



# LINC00178 promotes cell growth, migration, and invasion in colorectal cancer *in vitro* and *in vivo*

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**Background:** This study aimed to determine the role of LINC00178 in colorectal cancer (CRC) cell invasion and migration by examining its expression in CRC cells and tissues.

**Methods:** Cancer tissues and corresponding adjacent tissue specimens were collected from 45 patients who experienced radical CRC resection in the hospital from March to September 2021. The expression of LINC00178 was measured in both CRC cells and tissues and normal human colorectal mucosal cells using quantitative fluorescence polymerase chain reaction (QF-PCR). Cell Counting Kit-8 (CCK-8), clonogenic, and transwell assays were used to assess the impact of LINC00178 overexpression or knockdown on the CRC cells invasion and proliferation. In addition, the expression levels of vimentin, E-cadherin, and N-cadherin in CRC cells were determined after either LINC00178 knockdown or overexpression was performed using western blotting.

**Results:** The experiments revealed that LINC00178 was over expressed in CRC cells and tissues. Overexpression of LINC00178 could significantly promote the propagation, clone formation, invasion, and transportation of CRC, whereas knockdown of LINC00178 had the opposite function. When LINC00178 was expressed at high levels, it suppressed the vimentin and N-cadherin expression and prevented the upregulation of E-cadherin. *In vivo* (nude mouse) studies showed that the over expression of LINC00178 could significantly promote the propagation in CRC cells.

**Conclusions:** LINC00178 is overexpressed in CRC cells and tissues. *In vivo* and *in vitro* experiments showed that LINC00178 can significantly promote the propagation of CRC cells, so it may develop a potential biological site for targeted therapy of CRC patients.

**Keywords:** LINC00178; colorectal cancer (CRC); invasion; migration

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## Introduction

Colorectal cancer (CRC) is a very common gastrointestinal malignant tumor for which the incidence is increasing annually and the age at diagnosis is becoming younger (1,2). At present, surgical resection is mainly used in clinical

practice, supplemented by preoperative or postoperative chemotherapy. Although CRC diagnosis and treatment methods have made significant progress, due to the hidden onset of colon cancer, most patients have already reached the middle or late stage at the time of diagnosis. The shortage of sensitive analytical methods to diagnose CRC

in the early stage leads to poor prognosis and around more than half of the patients with a continuous progression eventually die due to metastasis and recurrence (3,4). Molecularly targeted therapeutic drugs have recently played a significant role in the comprehensive treatment of patients with advanced CRC. Molecularly targeted therapeutic drugs with different targets have different effects, but the clinical application of molecularly targeted drugs is still unclear. There are many problems that need to be further clarified (5-7). For that reason, the discovery of molecular targets for early detection and novel targets for treatment, as well as the identification of molecular pathways impacting the growth of CRC, are crucial.

Any RNA molecule longer than 200 nucleotides is considered a long non-coding RNA (lncRNA), and these molecules play important roles in pathophysiology. They regulate gene expression at the epigenetic, transcriptional, and post-transcriptional stages (8,9). Studies have found that a diversity of lncRNAs are strangely expressed in a variety of species and play different biological roles. An example is lncRNAs with tumor-promoting functions, the overexpression of which promotes tumor growth and cancer cell proliferation and metastasis, or exerts tumor suppressor functions. The over expression of lncRNA can inhibit the progression, migration or transportation, and metastasis of tumor cells (10-12). In addition to a high prevalence of metastasis and recurrence, previous research has shown that lncRNAs play a crucial role in the development and spread of CRCs (13); CRC tissues showed markedly elevated expression of lncRNA and *NEAT1* genes. Further, *in vivo* cum *in vitro* experiments have shown that overexpression of lncRNA, and *NEAT1* genes promoted the progression and metastasis of CRCs by activating the Wnt signaling pathway (14). In addition, the lncRNA, and *SNHG11* in peripheral blood serve as a unique biological marker for an early prognosis and timely diagnosis of CRC (15). Many lncRNAs, including LBX2-AS1, have been linked to increased tumor cell proliferation and resistance to the chemotherapy medication 5-fluorouracil in CRC (16).

New evidence suggests that the non-coding RNA LINC00178, commonly known as urothelial carcinoma associated 1 (UCA1), has a role in controlling tumor growth, apoptosis, and invasion (17,18). Thyroid cancer cells' proliferation and metastasis were discovered to be induced by LINC00178 via the elimination of miR-497-3p (19). However, the mechanism of LINC00178 in CRC is unclear. Therefore, in-depth research into the molecular

level mechanisms of CRC cell invasion and metastasis, as well as the identification of potential biomarkers, are critical for disease diagnosis and treatment. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-940/rc>).

