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LncRNA MATN1-AS1/miR-200c-3p/HAS-2 Axis Modulates Proliferation, Invasiveness, Migration and Epithelial-Mesenchymal Transition of Colorectal Cancer Cells

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Background: The Long noncoding RNAs (lncRNAs) have been recognized as pivotal regulators in the development of colorectal cancer (CRC). In this study, we aim to evaluate the impact of lncRNA matrilin 1 (MATN1)-Antisense RNA 1 (AS1)/micro RNA (miRNA, miR)-200c-3p/hyaluronan synthase-2 (HAS-2) axis on CRC cells and *in vivo* CRC model.

Methods: The expression levels of LncRNA MATN1-AS1 in CRC tissues were assessed utilizing the Gene Expression Profiling Interactive Analysis (GEPIA) platform. The expressions of LncRNA MATN1-AS1 and miR-200c-3p were validated through reverse transcription quantitative polymerase chain reaction, while HAS-2 protein and epithelial-mesenchymal transition (EMT)-related protein expressions were examined using western blot analysis. The enrichment of lncRNA MATN1-AS1 and miR-200c-3p was evaluated using RNA Binding Protein Immunoprecipitation (RIP). The binding sites between miR-200c-3p and lncRNA MATN1-AS1 or HAS-2 were predicted by StarBase and confirmed through dual-luciferase reporter assay. Additionally, colony formation assay was performed to assess CRC cell proliferation. Transwell migration assay and invasion assay were conducted to evaluate the migratory and invasive abilities of CRC cells. An *in vivo* model was established by subcutaneous injection in BALB/c nude mice, while lung metastasis models were created through caudal vein injection. Immunohistochemistry was employed for the detection of HAS-2 protein *in vivo*.

Results: The expression of LncRNA MATN1-AS1 was found to be upregulated in both CRC tissues and cells. Subsequently, dual-luciferase reporter assay was performed to demonstrate the interaction between miR-200c-3p and lncRNA MATN1-AS1, which was further confirmed by RNA immunoprecipitation (RIP) assay. Suppression of miR-200c-3p rescued low CRC cell proliferation, migratory and invasive abilities. EMT caused by the knockdown of lncRNA MATN1-AS1. HAS-2 is identified as a target that is negatively regulated by miR-200c-3p, highlighting a critical interaction within the CRC molecular landscape. As for *in vivo* detections, tumor weight and volume were suppressed via the downregulation of lncRNA MATN1-AS1. Additionally, lncRNA MATN1-AS1 downregulation inhibited lung metastasis and suppressed HAS-2 protein levels.

Conclusion: The MATN1-AS1/miR-200c-3p/HAS-2 axis was shown to regulate CRC cell proliferation, invasiveness, migration, EMT, and tumorigenesis *in vivo*.

Keywords: long noncoding RNA MATN1-AS1; MicroRNA-200c-3p; hyaluronan synthase 2; colorectal cancer

Introduction

Colorectal cancer (CRC), an aggressive malignancy, exhibits a progressively rising global incidence rate and mortality, posing a significant threat to human health [1,2]. Recently, aberrantly expressed genes and epigenetic alternations have emerged as pivotal regulators in the development of colorectal cancer (CRC) [3]. Therefore, investigating the pathogenesis and identifying molecular therapeutic targets of colorectal cancer (CRC) represent promising strategies for the diagnosis and treatment of CRC.

Metastasis, a complex and sequential process influenced by both intrinsic and extrinsic factors, plays a pivotal role in cancer progression [4,5]. Epithelial cells gained migratory and invasive capacities to diffuse from primary tumors [6], which is aggravated by a switch from migration mode to proliferation mode of tumor cells to form metastasis [7]. The epithelial-mesenchymal transition (EMT) represents a crucial step in the metastasis of colon cancer, as it facilitates the acquisition of an invasive phenotype by migratory mesenchymal cells [8]. Revealing the underlying mechanisms of epithelial-mesenchymal transition (EMT)

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during metastasis is imperative for the development of innovative therapeutic strategies targeting colorectal cancer (CRC).

Long noncoding RNAs (lncRNAs), comprising more than 200 nucleotides and devoid of protein-coding potential, function as indispensable noncoding RNA molecules that intricately regulate a cascade of downstream biomarkers, thereby exerting influence on the progression of colorectal cancer (CRC) [9]. Numerous studies have substantiated the association between dysregulated lncRNA expression and the progression of colorectal cancer (CRC) [10]. It has been observed that LncRNA HNF1 homeobox A-Antisense RNA 1 (AS1) promotes the proliferation, invasiveness, and migration of CRC cells by upregulating c-Myc, cyclin D1, and β-catenin [11]. These processes are mediated through the inhibition of protein kinase B phosphorylation, extracellular signal-regulated kinases, and signal transducer and activator of transcription 3, which enhance tumorigenesis as observed in an *in vivo* model [12]. The expression of long non-coding RNA matrilin 1 (MATN1)-AS1 was upregulated in osteosarcoma tissues and cells, leading to the suppression of osteosarcoma cell proliferation, invasiveness, and migratory ability through miR-1299 sponging [13]. Nevertheless, its role in CRC have not yet been explored.

Additionally, it has been reported that long non-coding RNAs (lncRNAs) participate in cancer biology through their interaction with microRNAs (miRNAs, miR). MiRNAs can function as either suppressors or promoters of colorectal cancer (CRC), exerting a significant influence on various biological processes associated with CRC [14]. Previous evidence has verified that miR-200c-3p was suppressed in CRC while its upregulation accelerated CRC cell apoptosis [15]. Furthermore, lncRNA MATA1 acted as a competitive endogenous RNA to sequester miR-200c-3p, resulting in the upregulation of ERCC4 in colorectal cancer (CRC) cells. This molecular mechanism ultimately promoted CRC progression and negatively impacted the prognosis of CRC patients [16]. Moreover, lncRNA MATN1-AS1 was observed, using gene expression profiling interactive analysis (GEPIA) (http://gepia.cancer-pku.cn/), to be upregulated in CRC, revealing an oncogenic role of lncRNA. Hence, lncRNA MATN1-AS1 might modulate CRC cell progression via interacting with miR-200c-3p.

