

RETRACTED ARTICLE: Long Noncoding RNA MALAT1 Promotes the Development of Colon Cancer by Regulating *miR-101-3p*/STC1 Axis

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Purpose: Colon cancer (CC) is a leading cause of cancer-related death worldwide. This study aimed to clarify the effect of long noncoding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) on CC progression and the potential mechanism.

Methods: CC cell lines HCT116 and HT29 were selected for functional analysis. The expression of MALAT1, *microRNA* (*miR*)-101-3p, and stathocalcin 1 (STC1) in CC tissues and cells were measured by quantitative reverse transcription PCR (qRT-PCR). Cell proliferation, apoptosis, migration and invasion were measured by Cell Counting Kit-8 (CCK-8), flow cytometry, wound scratch and transwell assay, respectively. The target relationships (MALAT1 and *miR*-101-3p, *miR*-101-3p and STC1) were validated by dual-luciferase reporter and RNA pull-down assay.

Results: The expression of MALAT1 was elevated in CC tissues compared with adjacent normal tissues and was associated with lymph node metastasis, depth of invasion and tumor-node-metastasis (TNM) stage. Up-regulation of MALAT1 promoted the proliferation, migration, and invasion and inhibited the apoptosis of CC cells; while MALAT1 knockdown exhibited opposite results. *MiR*-101-3p was a target of MALAT1, which was negatively regulated by MALAT1. Silencing of *miR*-101-3p reverses the anti-tumor effect of MALAT1 knockdown on CC cells. STC1 was a target of *miR*-101-3p, which was negatively regulated by *miR*-101-3p. Silencing of STC1 reverses the tumor promoting effects of MALAT1 up-regulation and *miR*-101-3p down-regulation on CC cells.

Conclusion: MALAT1 may function as an oncogene in CC progression by affecting the *miR*-101-3p/STC1 axis, providing a hopeful therapeutic option for CC.

Keywords: colon cancer, MALAT1, *miR*-101-3p, proliferation, apoptosis, migration, invasion

Introduction

Colon cancer (CC) and rectal cancer (RC) are collectively called colorectal cancer (CRC).¹ CC is the fourth frequent cancer and the fifth cause of cancer-related deaths all over the world, leading to approximately 1,096,601 new cases and 551,269 deaths in 2018.² During the past two decades, the incidence and mortality of CRC in Chinese are increased.³ The common risk factors of CRC are family history of CRC,⁴ inflammatory bowel disease (IBD),⁵ consumption of red and processed meat,⁶ sedentary lifestyles,⁷ smoking⁸ and bibulosity.⁹ The therapeutic strategies for CC mainly include surgical resection, radiotherapy, chemotherapy, targeted therapy, and immunotherapy.¹⁰ It is very important to study the underlying mechanisms involving the occurrence and development of CC.

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Long noncoding RNAs (lncRNAs) are non-coding RNA molecules of more than 200 nucleotides in length without protein-coding capacity.¹¹ lncRNAs function as tumor suppressors or oncogenic genes by modulating multiple cellular and biological processes in human cancers.¹² For examples, the up-regulation of lncRNA TUG1 promotes the proliferation of CC cells.¹³ lncRNA ATB is up-regulated in CC tissues and serves as a predictor for the poor prognosis of CC patients.¹⁴ lncRNA-ZEB2-AS1 is up-regulated in CC and exerts an oncogenic role in CC via regulating the *miR-143/bcl-2* axis.¹⁵ On the contrary, Xiong et al have validated that LINC01082 suppresses the development of CC.¹⁶ lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) plays an oncogenic role in CRC through regulating tumor growth and metastasis.¹⁷ Previous studies have proved that MALAT1 exerts important regulatory role on CRC progression through targeting certain microRNAs (miRNAs), such as *miR-194-5p*,¹⁸ *miR-145*,¹⁷ *miR-129-5p*¹⁹ and *miR-106b-5p*.²⁰ The potential regulatory mechanisms of MALAT1 in CC still need to be elucidated.