## Methods

### *Clinical sample collection*

This experiment included 45 patients with CRC in the Affiliated Hospital of Nanjing University of Chinese Medicine, from March to September 2021. All the patients were pathologically diagnosed with CRC; immune- and other related underlying diseases were excluded. No chemotherapy or other treatments were given before surgery. Cancer tissue and matched non-cancer colorectal tissue samples were obtained within half an hour of tumor excision and stored in liquid nitrogen. Each participant and each examining physician completed an informed consent form. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (No. 2021NL-034-01).

### *Cell culture and transfection*

This experiment used CRC cell lines (LoVo, SW620, SW480, HT-29, and HCT116), and normal or control human colorectal mucosal cells (FHC) were purchased from the Shanghai Institute of Biological Sciences (Shanghai, China). These cells were grown in a 5% CO<sub>2</sub> incubator in a medium containing 15% fetal bovine serum (FBS) from either Roswell Park Memorial Institute (RPMI)-1640 or Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA). Passaging was carried out at a ratio of 1:3. The CRC cells were selected in logarithmic phase and then inoculated into 6-well plates and cultured overnight. When the confluency of cells attached to the wall reached about 50%, the pcDNA-178 overexpression recombinant vector or empty vector plasmid was transfected with lentivirus, short hairpin (sh)RNA-Control, shRNA-1, and shRNA-2 were transferred to the cells. For specific steps, refer to the instructions of the transfection reagent. The relevant indicators were detected 48 hours after the cells were transfected.

### *Fluorescence quantitative polymerase chain reaction*

The total RNA in the transfected CRC cells was isolated using the one-step TRIzol method, then the RNA was purified. Total RNA was then analyzed for concentration and purity using NanoDrop (Thermo Fisher, Waltham, MA, USA). Complementary DNA (cDNA) was prepared using a reverse transcription (RT) kit. We took 2  $\mu$ L of the RT product as template, GAPDH as the internal reference, and used SYBR Green PCRMaster Mix kit (Thermo Fisher) for quantitative polymerase chain reaction (qPCR) detection. Each reaction system in the 20  $\mu$ L vial included 2  $\mu$ L of RT product, 10  $\mu$ L of 2 $\times$  SYBR Green dye, 0.8  $\mu$ L of both primers (forward and reverse), and 6.4  $\mu$ L of double distilled water (ddH<sub>2</sub>O). The 45 cycles of the reaction consisted of 95 °C for 5 minutes, 60 °C for 30 seconds, and at 72 °C. for 10 seconds. After the reaction, the relative quantitative  $2^{-\Delta\Delta C_t}$  method was used for analysis.

### *Cell Counting Kit-8 assay*

After transfection, CRC cells were added to a 96-well plate,  $2 \times 10^3$  cells were allocated to each well, and 6 duplicate (2x) wells were set up per group and placed in a 37 °C incubator for different durations of time. The Cell Counting Kit-8 (CCK-8) mixture was poured into each vial, which was then normally incubated at 37 °C for 4 hours in order to carry out the detection at 72 hours. The optical density (OD) value of each vial was then determined at a wavelength ( $\lambda=450$  nm) using a microplate reader.

### *Clonogenic assay*

After transfection, the CRC cells were digested by trypsin and then centrifuged for 3 minutes. After that, the supernatant was discarded, and the pellets were washed thoroughly using 3 mL of sterile phosphate-buffered saline (PBS) solution. Next, centrifugation was conducted again, and repeated 3 times. The culture medium was then added and a single cell suspension was made to adjust cell concentration to 600 cells/mL, and the prepared single-cell suspension was evenly planted in a 6-well plate for colony formation experiments. After every 3rd day, the culture media was monitored and changed, colonies were fixed with 4% paraformaldehyde and stained by crystal violet for 15 min at room temperature and photographed by a camera.