Hyaluronan synthase-2 (HAS-2) is one of the synthases of hyaluronan (HA), which is essential in tumorigenesis [17]. Dysregulated HAS-2 participated in the development of different types of malignancies, including breast cancer, ovarian cancer and gastric cancer [18–20]. Furthermore, it has been reported that HAS-2 plays a pivotal role in driving the process of epithelial mesenchymal transition (EMT) in thyroid cancer [21]. Therefore, this study postulates that the lncRNA MATN1-AS1/miR-200c-3p/HAS-2 axis potentially modulates the progression of colorectal cancer (CRC) cells. Their possible molecular mechanisms were explored to provide a novel theoretical aspect to treat CRC.

Methods

Online Database Analysis

LncRNA MATN1-AS1 expressions in CRC tissue samples were validated using GEPIA2 (http://gepia.cancer-pku.cn/) based on data from HiSeq RNA-Illumina HiSeq 2000 RNA sequencing platform (Illumina Inc., CA, USA). MATN1-AS1 expressions were analyzed in 275 CRC (COAD) tissue samples, 349 normal and 92 rectal cancer (READ) tissue samples, based on the criteria of excluding all samples with less than 70% tumor purity. In addition, 318 normal samples from The Cancer Genome Atlas (TCGA) database and the Genotype-Tissue Expression (GTEx) projectwere also included in the analysis. To ensure data quality, samples with a sequencing depth of less than 10 million reads and a mapping quality score of less than 30 were excluded. RNA-Seq data were normalized and processed using the Trimmed Mean of M-values method for normalization and the DESeq2 algorithm (Version 1.26.0, Bioconductor, WA, USA) for differential expression analysis, with statistical significance of differential expression was determined at p-value < 0.05 and fold change ≥ 2.

Cell Culture

SW1116 (Cat. No. TCHu174), HCT-8 (Cat. No. TCHu 18), SW480 (Cat. No. TCHu172), SW620 (Cat. No. TCHu101), HCT116 (Cat. No. TCHu 99) and Hce-8693 (Cat. No. TCHu 70) cells (Cell Bank of the Chinese Academy of Sciences, Shanghai, China), and NCM460 (Cat. No. BFN608006385, Qingqi Biotechnology Development Co., LTD, Shanghai, China) were cultivated using RPMI-1640 medium (Cat. No. 11875093, Gibco, Thermo Scientific, Waltham, MA, USA), containing 10% fetus bovine serum (Cat. No. 16010159, FBS; Gibco) and double-resistant penicillin (100 U/mL)-streptomycin (100 µg/mL, Cat. No. 15140148, Gibco) at 37 °C, 5% CO_2. All the cell lines were authenticated by short tandem repeat methods and mycoplasma free.

Cell Transfection

HCT-8 and SW1116 cells in log phase were gathered followed by cell inoculation onto 6-well plates (1×10^5 cells/well). GenePharma (Shanghai, China) provided small interfering RNA of lncRNA MATN1-AS1 (si-MATN1-AS1) and the negative control (si-NC), mimic and inhibitor of miR-200c-3p (miR-200c-3p mimic/inh, 100 nmol/µL) and their corresponding controls (mimic-NC/NC inh, 100 nmol/µL). After cell confluence reached 80%, transfection was conducted using Lipofectamine 3000 (Cat. No. L3000150, Invitrogen, Thermo Scientific). The cells were subsequently cultured for an

additional 24 hours. RNA expressions were evaluated using reverse transcription quantitative polymerase chain reaction (RT-qPCR) while HAS-2 protein expressions were assessed using western blot. The sequences for these transfection vectors are as follows: si-MATN1-AS1: 5′-CUCUGAAUGGCAGUUCUAUGG-3′; si-NC: 5′-UAUUCGACGGGUUAAUCGGCU-3′; miR-200c-3p mimic: 5′-UAAUACUGCCGGGUAAUGAUGGA-3′; miR-200c-3p inhibitor: 5′-UCCAUCAUUACCCGGCAGUAUUA-3′; mimic-NC: 5′-GUCCAUCUUACAUCAAUCAGCUG-3′; NC inhibitor: 5′-CAGUACUUUUGUGUAGUACAA-3′.

RT–qPCR

RNAiso Plus Reagent (Cat. No. 9108Q, Takara, Shiga, Japan) was applied for isolating total RNA from HCT-8 and SW1116 cells. Thereafter, cDNA was synthesized through reverse transcription using a reverse transcription kit followed by a PCR with the SYBR Green Master Mix (Cat. No. A46110, Applied Biosystems, Thermo Scientific) and CFX Opus 96 Real-Time PCR System (Cat. No. #12011319, Bio-Rad, Hercules, CA, USA). The $2^{-\Delta\Delta CT}$ formula was used to calculate and statistically analyze the lncRNA MATN-AS1 and miR-200c-3p expressions. Sequences of the PCR primers (Suzhou Jima gene Co., Ltd., Suzhou, China) were as follows: lncRNA MATN1-AS1: F (forward), 5′-ACTTGACACCAATGAACCCAGGTG-3′, and R (reverse), 5′- ACACTAACCAGTGAGTGATTAGAACCG-3′; miR-200c-3p: F, 5′-ACACCAGGAATTGGGTGGCTGT-3′, and R, 5′-CTCAACTGGTGTCGTGGAG-3′; U6: F, 5′-CGGTTTCACCCGCAGCA-3′, and R, 5′-AATCTTCACCGATGCGAGTT-3′ and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): F, 5′-AGCCACATCGCTCAGACAC-3′, and R, 5′-GCCCAATACGACCAAATCC-3′. GAPDH and U6 were used as internal controls of lncRNA MATN1-AS1 and miR-200c-3p, respectively.