MiRNAs are a category of noncoding RNAs with about 22 nucleotides long, which exert key regulatory functions in animals and plants by modulating message RNAs (mRNAs).²¹ Abnormal expression of miRNAs has been shown to be involved in the occurrence of CC.^{22,23} For examples, *miR-378* inhibits the proliferation, migration and invasion of CC cells by inhibiting SDAF.²⁴ *MiR-195* is a potential diagnostic marker of CC, which can inhibit proliferation and metastasis of CC cells.²⁵ In contrast, *miR-27a* promotes the proliferation and invasiveness of CC cells by targeting SFRP1.²⁶ *MiR-101-3p* promotes the development of CRC by targeting FNHG6.²⁷ Because the regulatory mechanisms of miRNAs in CC are complex, we intended to explore more about the regulatory effects and underlying mechanisms of *miR-101-3p* on CC progression.

In this study, the clinical significance of MALAT1 in CC was analyzed. The regulatory effects of MALAT1 on the proliferation, apoptosis, migration, and invasion of CC cells were explored. Additionally, the potential regulatory mechanism of MALAT1/*miR-101-3p*/STC1 in CC was determined. Our findings may provide a potential therapeutic target for CC.

Materials and Methods

Clinical Samples

CC tissues and adjacent normal tissues were collected from 62 CC patients (33 males and 29 females, 38~72 years old,

median 52.6 years old) who had undergone surgery in our hospital from Jan 2017 to Oct 2018. All the samples were histologically confirmed and preserved in liquid nitrogen for further analysis. All the patients involved in this study did not receive any preoperative chemotherapy or radiotherapy. The current research obtained approval from the Ethics Committee of our hospital. Written informed consent was obtained from each patient.

Cell Culture and Transfection

CC cell lines HCT116 (ATCC® CCL-247) and HT29 (ATCC® HTB-38) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), and human normal colon epithelial cell line NCM460 (CM-H203) was purchased from CHINING BIOLOGICAL (Shanghai, China). All cells were cultured in Roswell Park Memorial Institute 1640 Medium (RPMI1640; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37°C with 5% CO₂.

The overexpression plasmid of MALAT1 (LV-MALAT1), the small interfering RNA (si-RNA) against MALAT1 (si-MALAT1-1 and si-MALAT1-2), si-RNA against STC1 (si-STC1), and the negative controls (LV-NC and si-NC) were purchased from GenePharma (Shanghai, China). The targeting sequences for si-MALAT1-1, si-MALAT1-2, si-STC1 and si-NC were 5'-GGCAAUGUUUACACUAUUTT-3', 5'-GACACAGGGAAAGCGAGTGGTTGGTAA-3', 5'-CTGCTTAAACAAAGCAGTATA-3' and 5'-UUCUCCGAACGUGUCACGUTT-3', respectively. In addition, *miR-101-3p* mimics, *miR-101-3p* inhibitor and the negative controls (mimics NC and inhibitor NC) were purchased from Ribobio (Guangzhou, China). When reaching 80% confluence, cells were transfected with the above agents using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) for 48 h.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from CC tissues and cells using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) based on the instructions. Subsequently, RNA was used for the synthesis of complementary DNA (cDNA). The qRT-PCR assay was conducted using SYBR Green Master Mix (Roche Diagnostics, Basel, Switzerland) on a 7500 PCR System (Thermo Fisher Scientific). The relative expression of MALAT1, STC1 or *miR-101-3p* was normalized to Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6, respectively. The expression level was

calculated using $2^{-\Delta\Delta Ct}$ method. Primers used in this research were shown as follows:

MALAT1, forward primer: 5'-AAAGCAAGGTCTC CCCACAAG-3', reverse primer: 5'-GGTCTGTGCTAGA TCAAAAGGCA-3';

STC1, forward primer: 5'- GCAGGAAGAGTGCTA CAGCAAG-3', reverse primer: 5'- CATTCCAGCAGGC TTCGGACAA-3';

miR-101-3p, forward primer: 5'-TCCGAAAGTCAA TAGTGTC-3', reverse primer: 5'-GTGCAGGGTCCGAG GT-3';

GAPDH, forward primer: 5'-AGAAGGCTGGGGCTC ATTTG-3', reverse primer: 5'-AGGGGCCATCCACAGT CTTC-3';

U6, forward primer: 5'-CTCGCTTCGGCAGCACA-3', reverse primer: 5'-AACGCTTCACGAATTTGCGT-3'.