### *Transwell migration assay*

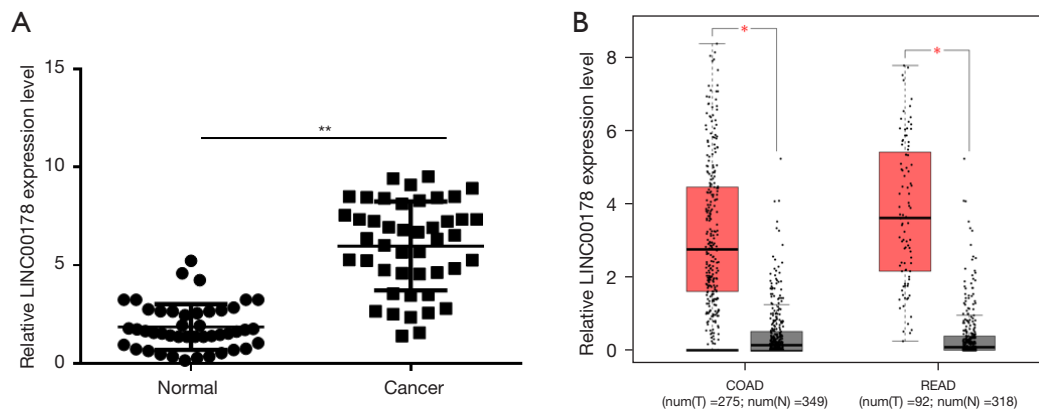
After adjusting to  $1 \times 10^5$  cells/mL in serum-free DMEM, 600  $\mu$ L of DMEM with 10% FBS was introduced to the transwell's bottom chamber. The upper chamber contained 200  $\mu$ L of test cell suspension. The whole chamber was cultured overnight in a conventional incubator, a cotton swab was used to clean the cells in the upper chamber, and cells adhering to the lower membrane surface of the transwell's lower chamber were fixed with 4% paraformaldehyde solution for 15 minutes before being stained in 0.05% crystal violet dye for 40 minutes. The cells in transit were tracked and counted under a microscope.

### *Western blot detection*

We digested, centrifuged, and collected the cells 48 hours after transfection, added cell lysate to resuspend the cells, centrifuged and collected the supernatant containing total protein, and used a bicinchoninic acid (BCA) kit to quantify the protein. A solution of 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared, 50  $\mu$ g of protein was loaded, and then transferred to a polyvinylidene fluoride (PVDF) membrane after electrophoresis separation. The solution was blocked in 5% non-fat dry milk for 120 minutes at 25 °C, and diluted with E-cadherin, N-cadherin, Vimentin, and GAPDH primary antibodies at 1:600 according to the antibody instruction manual. The corresponding primary antibodies were added and incubated for 24 hours at 4 °C on a shaker. The next day, after being rinsed 3 times in tris-buffered saline with Tween 20 (TBST) solution, the membrane had secondary antibodies diluted 1:3,000 added to it and incubated at 25 °C for 1 hour. Images were captured by exposing the membrane to a chromogenic solution, washing it again, and then exposing it to darkness in the dark room. Using GAPDH as the internal reference, Quantity One software (Bio Rad Laboratories, Hercules, CA, USA) was used to analyze the gray value of protein bands.

### *Tumorigenic experiment*

Male BALB/c nu/nu mice (weight: 16–18 g, 4–5 weeks old) purchased from the Laboratory Animal Center of Shanghai, Academy of Science Chinese (Shanghai, China). The transfected CRC cells were re-suspended in serum-free media, the cellular density of suspension was adjusted to  $1 \times 10^7$ /mL, and 0.1 mL of the suspended tumor cell fluid



**Figure 1** Figure shows the tissues from colorectal tumors at which LINC00178 is expressed. (A) QF-PCR identified the expression of LINC00178 in 45 early-stage colorectal tumor samples and associated paracancerous tissues. (B) Colon cancer in TCGA database. Transcriptome data analysis related to rectal adenocarcinoma LINC00178, \* $P < 0.05$ , \*\* $P < 0.01$ . QF-PCR, quantitative fluorescence-polymerase chain reaction; TCGA, The Cancer Genome Atlas. COAD, colon adenocarcinoma; READ, Rectum adenocarcinoma.

was injected hypodermically in the right lower limb of the nude mice. After 10 days, a vernier calliper (0.01 mm) was used to measure the length (L) and breadth (W) of tumor-bearing tissues at the site of inoculation, and the tumor's volume (V) was computed using the formula  $V = (L \times W^2) / 2$ . A tumor's volume was assessed once every 5 days. After 30 days, the naked mice were euthanized, their tumor-bearing tissues were harvested and photographed, and then the total messenger RNA (mRNA) in the tissues was extracted for subsequent detection and analysis.

The animal experiment was conducted in the Laboratory Animal Center of Nantong University. Animal experiments were approved by the Laboratory Animal Ethics Committee of Nantong University (No. S20210317-501), in compliance with national guidelines for the care and use of animals. A protocol was prepared before the study without registration.

### Statistical analysis

All measurement data obtained in this experiment are expressed as  $\bar{x} \pm s$ . The difference between the 2 samples was analyzed by the analysis of variance (ANOVA) variance SNK-q method, and  $P < 0.05$  was considered statistically significant.