Western Blot Assay

Segregation of protein from HCT-8 and SW1116 cells was assessed using radio immunoprecipitation assay (RIPA) buffer (Cat. No. PP1901, BioTeke, Wuxi, China). Protein quantification was performed with a bicinchoninic acid assay protein determination kit (Cat. No. 23227, Thermo Scientific). Afterwards, protein was isolated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Cat. No. P0012A, Beyotime, Shanghai, China) and polyvinylidene difluoride membranes (Cat. No. 03010040001, Merck, Kenilworth, NJ, USA) with the intention of transferring the protein. After blocking nonspecific signals with 5% non-fat milk powder, primary antibodies, specific for anti-occludin (1:500, Cat. No. bs-10011R, Bioss, Beijing, China), anti-GAPDH (1:5000, Cat. No. bs-41373R, Bioss), anti-N-cadherin (1:000, Cat. No. bs-55138R, Bioss), anti-Snail1 (1:000, Cat. No. bs-1371R, Bioss), anti-vimentin (1:000, Cat. No. bs-8533R, Bioss) and anti-E-cadherin (1:1000, Cat. No. bs-1519R, Bioss) were cultured with membranes at 4 °C overnight, followed by HRP-coupled incubation with secondary antibody Goat Anti-Rabbit IgG (1:5000, Cat. No. bs-80295G-HRP, Bioss) at 25 °C for 1.5 h. Finally, western blot analysis was visualized using enhanced chemiluminescence reagents. Bands were examined using Image Lab software (Version 6.1, Bio-Rad).

Colony Formation Assay

HCT-8 and SW1116 cells were digested by 0.25% trypsin (Cat. No. 25300054, Gibco) and suspended in RPMI-1640 medium with 10% FBS. After dilution, cells were seeded into 10ml Petri dishes and cultivated at 37 °C, 5% CO_2 for 2~3 weeks. Thereafter, the cells were fixed with 4% paraformaldehyde (Cat. No. P0099-500ml, Beyotime) and stained with 0.1% crystal violet. Finally, clones with more than 10 cells were counted using microscope (at low magnification, Cat. No. Nikon Eclipse E100, Nikon, Tokyo, Japan).

Transwell Assay

CRC cell migratory and invasive capacities after transfection were examined by transwell method. Matrigel (Cat. No. 354234, Corning Incorporated, Corning, NY, USA) was applied during invasion or migration detections. Cells were planted into the upper chamber with serum-free medium (Cat. No. 11875093, Gibco) followed by the addition of RPMI-1640 medium containing 10% FBS in the basal compartment. After cultured for 8 h, 4% paraformaldehyde (Cat. No. P0099, Beyotime) was used to fix migrated or invaded cells. Then cells were stained using 0.5% crystal violet. Finally, migrated or invaded cells were observed by microscope (Cat. No. IX73, Olympus, Tokyo, Japan). The average number of cells were randomly selected from six fields.

Dual-Luciferase Assay

The lncRNA MATN1-AS1 sequence or HAS-2 sequence containing the predicted binding sites with miR-200c-3p, and their mutated types were cloned into psiCHECK-2 plasmid (Cat. No. C8021, Promega, Madison, WI, USA) and designated as MATN1-AS1-Wild type (WT)/-mutant (MUT) or HAS-2-WT/-MUT. Then, MATN1-AS1-WT/-MUT or HAS-2-WT/-MUT were transfected into HCT-8 and SW1116 cells together with miR-200c-3p mimic/mimic-NC using the Lipofectamine 3000. Fluorescence was validated using a dual luciferase reporter gene detection system (Cat. No. E1910, Promega), with *Renilla* luciferase activity serving as the internal reference to normalize for transfection efficiency and cell viability.

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RNA Immunoprecipitation (RIP)

The bound of lncRNA MATN1-AS1 with miR-200c-3p to Argonaute 2 (Ago2) protein was assessed by RNA immunoprecipitation. After lysing with RIPA buffer (Cat. No. P0013D, Beyotime), protein supernatant was divided in two groups: one was the input and the other was for immunoprecipitation Then, anti-Ago2 antibody (Cat. No. 1:2000, ab186733, Abcam, Cambridge, UK) or anti-mouse IgG (Cat. No. 1:200, ab205718, Abcam) negative control magnetic beads were added and incubated with the lysis overnight at 4 °C. Protein A/G Agarose (Cat. No. P2055-10mL, Beyotime) was added and cultured for 1 h at 4 °C. After washed by phosphate buffer solution (PBS), beads were resuspended by RNAiso Plus Reagent to isolate RNA. Then, RT-qPCR was used to examine RNA expressions.

Tumor Xenotransplantation

All animal procedures were performed complying with the guidelines for care and use of experimental animals in Chinese Academy of Medical Sciences and Peking Union Medical College. The study was approved by the ethics committee of Chinese Academy of Medical Sciences and Peking Union Medical College (Approval Number: AEEI-2024-015). Six-week-old male BALB/c nude mice (22 ± 2 g) were purchased from the Weitonglihua Experimental Animal Technology Company (Beijing, China). Before *in vitro* experiments, GenePharma provided lentivirus vectors containing siRNA sequences of lncRNAs MATN1-AS1 (CCG GGC GCT CCT GTT TAT GTA CTT ACT CGA GTA AGT ACA TAA ACA GGA GCG CTT TTT TG) or its negative control (CCG CCC TTT TTT GGG CCT AAA ACC CCT GGA GGA AAA ATT GTT TTC GGC GGG GTA GTC CG). After stable transfection, SW1116 cells were collected for following experiments. Thereafter, in the xenograft tumor model experiment, mice were randomly divided in two groups. In each group, three mice were injected in the right flank with SW1116 cells (6×10^6) suspended in 100 µL of PBS after transfection of si-NC or siMATN1-AS1, respectively. After 45 days, the mice were euthanized by intraperitoneal injection of pentobarbital sodium (150 mg/kg). The criteria for successful model construction were: palpable tumor in xenograft tumor model and visible metastatic nodules in lung metastatic tumor model. The tumor was weighed, and volumes were calculated following the next equation:

Tumor volume = 0.5 × length × width × height.

Mice were randomly divided in two groups with three mice in each group to assess the lung metastasis model. SW1116 cells (5×10^5) stably transfected with si-NC or si-MATN1-AS1 were injected through the caudal vein with 200 µL PBS. Mice were euthanized 45 days after injection. Lungs were removed, dissected, observed for lung metastasis, and photographed.