Cell Counting Kit-8 (CCK-8) Assay

Cell viability was measured using CCK-8 assay kit (Beyotime, Shanghai, China) according to the instructions. HCT116 and HT29 cells (5×10^3) were seeded into 96-well plate, and cultured for 24, 48, 72, and 96 h, respectively. 10 μ L CCK-8 solution was then added into each well. After the reaction, the optical density at 450 nm (OD450) was measured using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to assess the cell viability.

Cell Apoptosis Assay

Apoptotic cells were analyzed using Annexin V-propidium iodide (PI) kit (Keygen, Jiangsu, China). In brief, the transfected HCT116 and HT29 cells were double-stained with Annexin V-EGFP and PI for 10 min in the dark. The apoptosis rate (percentage of cells in the right quadrants) was examined by flow cytometry (BD Biosciences, San Jose, CA, USA).

Migration Assay

Wound scratch assay was applied to assess the migration of HCT116 and HT29 cells in vitro. The transfected CC cells (2×10^5) were seeded into 6-well plates, and cultured overnight. A 10-microliter sterile pipette tip was used to generate a scratch across the diameter of each well. After three times of washing with PBS to remove the suspended cells, the plates were continually incubated for 48 h at 37°C. The wound scratches were photographed using a digital camera system at 0 h and 48 h. The wound width was measured using Image J software (NIH, Bethesda, MD, USA). Cell migration rate was calculated by the following formula:

Cell migration rate = (0 h scratch width - 48 h scratch width)/0 h scratch width \times 100%.

Transwell Invasion Assay

Transwell invasion assay was applied to assess the invasion of HCT116 and HT29 cells in vitro. The transfected CC cells in non-serum medium were placed into the upper chamber pre-coated with 100 μ L Matrigel (Corning, Corning, NY, USA). Complete medium was placed in the lower chamber. At 24 h post-incubation, cells in the lower chamber were fixed and stained with 0.2% crystal violet. Positive stained cells were counted in 4 randomly selected fields under a light microscope ($\times 200$; Nikon, Tokyo, Japan) using ImageJ (National Institutes of Health, Bethesda, MD, USA).

Dual-Luciferase Reporter Assay

The binding sites between MALAT1 and *miR-101-3p* and between STC1 and *miR-101-3p* were predicted by StarBase (<http://starbase.sysu.edu.cn/>). To further confirm the target relationship, the binding sequences of MALAT1 and STC1, and the mutant sequences were inserted into pGL3 (Promega, Madison, WI, USA) to generate MALAT1-WT (wild-type), MALAT1-MUT (mutant-type), STC1-WT, and STC1-MUT (Ribobio). HCT116 cells were co-transfected with MALAT1-WT or MALAT1-MUT and *miR-101-3p* mimics or mimics NC. HT29 cells were co-transfected with MALAT1-WT or MALAT1-MUT and *miR-101-3p* inhibitor or inhibitor NC. HCT116 and HT29 cells were further co-transmitted with STC1-WT or STC1-MUT and *miR-101-3p* mimics or *miR-NC*. Cell transfection was performed using Lipofectamine 3000 (Invitrogen). After 48 h of transfection, luciferase activity was detected by Dual-Luciferase Reporter Assay Kit (Promega).

RNA Pull-Down Assay

HCT116 cells were lysed using RIPA Lysis buffer (Invitrogen), and then incubated with Biotinylated (Bio)-NC, Bio-MALAT1-Wt or Bio-MALAT1-Mut (GenePharma Company) for 1 h. The cell lysate was then incubated with Dynabeads M-280 Streptavidin (Invitrogen) at 4°C for 3 h. The eluants were used for the detection of *miR-101-3p* expression via qRT-PCR assay.

Statistical Analysis

All data derived from 3 parallel repetition experiments were expressed as mean \pm standard deviation (SD). Statistical analysis was performed by SPSS 22.0 (SPSS Inc., Chicago, IL, USA). *T*-test (two groups) and One-way analysis of

variance (ANOVA) followed by Tukey's multiple comparisons test (multi-groups) were used for comparison. Pearson χ^2 test was used to evaluate the differences of clinicopathological variables between high and low groups (MALAT1 expression). Spearman correlation analysis was used to confirm the correlation between MALAT1 and *miR-101-3p* expression. $P < 0.05$ was considered statistically significant.