## Results

### CRC tissues are characterized by high levels of LINC00178 expression

The expression of LINC00178 was first discovered by

quantitative fluorescence (QF)-PCR in 45 early-stage colon cancer tissues and matched para neoplastic tissues to study its presence and relative abundance in tumors of the digestive tract. Thus, the results linked normal para cancerous tissues with LINC00178 and found that it was over expressed in cancer tissues (Figure 1A). Through the analysis of the transcriptome data related to colon cancer and rectal adenocarcinoma in The Cancer Genome Atlas (TCGA) database, in which, colon adenocarcinoma (COAD): 275 cases of cancer tissue and 349 cases of adjacent cancer; rectal adenocarcinoma (READ): 92 cases of cancer tissue and carcinoma 318 cases. It was shown that LINC00178 was differentially expressed in both COAD and READ (Figure 1B). The above results indicate that LINC00178 is over expressed in tissues.

### Relationship between LINC00178 level and clinical case characteristics of colorectal cancer patients

To analyze the relationship between the expression level of LINC00178 and clinical characteristics of colorectal cancer patients. The results showed that LINC00178 expression was correlated with TNM stage and differentiation degree (all  $P < 0.05$ ) (Table 1).

### LINC00178 knockdown or overexpression influences CRC cells

Additionally, to explore the role of LINC00178 in colorectal tumors, commonly used CRC cell-lines (LoVo,

**Table 1** Relationship between LINC00178 level and clinical case characteristics of colorectal cancer patients (mean  $\pm$  SD)

Clinicopathological features	n	LINC00178 level	t	P
Gender				
Male	25	5.67 $\pm$ 1.73	0.7984	0.4290
Female	20	5.28 $\pm$ 1.49		
Age (years)				
<65	11	5.64 $\pm$ 1.64	0.6047	0.5486
$\geq$ 65	34	6.01 $\pm$ 1.80		
Tumor diameter				
<3 cm	19	6.15 $\pm$ 1.92	1.893	0.0651
$\geq$ 3 cm	26	5.21 $\pm$ 1.45		
TNM stage				
I–II	16	5.16 $\pm$ 1.17	2.120	0.0398
III–IV	29	6.29 $\pm$ 1.94		
Differentiation degree				
Poorly	24	6.19 $\pm$ 1.55	2.362	0.0228
High	21	5.18 $\pm$ 1.28		

Data are expressed as number and Mean  $\pm$  SD. SD, standard deviation. TNM, tumor/lymph nodes/metastasis.

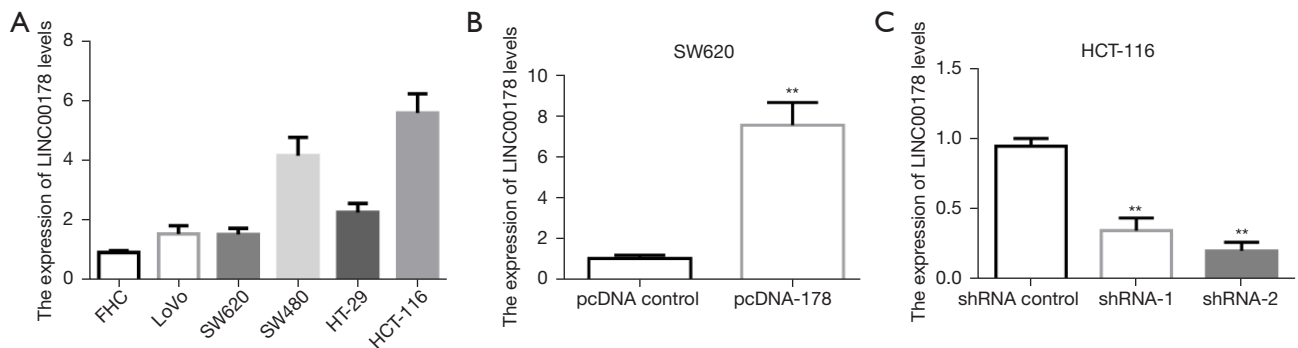
SW620, SW480, HT-29, and HCT116) and normal or control human colorectal mucosal cells (FHC) were first selected. The results of q-PCR compared human normal colorectal mucosal cells (FHC) with LINC00178 which was over expressed in CRC cell lines, with over expression in SW480 and HCT116, and relatively low expression in LoVo, SW620, and HT-29 (*Figure 2A*). According to the above results, the over expression vector (pcDNA-178) and blank control vector (pcDNA-control) of LINC00178 were synthesized and transfected into SW620 (with low expression of LINC00178). Compared with other groups, transfection of LINC00178 over expression vector (pcDNA-178) could significantly enhance the level of the expression level of LINC00178 in cells (*Figure 2B*). In addition, HCT-116 cells were transfected with 2 blank/control vector (shRNA-control) and LINC00178 knockdown vectors (shRNA-1 and shRNA-2). Transfection of LINC00178 knock down vectors (shRNA-1 and -2) dramatically decreased LINC00178 expression in cells than

in the control group (*Figure 2C*).