Immunohistochemistry (IHC)

IHC was performed to examine HAS-2 expressions in lung metastasis models. After fixed by 4% paraformaldehyde, lung tissue samples were embedded by paraffin, cut in 4 µm slices and dried at 60 °C for 20 min. Xylene dewaxed and ethanol gradient dehydrated and high-pressure antigen repair. The primary antibody against HAS-2 (1:200, Cat. No. PA5-115388, Invitrogen) was added to incubate the slices at 4 °C overnight. Afterward, secondary antibody (HRP) was added to the slices followed by 1 h incubation at 25 °C. DAB chromogenic (Cat. No. P0202, Beyotime) solution was used for color development. After hematoxylin (Cat. No. C0107-100 mL, Beyotime) counterstaining, ethanol gradient dehydrating and neutral balsam sealing, microscopic examination was performed (200×). Five fields were chosen randomly, and ImageJ software (National Institutes of Health [NIH], Bethesda, MD, USA) was used to determine the positive area score statistics. No staining was a score of 0; ≤25% staining was a score of 1; 26% to 50% staining was a score of 2; 51%~75% staining was a score of 3; >75% staining was a score of 4.

Statistical Analysis

Data analysis was conducted using IBM SPSS 22.0 (IBM Corp., Armonk, NY, USA), and graphical representations were generated with GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA) All experiments in this study were triplicated. Data is reported as the mean ± SD. Independent *t* tests was used to analyse differences between two groups, while one-way ANOVA and Tukey's post hoc test were used to examine differences in multiple groups. $p < 0.05$ was used to consider statistically meaningful result.

Results

LncRNA MATN1-AS1 Increased in CRC Tumor Tissue Samples and Cells

The expression levels of lncRNA MATN1-AS1 were found to be significantly upregulated in colorectal cancer (CRC) tissue samples compared to normal counterparts, based on comprehensive data analysis from the TCGA database and GTEX project (Fig. 1A). Additionally, lncRNA MATN1-AS1 expressions were also elevated in SW1116, HCT-8, SW480, SW620, HCT116 and Hce-8693 cells (Fig. 1B).

LncRNA MATN1-AS1 Sponged miR-200c-3p in CRC Cells

Using StarBase (http://starbase.sysu.edu.cn/), putative binding sites of miR-200c-3p with lncRNA MATN1-AS1 were found (Fig. 2A). Furthermore, the results of the dual-luciferase assay demonstrated that HCT-8 and SW1116 cells transfected with wild-type MATN1-AS (MATN1-AS-WT) and miR-200c-3p mimic exhibited reduced fluorescence intensity compared to other experimental groups

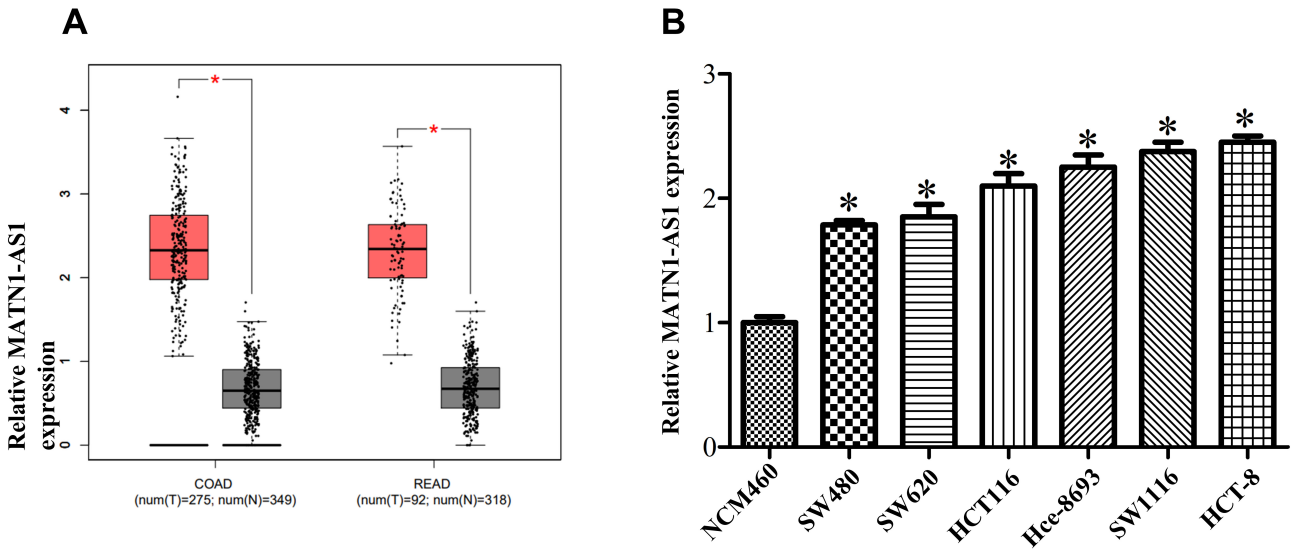

Fig. 1. LncRNA MATN-AS1 increased in CRC tumor tissue samples and cells. (A) LncRNA MATN1-AS1 expressions in CRC tissue samples and normal ones evaluated using the GEPIA database, *Denotes significant difference, $p < 0.05$. (B) LncRNA MATN1-AS1 expressions in normal and colorectal cell lines (SW1116, HCT-8, SW480, SW620, HCT116 and Hce-8693 cells), *Denotes significant difference, $p < 0.05$ vs. NCM460 cells. LncRNA, Long noncoding RNA; CRC, colorectal cancer; GEPIA, Gene Expression Profiling Interactive Analysis.