Results

The Expression of MALAT1 Is Up-Regulated in CC Tissues

The expression of MALAT1 in CC tissues and adjacent normal tissues was measured by qRT-PCR. The expression of MALAT1 was upregulated in CC tissues compared with adjacent normal tissues ($P < 0.001$, Figure 1A). As shown in Figure 1B, the expression of MALAT1 in CC tissues with lymph-node (LN) metastasis was significantly higher than that in tissues with non-metastasis ($P < 0.01$). The expression of MALAT1 expression was significantly higher in CC tissues with invasive depth (T3 + T4) compared with tissues

with invasive depth (T1 + T2) ($P < 0.05$, Figure 1C). In addition, the expression of MALAT1 was significantly higher in CC tissues with tumor-node-metastasis (TNM) III + IV than tissues with TNM I + II ($P < 0.01$, Figure 1D). The clinicopathological characteristics of 62 patients with CC were shown in Table 1. According to the median expression level of MALAT1 in CC tissues, CC patients were divided into high (MALAT1 expression $>$ median, $N = 31$) and low groups (MALAT1 expression \leq median, $N = 31$). The expression of MALAT1 was associated with the depth of invasion, lymph node metastasis and TNM stage, but not associated with gender, age, tumor size, differentiation and tumor site. Taken together, these results suggested that MALAT1 might be involved in CC progression.

LncRNA MALAT1 Promotes the Proliferation and Inhibits the Apoptosis of CC Cells

With the application of qRT-PCR assay, the up-regulated expression of MALAT1 was detected in HCT116 and