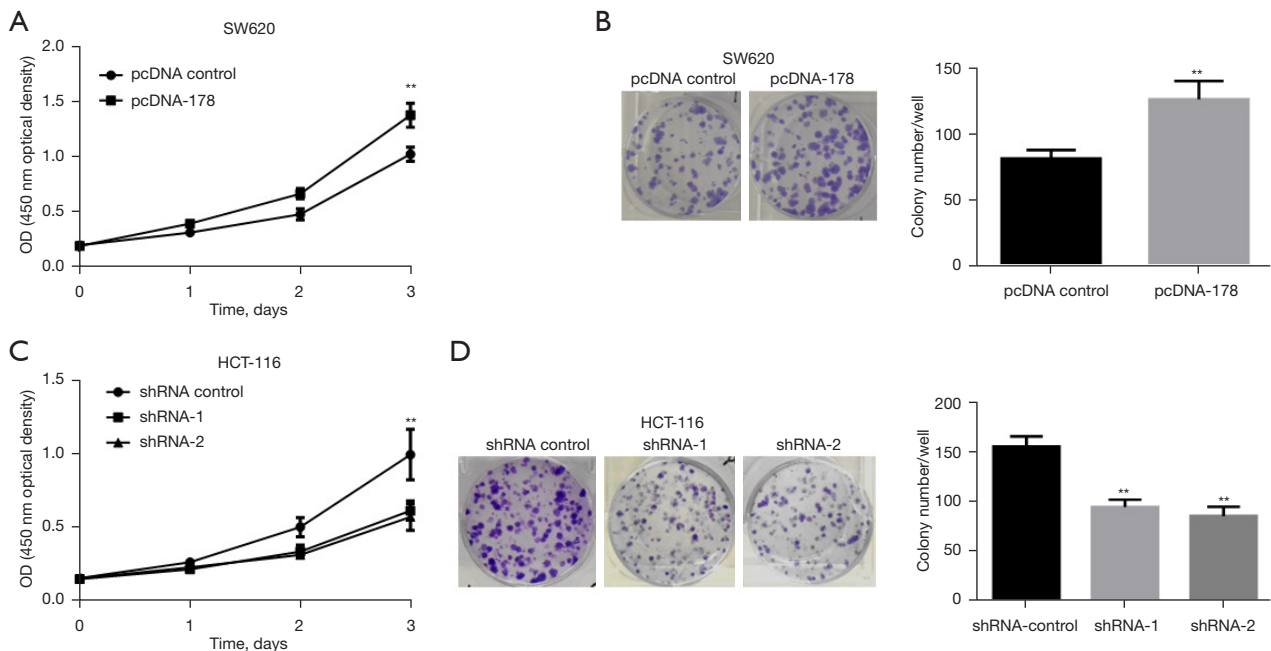
#### ***CCK-8 measured the impact of LINC00178 overexpression and knockdown on the growth of CRC cells***

The results compared the blank control vector (pcDNA-control) group with transfection of the LINC00178 overexpression vector (pcDNA-178), which significantly promoted cellular proliferation (*Figure 3A*). In comparison with the control group, transfection of the LINC00178 overexpression vector (pcDNA-178) could significantly enhance the clonogenic ability of the cells (*Figure 3B*). Comparing the transfection of LINC00178 blank/control vector group (shRNA-control) and knockdown vectors (shRNA-1 and -2) showed that the latter could more effectively suppress cell growth (*Figure 3C*). Furthermore, the clonogenic experiment demonstrated a substantial reduction in clone formation capacity between the shRNA-control group and the transfection of LINC00178 knock