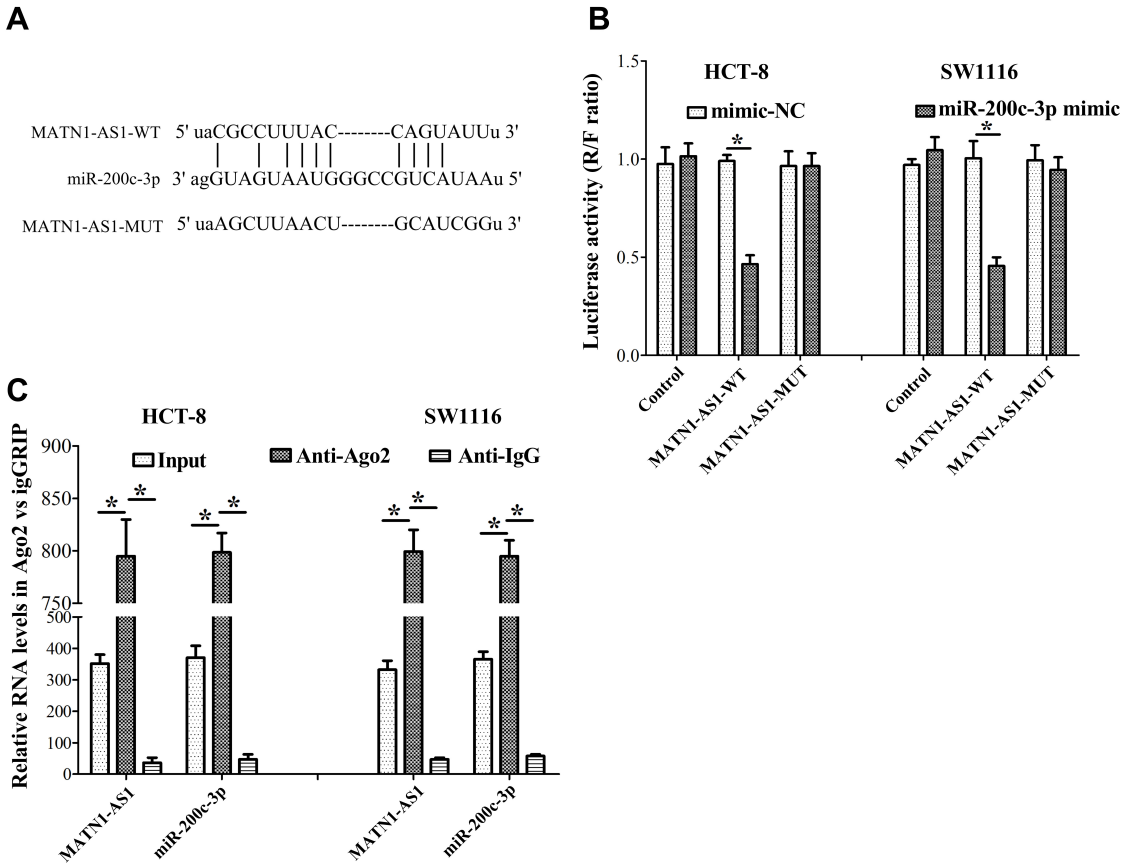

Fig. 2. LncRNA MATN1-AS1 sponged miR-200c-3p in CRC cells. (A) Binding sites of lncRNA MATN1-AS1 with miR-200c-3p analyzed using StarBase. (B) Fluorescence in MATN1-AS1-WT/MUT with mimic NC or miR-200c-3p mimic verified using dual-luciferase reporter test. (C) RIP with Ago2 antibody applied for examining enrichment of lncRNA MATN1-AS1 or miR-200c-3p. *Denotes significant difference, $p < 0.05$.

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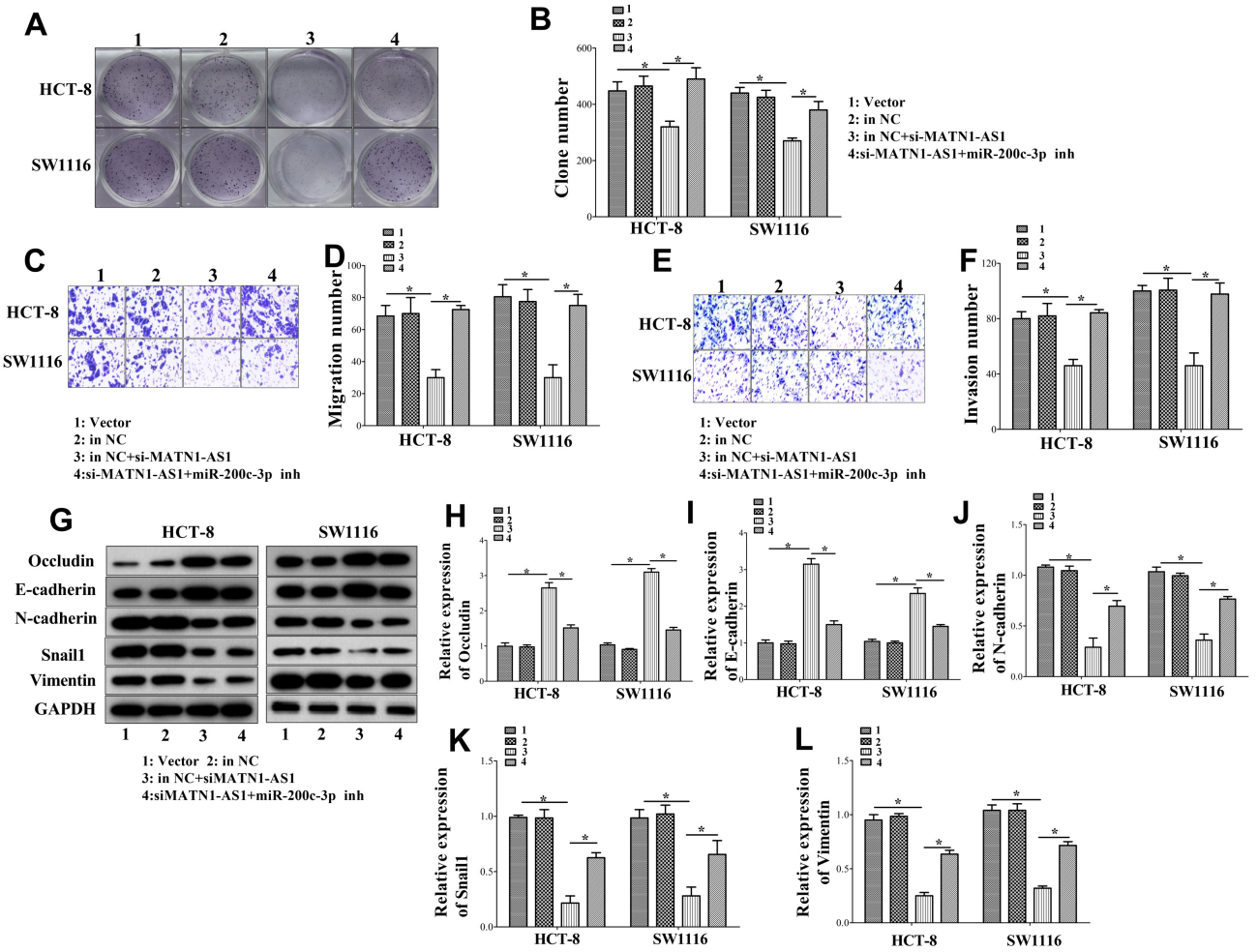

Fig. 3. LncRNA MATN1-AS1 facilitated CRC cell proliferation, invasiveness, migration and EMT via interaction with miR-200c-3p. (A,B) Proliferative abilities of SW1116 and HCT-8 cells examined using colony formation. (C–F) Effect of MATN1-AS1 on modulating migratory ability and invasiveness of HCT-8 and SW1116 cells examined by Transwell (×100 magnification). (G–I) Occludin, E-cadherin, Snail1, Vimentin and N-cadherin protein expression in HCT-8 and SW1116 cells validated using western blot.

