and cancer. Einstein. 2021;19:eRB996.
8. Vaghf A, Khansarinejad B, Ghaznavi-Rad E, Mondanizadeh M. The role of microRNAs in diseases and related signaling pathways. Mol Biol Rep. 2022;49(7):6789–801.
9. Sileno S, Beji S, D'Agostino M, Carassiti A, Melillo G, Magenta A. microRNAs involved in psoriasis and cardiovascular diseases. Vascul Bio. 2021;3(1):R49-r68.
10. Balacescu O, Sur D, Cainap C, Visan S, Cruceriu D, Manzat-Saplacan R, et al. The impact of miRNA in colorectal cancer progression and its liver metastases. Int J Mol Sci. 2018;19(12):3711.
11. Wang H. MicroRNAs and apoptosis in colorectal cancer. Int J Mol Sci. 2020;21(15):5353.
12. Liu H, Liu Y, Sun P, Leng K, Xu Y, Mei L, et al. Colorectal cancer-derived exosomal miR-106b-3p promotes metastasis by down-regulating DLC-1 expression. Clin Sci. 2020;134(4):419–34.
13. Zhang D, Yang N. MiR-335-5p inhibits cell proliferation, migration and invasion in colorectal cancer through downregulating LDHB. J BUON. 2019;24(3):1128–36.
14. Joseph NA, Chiou SH, Lung Z, Yang CL, Lin TY, Chang HW, et al. The role of HGF-MET pathway and CCDC66 cirRNA expression in EGFR resistance and epithelial-to-mesenchymal transition of lung adenocarcinoma cells. J Hematol Oncol. 2018;11(1):74.
15. Yang Y, Meng WJ, Wang ZQ. MicroRNAs (miRNAs): novel potential therapeutic targets in colorectal cancer. Front Oncol. 2022;12:1054846.
16. Su Y, Zhang M, Zhang L, Chen S, Zhang D, Zhang X. Construction of an miRNA-mRNA regulatory network in colorectal cancer with bioinformatics methods. Anticancer Drugs. 2019;30(6):588–95.
17. Zhou J, Sun X, Zhang X, Yang H, Jiang Z, Luo Q, et al. miR-107 is involved in the regulation of NEDD9-mediated invasion and metastasis in breast cancer. BMC Cancer. 2022;22(1):533.
18. Sun X, Li W, Li G, Yang H, Jiang Z, Shen L, et al. A novel long non-coding RNA LINC00524 facilitates invasion and metastasis through interaction with TDP43 in breast cancer. J Cell Mol Med. 2024;28(8): e18275.
19. Zaborowski AM, Winter DC, Lynch L. The therapeutic and prognostic implications of immunobiology in colorectal cancer: a review. Br J Cancer. 2021;125(10):1341–9.
20. Keenan JI, Frizelle FA. Biomarkers to detect early-stage colorectal cancer. Biomedicines. 2022;10(2):255.
21. Al-Akhrass H, Christou N. The clinical assessment of MicroRNA diagnostic, prognostic, and theranostic value in colorectal cancer. Cancers. 2021;13(12):2916.
22. Yang L, Zhang X, Hu G. Circulating non-coding RNAs as new biomarkers and novel therapeutic targets in colorectal cancer. Clin Translat Oncol. 2021;23(11):2220–36.
23. Kim H, Kim T, Jaygal G, Woo J, Kim CJ, Baek MJ, et al. Downregulation of miR-9 correlates with poor prognosis in colorectal cancer. Pathol Res Pract. 2020;216(8): 153044.
24. Kim TW, Lee YS, Yun NH, Shin CH, Hong HK, Kim HH, et al. MicroRNA-17-5p regulates EMT by targeting vimentin in colorectal cancer. Br J Cancer. 2020;123(7):1123–30.
25. Zhang W, Zhu Y, Zhou Y, Wang J, Jiang P, Xue L. miRNA-31 increases radiosensitivity through targeting STK40 in colorectal cancer cells. Asia Pac J Clin Oncol. 2022;18(3):267–78.