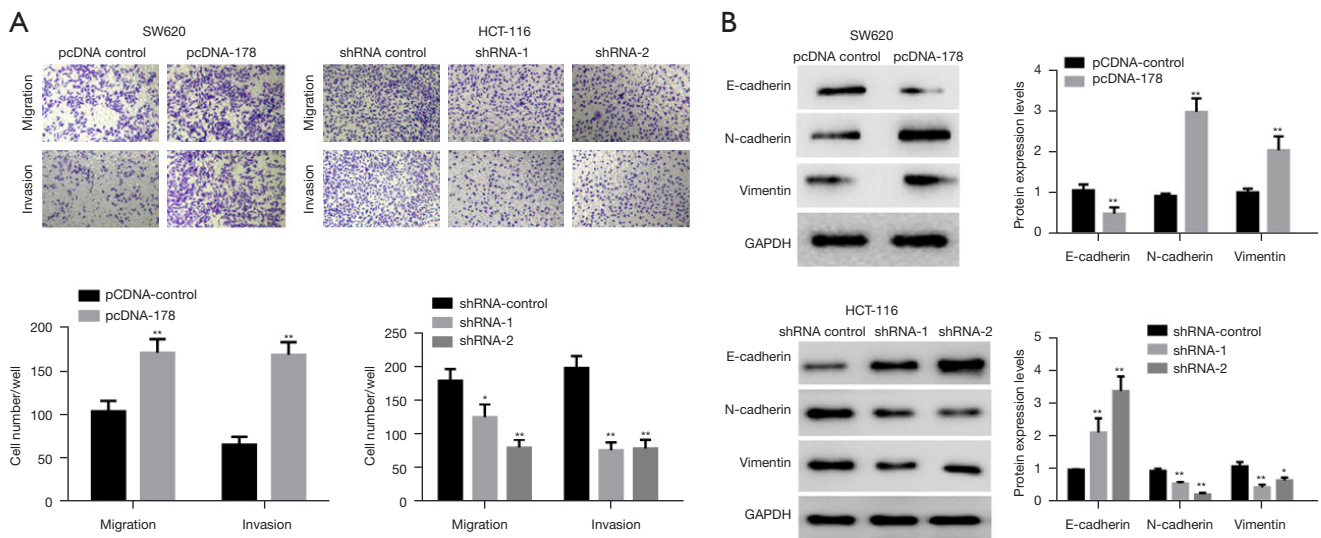




**Figure 2** Expression, overexpression level and knockdown vector construction of LINC00178 in colorectal tumor cell-lines. (A) qPCR to detect the LINC00178 expression in colorectal cancer cell-lines, (B) RT-qPCR to detect the overexpressed LINC00178 expression in SW620 cells, (C) qPCR detection of the expression of knockdown of LINC00178 in HCT-116 cells, \*\* $P < 0.01$ . qPCR, quantitative polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction.



**Figure 3** Knockdown or overexpression of LINC00178 affect the proliferation of CRC cells *in vitro*. (A) CCK-8 detected the effects of LINC00178 overexpression on the proliferation of CRC cells (SW620). (B) Effects on the clonogenic capacity of colorectal malignant cells (SW620) of LINC00178 overexpression level (Crystal violet staining). (C) CCK-8 detected the effects of LINC00178 knockdown on the proliferation of CRC cells (HCT-116). (D) Effects on the clonogenic capacity of colorectal malignant cells (HCT-116) of LINC00178 overexpression level (Crystal violet staining). \*\* $P < 0.01$ . CCK-8, Cell Counting Kit-8; CRC, colorectal cancer.



**Figure 4** The cumulative effects of LINC00178 overexpression or knockdown on the ability of CRC cells to invade and migrate. (A) The impact of LINC00178 overexpression or knockdown on the ability of CRC cells to invade and migrate (Crystal violet staining, 100 $\times$ ). (B) The impact of LINC00178 overexpression or knockdown on the expression of molecular markers of EMT in CRC cells, \* $P < 0.05$ , \*\* $P < 0.01$ . CRC, colorectal cancer; EMT, epithelial-mesenchymal transition.

down vectors (shRNA-1 and -2) (Figure 3D).