(Fig. 2B). Moreover, results of RIP indicated enrichments of MATN1-AS1 and miR-200c-3p compared to the input (Fig. 2C).

LncRNA MATN1-AS1 Facilitated CRC Cell Proliferation, Invasiveness, Migration and EMT by Interacting with miR-200c-3p

To further elucidate the impact of lncRNA MATN1-AS1 in colorectal cancer (CRC) cells, we investigated its interaction with miR-200c-5p. The colony formation assay results demonstrated that the proliferative abilities of HCT-8 and SW1116 cells were impaired upon knockdown of lncRNA MATN1-AS1. However, this impact was reversed when miR-200c-3p suppression was applied (Fig. 3A,B). Furthermore, the migratory and invasive capabilities of CRC cells, which were suppressed by lncRNA MATN1-AS1 inhibition, exhibited enhanced acceleration following downregulation of miR-200c-3p (Fig. 3C–F). The western blot analysis results revealed that the inhibition of lncRNA MATN1-AS1 led to an upregulation of Occludin and E-cadherin, although this upregulation was partially reversed upon suppression of miR-200c-3p In contrast, N-cadherin, Snail1 and Vimentin were decreased by suppression of lncRNA MATN1-AS1 but the decrease was partly reversed after miR-200c-3p inhibition (Fig. 3G–I).

HAS-2 was Targeted by miR-200c-3p

The underlying binding sites of miR-200c-3p with HAS-2 were predicted by StarBase, as depicted in Fig. 4A. In HCT-8 and SW1116 cells, the fluorescence intensity was significantly reduced in the HAS-2-WT group transfected with miR-200c-3p mimic compared to other experimental groups (Fig. 4B). Moreover, in HCT-8 and SW1116 cells, HAS-2 protein expression was downregulated by miR-200c-3p mimic while its protein expressions were elevated by inhibited miR-200c-3p (Fig. 4C,D).

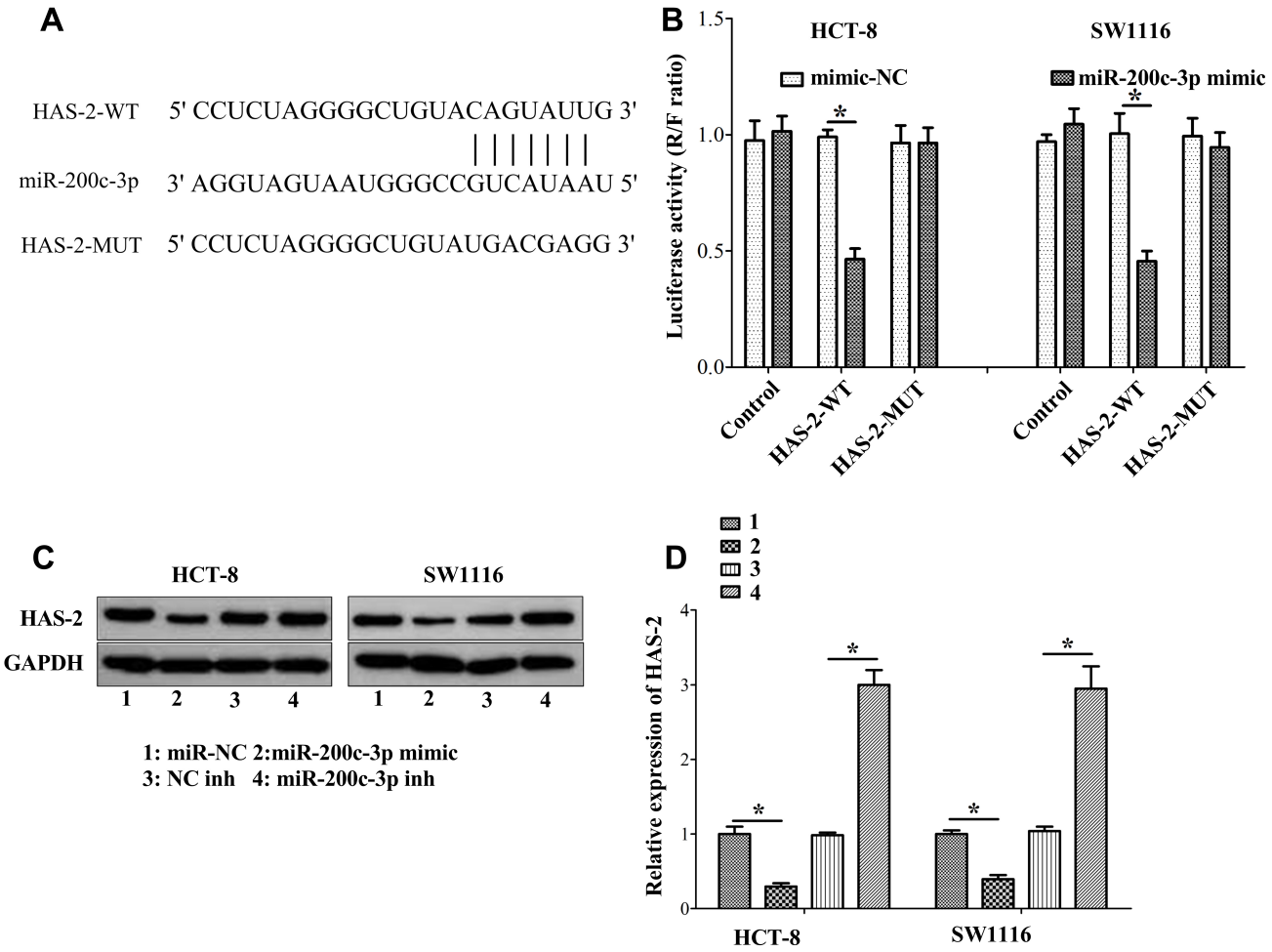

Fig. 4. HAS-2 was targeted by miR-200c-3p. (A) Binding sites of miR-200c-3p with HAS-2 provided by StarBase. (B) Dual-luciferase activities in miR-200c-3p mimic or mimic-NC with HAS-2-WT/MUT verified by a dual-luciferase reporter test. (C,D) HAS-2 protein expressions in HCT-8 and SW1116 cells transfected with miR-200c-3p mimic/inhibitor assessed using western blot. *Denotes significant difference, $p < 0.05$.