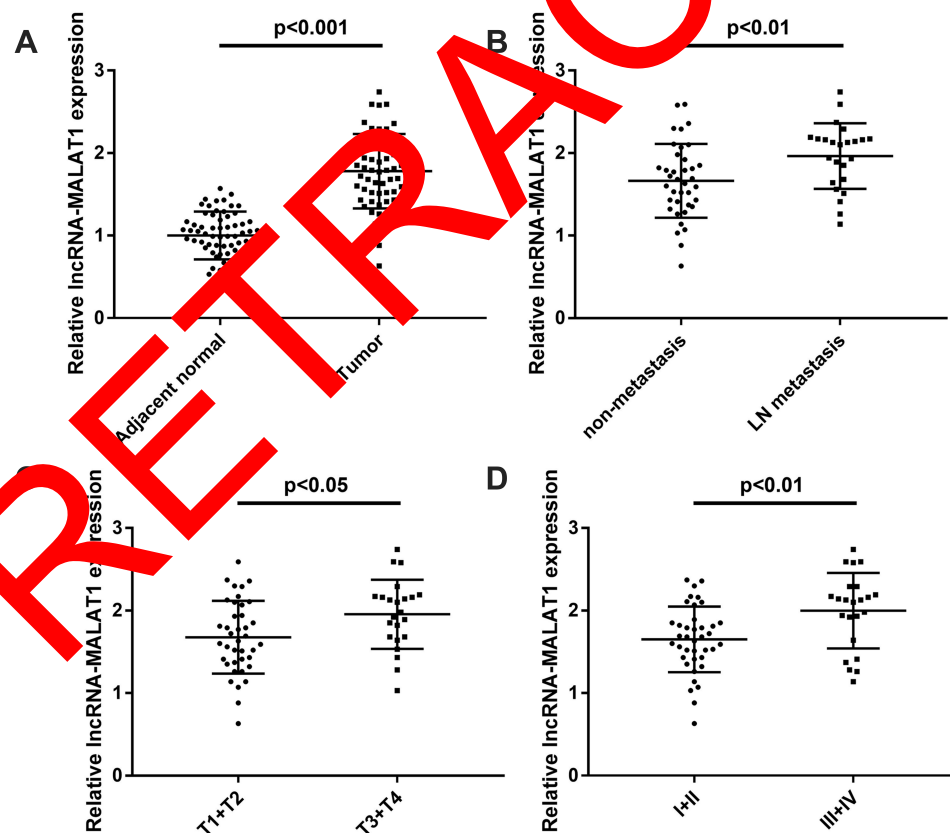


Figure 1 LncRNA MALAT1 is overexpressed in CC tissues. (A) Relative expression of MALAT1 in CC tissues and adjacent normal tissues ($N = 62$), $P < 0.001$. (B) Relative MALAT1 expression in CC tissues with and without lymph-node (LN) metastasis ($N = 62$), $P < 0.01$. (C) Relative expression of MALAT1 in CC tissues with depth of invasion T1 + T2 and T3 + T4 ($N = 62$), $P < 0.05$. (D) The relative expression of MALAT1 in CC tissues with TNM stage I + II and III + IV ($N = 62$), $P < 0.01$. Each experiment was performed in three replicates.

Table 1 Clinicopathologic Characteristics and MALAT1 Expression in 62 Patients with Colon Cancer

Characteristics	n = 62	MALAT1 Expression		p-value
		High (n=31)	Low (n=31)	
Gender				0.7991
Male	33	16	17	
Female	29	15	14	
Age, years				0.0754
≤60	31	12	19	
>60	31	19	12	
Tumor size, cm				0.2970
≤5	38	17	21	
>5	24	14	10	
Depth of invasion				0.0180*
T1+T2	39	15	24	
T3+T4	23	16	7	
Differentiation				0.3074
Well or moderate	34	15	19	
Poor	28	16	12	
Lymph node Metastasis				0.0091*
Negative	38	14	24	
Positive	24	17	7	
TNM stage				0.0038*
I-II	39	14	25	
III-IV	23	17	6	
Tumor site				0.6098
Colon	34	16	18	
Rectum	28	15	13	

Note: *Presented significantly different between high and low groups at $P < 0.05$.

HT29 cells compared with NCM-460 cells ($P < 0.01$, Figure 2A). In order to investigate the role of MALAT1 in the development of CC, MALAT1 was overexpressed in HCT116 cells and silenced in HT29 cells. HCT116 cells in the LV-MALAT1 group exhibited a higher MALAT1 expression than that in the LV-NC group ($P < 0.001$, Figure 2B); HT29 cells transfected with si-MALAT1-1 or si-MALAT1-2 exhibited a decreased MALAT1 expression compared with cells transfected with si-NC ($P < 0.01$, Figure 2B). Subsequently, cell proliferation and apoptosis were detected. Overexpression of MALAT1 promoted the proliferation ($P < 0.05$, Figure 2C) and repressed the apoptosis of HCT116 cells ($P < 0.01$, Figure 2D). Silencing of MALAT1 exhibited contrary results in HT29

cells. Altogether, our results indicated that MALAT1 could promote cell proliferation and inhibit cell apoptosis in CC.

MALAT1 Promotes the Migration and Invasion of CC Cells

The migration and invasion of transfected CC cells were measured by wound scratch and Transwell invasion assay, respectively. Overexpression of MALAT1 significantly enhanced the migration and invasion of HCT116 cells; meanwhile, knockdown of MALAT1 significantly inhibited the migration and invasion of HT29 cells ($P < 0.01$, Figure 3A and B). To sum up, MALAT1 promotes the migration and invasion of CC cells.

MALAT1 Targets *miR-101-3p*

To clarify the regulatory mechanism of MALAT1 in CC, the potential downstream targets of MALAT1 were predicted by StarBase (<http://starbase.sysu.edu.cn/>). A binding site between MALAT1 and *miR-101-3p* was predicted (Figure 4A). The target relationship between MALAT1 and *miR-101-3p* was further validated by a dual-luciferase reporter assay. Overexpression of *miR-101-3p* significantly reduced the luciferase activity of MALAT1-WT in HCT116 cells, whereas knockdown of *miR-101-3p* elevated the luciferase activity of MALAT1-WT in HT29 cells ($P < 0.05$, Figure 4B and C). RNA pull-down assay showed that *miR-101-3p* was pulled down by Bio-MALAT1-Wt, which further validated that MALAT1 could directly bind to *miR-101-3p* ($P < 0.05$, Figure 4D). Next, we explored the effect of MALAT1 on the expression of *miR-101-3p* in HCT116 and HT29 cells. As shown in Figure 4E, overexpression of MALAT1 significantly inhibited *miR-101-3p* expression in HCT116 cells, while silencing of MALAT1 promoted *miR-101-3p* expression in HT29 cells ($P < 0.05$). In addition, the expression of *miR-101-3p* was decreased in CC tissues compared with adjacent normal tissues ($P < 0.001$, Figure 4F). In CC tissues, there was a negative correlation between MALAT1 and *miR-101-3p* expression ($r = -0.3754$, $P = 0.0026$, Figure 4G). The above data manifested that MALAT1 may be a sponge of *miR-101-3p*, which could inversely modulate *miR-101-3p* expression.