#### **Knockdown or overexpression of LINC00178 can significantly affect CRC cell migration**

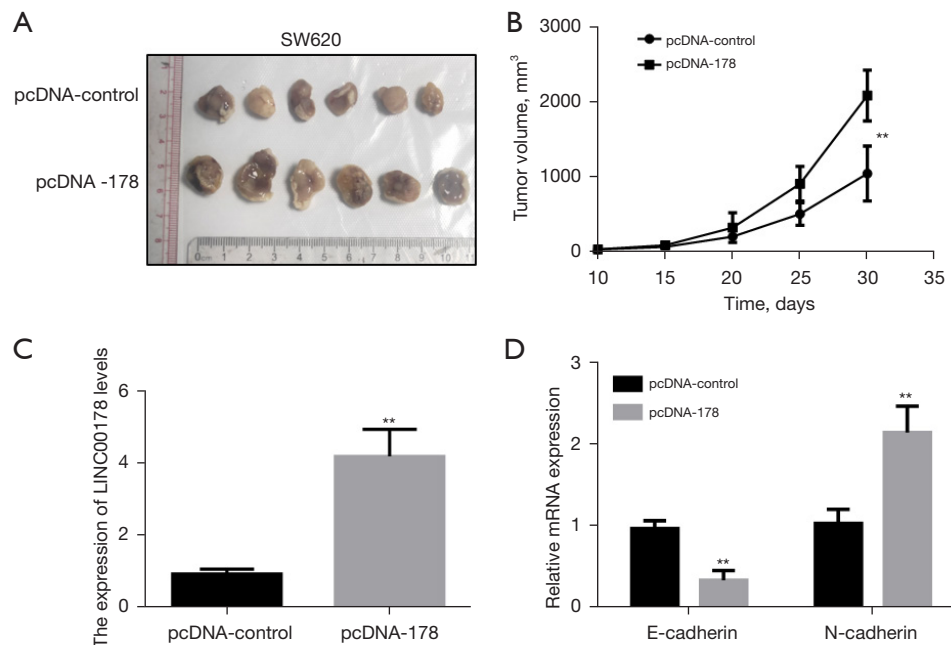
Transwell assay was used to assess the effects of LINC00178 knockdown or overexpression level on the ability migration in colorectal malignant cells. This study compared SW680 cells transfected with the LINC00178 overexpression vector (pcDNA-178) to a batch of control cells transfected with a non-targeting vector (pcDNA-control). Which significantly promotes the ability of cells to migrate from the upper chamber of the transwell to the lower chamber. As a means of simulating the matrix environment in vivo, Matrigel was streaked over the top chamber of a Transwell, and the cells' capacity to invade was then measured. Invasion of SW680 cells from Matrigel through the top chamber of transwell and into the bottom chamber was much improved after transfection with LINC00178 overexpression vector (pcDNA-178) compared to the (pcDNA-control) group (Figure 4A). After LINC00178 was knocked down, the same approach was used to detect HCT-116 cell movement and invasion. The HCT-116 cells were transfected with a knockdown vector for LINC00178 and compared to a group transfected with a blank/control vector (shRNA-1 and shRNA-control). Cell migration and invasion from

the upper transwell chamber to the lower chamber can be significantly inhibited (Figure 4A).

Western blot analysis revealed that the blank control vector (pcDNA-control) group surpassed the LINC00178 overexpression vector-transfected SW680 cells (pcDNA-178). E-cadherin expression was downregulated, but N-cadherin and vimentin levels were dramatically upregulated (Figure 4), through which cells underwent epithelial-mesenchymal transition (EMT) with favorable results. Transfection of LINC00178 knockdown vectors (shRNA-1 and -2) into HCT-116 cells led to a considerable up-regulation of E-cadherin expression compared to the control vector (shRNA-control) group, whereas it led to a significant down-regulation of vimentin expression. Vimentin and N-cadherin were significantly down-regulated (Figure 4B), inhibiting cells from undergoing EMT.

#### **LINC00178 overexpression affects CRC cell growth in vivo**

We investigated the impact of LINC00178 overexpression on CRC cell proliferation in vivo. First, nude mice were subcutaneously implanted with SW680 cells that had been transfected with an overexpression vector for LINC00178 (pcDNA-178). Mice were kept under specific pathogen-free conditions for 30 days before being slaughtered, at which point the tumors were removed and documented. The



**Figure 5** The effects of over expression of LINC00178 on the progression of CRC cells *in vivo*. (A) The morphology of tumor-bearing tissue after inoculation for 30 days. (B) The growth curve of tumor-bearing tissue after sub-cutaneous inoculation of CRC cells in nude mice for 10 days. (C) q-PCR detected expression levels of LINC00178 in tumor-bearing tissue. (D) q-PCR to detect the expression levels of E-cadherin and N-cadherin in tumor-bearing tissues. \*\* $P < 0.01$ . CRC, colorectal cancer; q-PCR, quantitative polymerase chain reaction.

results compared the blank control vector (pcDNA-control) group with the tumor-bearing tissue of the LINC00178 overexpression vector (pcDNA-178) group was significantly increased (Figure 5A). Tumor-bearing tissues grew faster in the vector (pcDNA-178) group than in the blank/control vector (pcDNA-control) group (Figure 5B). The QF-PCR detected the expression levels of LINC00178 in tumor-bearing tissues and the results showed that those of the blank control vector (pcDNA-control) group compared with the in the tumor-bearing tissues of the LINC00178 overexpression vector (pcDNA-178) group was significantly increased (Figure 5C). In addition, QF-PCR detected the levels of expression in E-cadherin and N-cadherin inside tumor-bearing tissues. The expression levels of E-cadherin were significantly lowered, while the variable level of N-cadherin was significantly enhanced (Figure 5D).