The Knockdown of lncRNA MATN1-AS1 Inhibited Tumorigenesis In Vivo via Interacting with HAS-2

The transplanted tumor with knockdown of lncRNA MATN1-AS1 exhibited significantly reduced weight and volume compared to the control group, as anticipated (Fig. 5A,B). RT-qPCR results further confirmed that MATN1-AS1 expression was reduced in tumors in the si-MATN1-AS1 group (Fig. 5C). Additionally, nodules were reduced in the lungs of mice with si-MATN1-AS1 xenografts compared to mice with si-NC (Fig. 5D). Results of the IHC analysis indicated that HAS-2 was downregulated by the suppression of lncRNA MATN1-AS1 (Fig. 5E).

Discussion

Investigating the underlying mechanisms governing the occurrence and progression of colorectal cancer (CRC) to identify potential biomarkers is critical to improve the prognosis of CRC patients. The elevated mortality rate observed in CRC patients can be attributed to the heightened invasiveness and migratory capacity exhibited by CRC cells, which is facilitated through a multistep process known as the metastasis cascade [22,23].

LncRNAs play pivotal roles in various biological processes, including chromatin remodeling, transcriptional control, posttranscriptional regulation and intercellular signal transduction [24]. They possess the ability to modulate cellular biological behaviors, thereby facilitating the progression of diverse types of cancers, including colorectal cancer (CRC) [25,26]. LncRNA colorectal neoplasia differentially expressed expression was elevated in CRC tissues, whose overexpression enhanced CRC cells proliferation, invasiveness and migration via sponging miR-181a-5p [27]. We conducted an investigation into the expression of lncRNA MATN1-AS1 in colorectal cancer (CRC) tissue samples and cells to elucidate its impact on the progression of CRC.. The expression of lncRNA MATN1-AS1 was upregulated in both colorectal cancer (CRC) tissue samples and cells, whereas the knockdown of lncRNA MATN1-AS1 suppressed the malignant biological behav-

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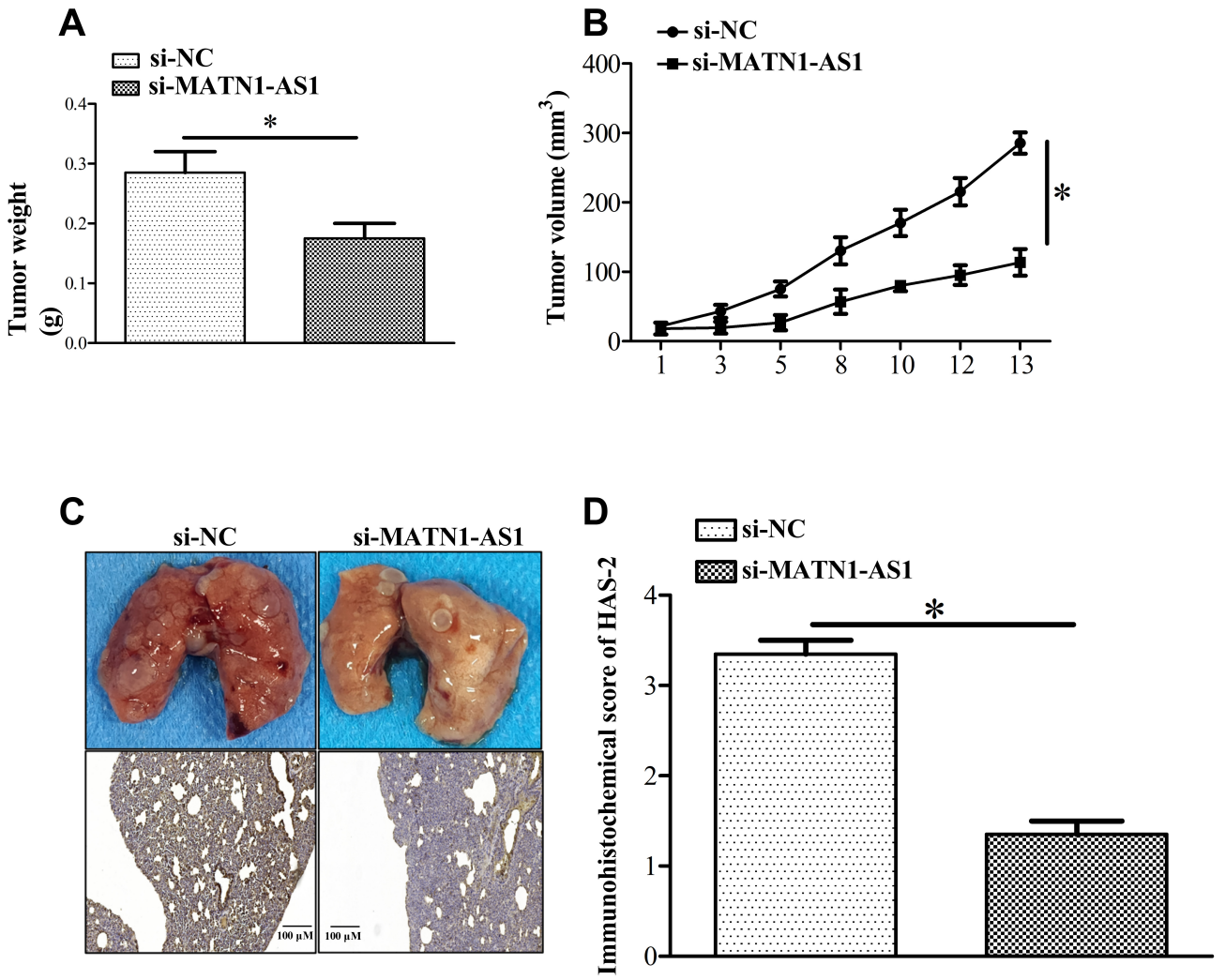