Silencing of *miR-101-3p* Reverses the Effect of MALAT1 Knockdown on CC Progression

To investigate whether MALAT1 is involved in the regulation of *miR-101-3p* on CC progression, rescue experiments were implemented. The qRT-PCR assay manifested that

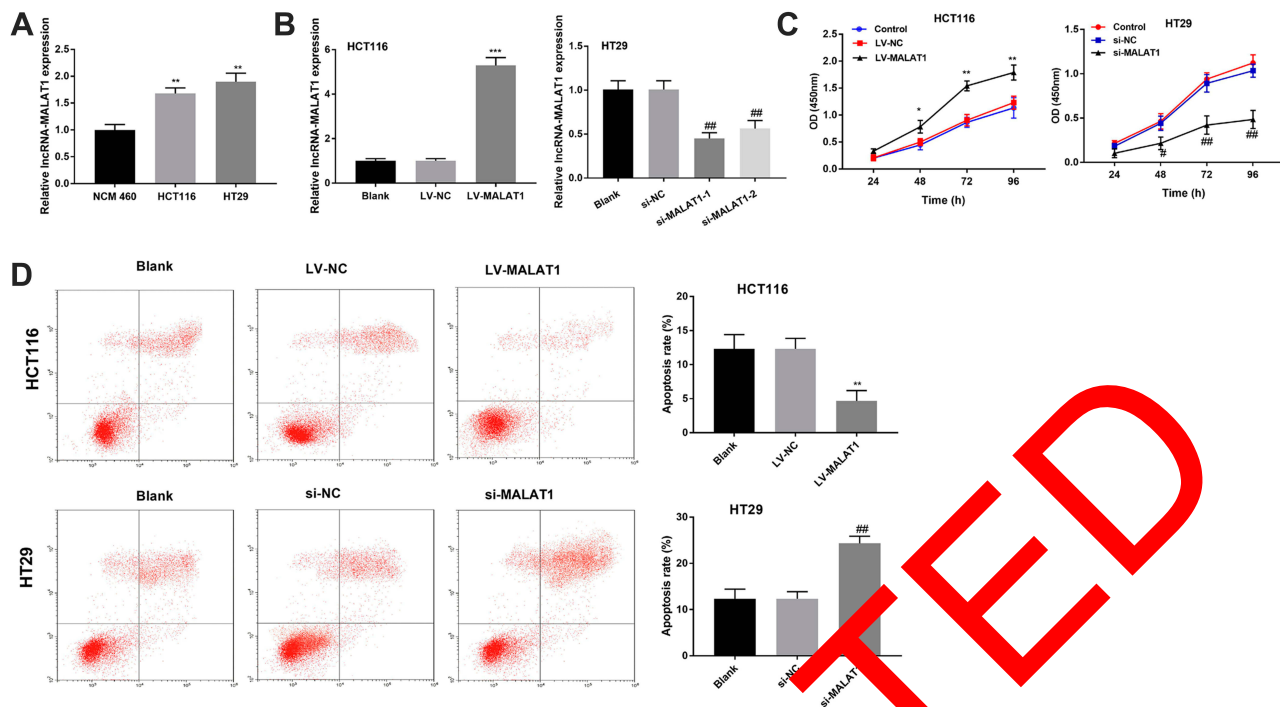


Figure 2 LncRNA MALAT1 promotes the proliferation and inhibits the apoptosis of CC cells. **(A)** Relative expression of MALAT1 in NCM 460 cells, HCT116 and HT29 cells. $^{**}P < 0.01$ vs NCM 460. **(B)** Relative expression of MALAT1 in transfected HCT116 and HT29 cells. **(C)** Cell proliferation of transfected HCT116 and HT29 cells. **(D)** The apoptosis of transfected HCT116 and HT29 cells. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs LV-NC; $^{\#}P < 0.05$, $^{###}P < 0.001$ vs si-NC. Each experiment was performed in three replicates.