## Discussion

Accumulating evidence suggests that dysregulated lncRNA expression is linked with tumor initiation, proliferation, and poor prognosis, and can be used as novel biomarkers for early diagnosis of tumors (11,13,20). LncRNA has potential

application value in the diagnosis, prognosis assessment and targeted therapy of CRC. LncRNA plays an important role in the occurrence and development of CRC by affecting key cancer signaling pathways, such as Wnt/ $\beta$ -catenin, PI3K/AKT, EGFR, NOTCH, m TOR and TP53 (21). Therefore, elucidating the function and regulatory mechanism of lncRNA in CRC has important potential to provide potential molecular targets for early correct diagnosis and targeted treatment of CRC. The results of this experiment revealed that LINC00178 is overexpressed in colorectal tumor tissues. LINC00178 expression is correlated with TNM stage and differentiation degree of colorectal cancer patients. The ability of CRC cells to proliferate and form clones is greatly aided by high levels of LINC00178 expression. Concurrently, the expression level of E-cadherin is dramatically lowered, and the number of cells that migrate and invade is significantly increased. Overexpression of N-cadherin and Vimentin regulates EMT pathways, which in turn affects the ability of CRC to invade and metastasize. Further, blocking LINC00178 expression reduced CRC cell growth *in vivo*.

Previous researches have shown that multiple genomic changes contribute to the development of CRC (22,23).



One of these changes is the presence of lncRNA, which may regulate gene expression at the transcriptional, post-transcriptional, and epigenetic levels, thereby playing a role in tumor proliferation, invasion, metastasis, and related pathological processes. The CRC cells, for instance, may be pushed forward by lncRNA-cCSC1, which does so by silencing miR-124-3p and, in turn, increasing CD44 expression (24). In addition, lncRNA and CCAT2 are overexpressed in colorectal tumors, and promote the development and induce proliferation of tumor cells in order to inhibit apoptosis (25). The results highlight the possibility that lncRNAs play crucial regulatory roles in the development and metastasis of CRC cells. Results from this research demonstrated that LINC00178 was substantially expressed in CRC tissues, as shown by both clinical samples and TCGA data. Further, cellular functional experiments showed that high expression of LINC00178 induced cell proliferation while knockdown of LINC00178 thus inhibited cellular proliferation.

Tumor metastasis is the propagation of tumor cells from their primary site and the development of secondary tumors at distant sites. In most cases, metastatic CRC remains incurable and frequently leads to death in most patients (26,27). It has been demonstrated, for instance (28) that the lncRNA FAS1 regulates the miR-34b/SOX4 signaling axis, hence promoting CRC metastasis and invasion. The lymphatic spread of CRCs is aided by the lncRNA GAS5's high expression (29). Therefore, early identification and site-directed therapy of CRC depend on pinpointing the essential regulatory molecules that control invasion and metastasis.

In this work, we found that LINC00178 induces EMT process in CRC cells. Overexpressing LINC00178 increased the expression of N-cadherin and vimentin and decreased expression of E-cadherin, all of which facilitated cell invasion and migration. These results indicated that LINC00178 could promote metastasis of CRC cells through EMT pathway. Overexpression of LINC00178 greatly increased the growth of CRC cells *in vivo* tumorigenic experiments. However, it is still unclear how LINC00178 functions. Therefore, further experiments will continue to explore the localization of LINC00178 in the cytoplasm and nucleus, which is due to the different localization of lncRNA in cells and different mechanisms of its effect (30).

In conclusion, overexpression of LINC00178 is seen in CRC cells and tissues. Knockdown of LINC00178 has a variety of effects, whereas overexpression of LINC00178

has been shown to promote CRC cell growth, proliferation, clonal expansion, and invasion. The protein expression levels of E-cadherin, N-cadherin, and vimentin can all be regulated by the overexpression of LINC00178. Experiments using nude mice demonstrated that LINC00178 overexpression dramatically increased tumor cell proliferation in colorectal malignancies, indicating that this gene may represent a promising target for the treatment of this disease.

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## Footnote

*Reporting Checklist:* The authors have completed the ARRIVE reporting checklist. Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-940/rc>

*Data Sharing Statement:* Available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-940/dss>

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <https://jgo.amegroups.com/article/view/10.21037/jgo-22-940/coif>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the ethics committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (No. 2021NL-034-01). Each participant and each examining physician completed an informed consent form. Animal experiments were approved by the Laboratory Animal Ethics Committee of Nantong University (No. S20210317-501), in compliance with national guidelines for the care and use of animals.

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