26. Perilli L, Tessarollo S, Albertoni L, Curtarello M, Pastò A, Brunetti E, et al. Silencing of miR-182 is associated with modulation of tumorigenesis through apoptosis induction in an experimental model of colorectal cancer. BMC Cancer. 2019;19(1):821.
27. Cheng K, Zhao Z, Wang G, Wang J, Zhu W. lncRNA GAS5 inhibits colorectal cancer cell proliferation via the miR-182-5p/FOXO3a axis. Oncol Rep. 2018;40(4):2371–80.
28. He W, Jin H, Liu Q, Sun Q. miR-182-5p contributes to radioresistance in nasopharyngeal carcinoma by regulating BNIP3 expression. Mol Med Rep. 2021;23(2):2.
29. Wu X, Chen H, Wu M, Peng S, Zhang L. Downregulation of miR-182-5p inhibits the proliferation and invasion of triple-negative breast cancer cells through regulating TLR4/NF-κB pathway activity by targeting FBXW7. Annals Translat Med. 2020;8(16):995.
30. Zheng J, Wu D, Wang L, Qu F, Cheng D, Liu X. mir-182-5p regulates cell growth of liver cancer via targeting RCAN1. Gastroenterol Res Pract. 2021;2021:6691305.

31. Jin Y, Zhang ZL, Huang Y, Zhang KN, Xiong B. MiR-182-5p inhibited proliferation and metastasis of colorectal cancer by targeting MTDH. Eur Rev Med Pharmacol Sci. 2019;23(4):1494–501.
32. Yan S, Wang H, Chen X, Liang C, Shang W, Wang L, et al. MiR-182-5p inhibits colon cancer tumorigenesis, angiogenesis, and lymphangiogenesis by directly downregulating VEGF-C. Cancer Lett. 2020;488:18–26.
33. Feng LY, Li L. Low expression of NCALD is associated with chemotherapy resistance and poor prognosis in epithelial ovarian cancer. J Ovar Res. 2020;13(1):35.
34. Shi X, Ma C, Zhu Q, Yuan D, Sun M, Gu X, et al. Upregulation of long intergenic noncoding RNA 00673 promotes tumor proliferation via LSD1 interaction and repression of NCALD in non-small-cell lung cancer. Oncotarget. 2016;7(18):25558–75.
35. Song Y, Zhang W, He X, Liu X, Yang P, Wang J, et al. High NCALD expression predicts poor prognosis of cytogenetic normal acute myeloid leukemia. J Transl Med. 2019;17(1):166.
36. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev Cell. 2009;17(1):9–26.
37. Postwala H, Shah Y, Parekh PS, Chorawala MR. Unveiling the genetic and epigenetic landscape of colorectal cancer: new insights into pathogenic pathways. Med Oncol. 2023;40(11):334.
38. Zhao H, Ming T, Tang S, Ren S, Yang H, Liu M, et al. Wnt signaling in colorectal cancer: pathogenic role and therapeutic target. Mol Cancer. 2022;21(1):144.
39. Yang X, Zou J, Cai H, Huang X, Yang X, Guo D, et al. Ginsenoside Rg3 inhibits colorectal tumor growth via down-regulation of C/EBPβ/NF-κB signaling. Biomed Pharmacoth. 2017;96:1240–5.
40. Xiao Z, Qu Z, Chen Z, Fang Z, Zhou K, Huang Z, et al. LncRNA HOTAIR is a prognostic biomarker for the proliferation and chemoresistance of colorectal cancer via MiR-203a-3p-MEDIATED WNT/ß-CATENIN SIGNALING PATHWay. Cell Physiol Biochem. 2018;46(3):1275–85.
41. Montorsi L, Guizzetti F, Alecci C, Caporali A, Martello A, Atene CG, et al. Loss of ZFP36 expression in colorectal cancer correlates to wnt/ß-catenin activity and enhances epithelial-to-mesenchymal transition through upregulation of ZEB1, SOX9 and MACC1. Oncotarget. 2016;7(37):59144–57.

Publisher's Note