miR-101-3p inhibitor effectively decreased the expression of STC1 and WT in HCT116 and HT29 cells ($P < 0.01$, Figure 6B). In addition, the mRNA expression of STC1 was significantly higher in CC tissues than that in adjacent normal tissues ($P < 0.001$, Figure 6C). The mRNA expression of STC1 was negatively correlated with the expression of *miR-101-3p* ($r = -0.361$, $P = 0.0039$, Figure 6D), and positively correlated with the expression of MALAT1 ($r = 0.3378$, $P = 0.0073$, Figure 6E). The mRNA expression of STC1 was also significantly higher in CC cell lines (HCT116 and HT29 cells) than that in NCM 460 cells ($P < 0.01$, Figure 6F). These results indicated that STC1 was a downstream target of MALAT1 and *miR-101-3p*.

MiR-101-3p Targets STC1

The potential downstream targets of *miR-101-3p* were further analyzed. A binding site between STC1 and *miR-101-3p* was predicted by StarBase (Figure 6A). The target relationship between STC1 and *miR-101-3p* was further confirmed by dual-luciferase reporter assay. Overexpression of *miR-101-3p* significantly decreased the luciferase activity of

STC1 and WT in HCT116 and HT29 cells ($P < 0.01$, Figure 6B). In addition, the mRNA expression of STC1 was significantly higher in CC tissues than that in adjacent normal tissues ($P < 0.001$, Figure 6C). The mRNA expression of STC1 was negatively correlated with the expression of *miR-101-3p* ($r = -0.361$, $P = 0.0039$, Figure 6D), and positively correlated with the expression of MALAT1 ($r = 0.3378$, $P = 0.0073$, Figure 6E). The mRNA expression of STC1 was also significantly higher in CC cell lines (HCT116 and HT29 cells) than that in NCM 460 cells ($P < 0.01$, Figure 6F). These results indicated that STC1 was a downstream target of MALAT1 and *miR-101-3p*.

Silencing of STC1 Reverses the Effects of MALAT1 Up-Regulation and miR-101-3p Down-Regulation on CC Progression

The regulatory relationship between STC1 and *miR-101-3p* was further identified by that overexpression of *miR-101-3p* significantly decreased the mRNA expression of STC1 in HCT116 and HT29 cells ($P < 0.01$, Figure 7A). Rescue experiments were then performed to investigate the regulatory mechanism of STC1 involving MALAT1 and *miR-101-3p* in CC cells. The viability, migration, and