Fig. 5. The downregulation of LncRNA MATN1-AS1 inhibits tumor growth via downregulating HAS-2. (A,B) Tumor weight and volume. (C) The expression difference of LncRNA MATN1-AS1 in Tumor was analyzed by RT-qPCR. (D) HAS-2 protein assessed by IHC staining after the knockdown of lncRNA MATN1-AS1 in lung metastasis models (n = 3). *Denotes significant difference, $p < 0.05$. RT-qPCR, reverse transcription quantitative polymerase chain reaction; HAS-2, hyaluronan synthase-2.

iors of CRC cells. Hence, lncRNA MATN1-AS1 might participate in the CRC cell progression. To the best of our knowledge, this is the first study detecting biological functions of lncRNA MATN1-AS1 in CRC cells.

Relevant studies have previously emphasized the pivotal role of EMT in governing the invasive and migratory capabilities of tumor cells, which are fundamental driversof tumor recurrence and metastasis [28–30]. To date, EMT is an attractive therapeutic target, as it is involved in the ability of cancer tissues to dedifferentiate to a more malignant state [31,32]. During the process of epithelial-mesenchymal transition (EMT), activation of specific transcription factors leads to downregulation of epithelial markers [33,34]. Meanwhile, the upregulation of N-cadherin, vimentin, and other interstitial markers is induced to facilitate the mediation of the EMT phenotype and expedite the process of epithelial-mesenchymal transition [35]. Therefore, the imbalance of N-cadherin, Snail1, vimentin, occludin and E-cadherin is crucial in EMT. In this study, downregulation of lncRNA MATN1-AS1 not upregulated the protein expression of occludin and E-cadherin but also suppressed Vimentin, Snail1 and N-cadherin. Therefore, it is plausible that MATN1-AS1 facilitates CRC cell invasiveness and migration by accelerating EMT.

Numerous long non-coding RNAs (lncRNAs) function as competitive endogenous RNA molecules, effectively sequestering miRNAs and thereby exerting regulatory control over cancer cell progression [36,37]. A previous study has previously demonstrated a significant association between MATN1-AS1 and miR-200c-3p, which is consistent with our findings [38]. As lncRNAs and miRNAs showed inhibition interaction, the expression of both was often negatively correlated in the same tumor [39,40]. The present study provides evidence supporting the nega-

tive interaction between lncRNA MATN1-AS1 and miR-200c-3p in colorectal cancer (CRC) cells. Previous findings have demonstrated the downregulation of miR-200c-3p in both colorectal cancer (CRC) tissue samples and cells, whereas upregulation of miR-200c-3p has been shown to inhibit malignant phenotypes in cancer cells [41]. Moreover, miR-200c-3p was observed to restrain EMT in epicardial mesothelial cells via targeting Follistatin-related protein 1 [42]. Moreover, the overexpression of miR-200c-3p has been experimentally validated to effectively suppress the epithelial-mesenchymal transition (EMT) process, thereby enhancing the sensitivity of epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer cells harboring epidermal growth factor receptor mutations [43]. In this study, the suppression of miR-200c-3p reversed the inhibitory effects of of downregulated lncRNA MATN1-AS1 on CRC cell proliferation, invasiveness, migration and EMT. Therefore, we present an underlying molecule that exhibits negative interplay with lncRNA MATN1-AS1 in CRC cells.

The downstream target gene of miR-200c-3p, as predicted by StarBase, was found to be HAS-2. Suppression or overexpression of miR-200c-3p resulted in elevated or inhibited expressions of HAS-2 in CRC cells, respectively. It has been observed that HAS-2 facilitate invasiveness and migration of luminal breast cancer cells, which also promote tumor growth and lung metastasis *in vivo* [44]. The activation of HAS-2 has also been observed to stimulate TGF-β and SMAD, thereby augmenting the invasiveness and migratory capabilities of CRC cells through the regulation of EMT. In this study, *in vivo* experiments showed that the metastatic lesions were reduced in mouse models after the knockdown of lncRNA MATN1-AS1. Meanwhile, HAS-2 protein expression was downregulated, indicating that lncRNA- MATN1-AS1 downregulation suppressed HAS-2 expression, causing the inhibition of EMT, decreasing lung metastasis *in vivo*. Hence, results suggested that MATN1-AS1 might promote CRC progression by upregulating HAS-2 expression via sponging miR-200c-3p.

Conclusion

LncRNA MATN1-AS1 was found to be upregulated in colorectal cancer (CRC) tissue samples and cells, thereby promoting CRC cell proliferation, invasion, migration and EMT through miR-200c-3p/HAS-2 signaling pathway what enhanced tumorigenesis and lung metastasis *in vivo*. However, due to the limited sample size *in vivo*, it is imperative to consider other potential confounding factors. Therefore, further investigations are warranted to enhance our comprehension of the functional role of lncRNA MATN1-AS1 in colorectal cancer (CRC).

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets generated and/or analysed during the current study are available in the Gene Expression Omnibus of the National Center for Biotechnology Information repository, [http://www.ncbi.nlm.nih.gov/geo/, accession number GSE 10474].

Author Contributions

X.C. conducted the study, analyzed the data, and drafted the manuscript. J.D. conducted the study and collected data. L.X analyzed the data and supported the conduction of the study. Q.Z. designed the study and revised the manuscript for important intellectual content.

Ethics Approval and Consent to Participate

All experimental protocols were approved by the Ethical Committee on the Use of Experimental Animals for Biomedical Research at Southern Medical University. (LAEC-2020-022). We confirm that all experiments were performed in accordance with relevant guidelines and regulations. Our manuscript reporting adheres to the ARRIVE guidelines (https://arriveguidelines.org) for the reporting of animal experiments.

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Conflict of Interest

The authors declare no conflict of interest.

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