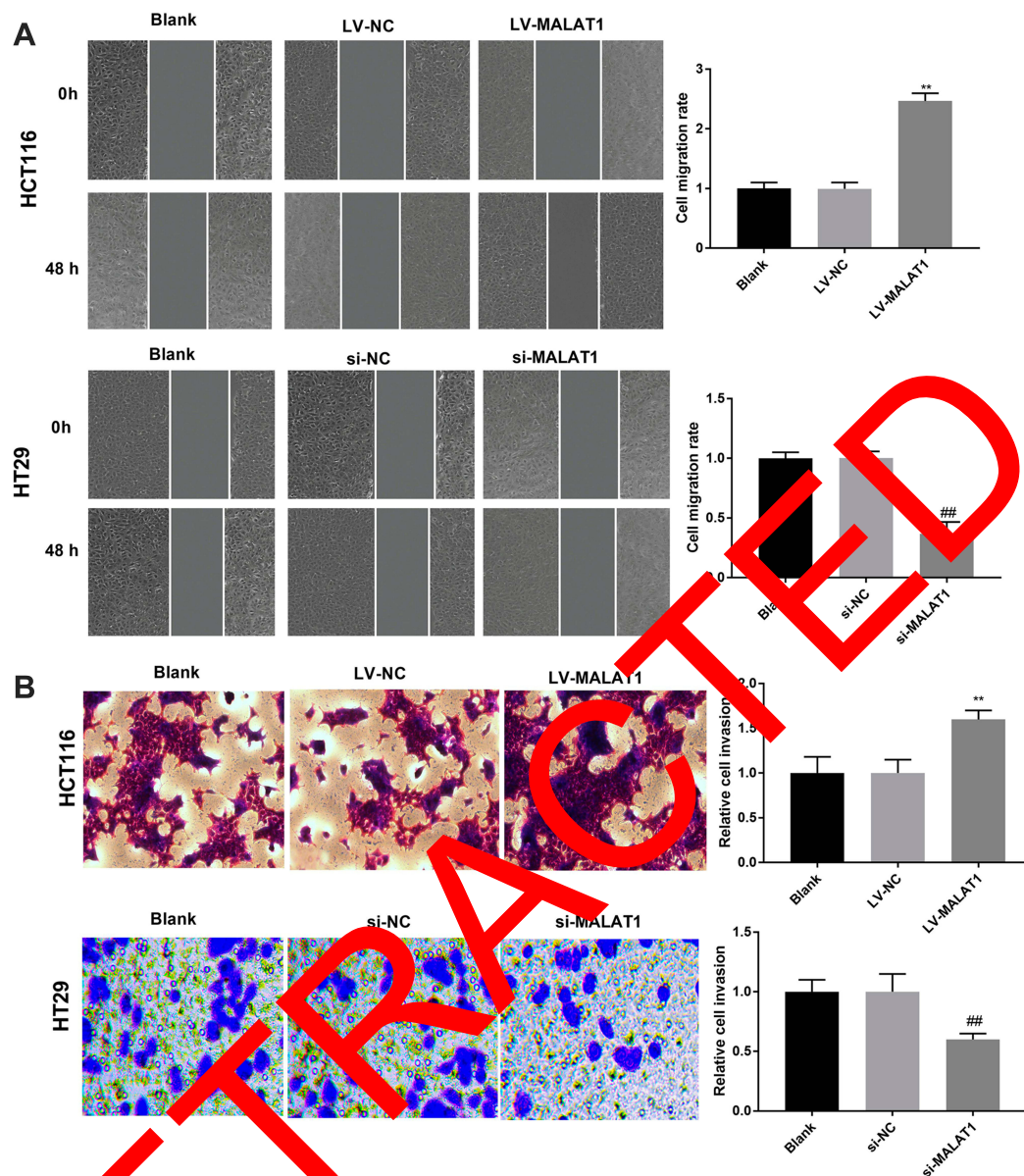


Figure 3 LncRNA MALAT1 promotes the migration and invasion of CC cells. **(A)** The migration of transfected HCT116 and HT29 cells. **(B)** The invasion of transfected HCT116 and HT29 cells. ** $P < 0.01$ vs LV-NC; ## $P < 0.01$ vs si-NC. Each experiment was performed in three replicates.

invasion of HT29 cells were significantly decreased in si-STC1 group compared with those in si-NC group ($P < 0.01$). In addition, silencing of STC1 significantly reversed the promoting effects of LV-MALAT1 and *miR-101-3p* inhibitor on the viability, migration, and invasion of HT29 cells ($P < 0.05$, Figure 7B–D).

Discussion

CC is one of the most prevalent and life-threatening cancers in the world.² LncRNA MALAT1 plays an important biological role in the development of CC.¹⁹ In this study, we found that the expression of MALAT1 was associated

with lymph node metastasis, depth of invasion and TNM stage. Furthermore, the up-regulation of MALAT1 contributed to the development of CC via targeting *miR-101-3p*/STC1 in vitro.

Previous studies have proved that MALAT1 is up-regulated in various types of cancers. The expression of MALAT1 is higher in ovarian cancer (OC) tissues and four OC cell lines compared with the normal ovary tissues and normal ovarian epithelial cell line, respectively.²⁸ MALAT1 is up-regulated in non-small cell lung cancer, and MALAT1 knockdown inhibits the cell metastasis.²⁹ MALAT1 is highly expressed in gastric cancer patients

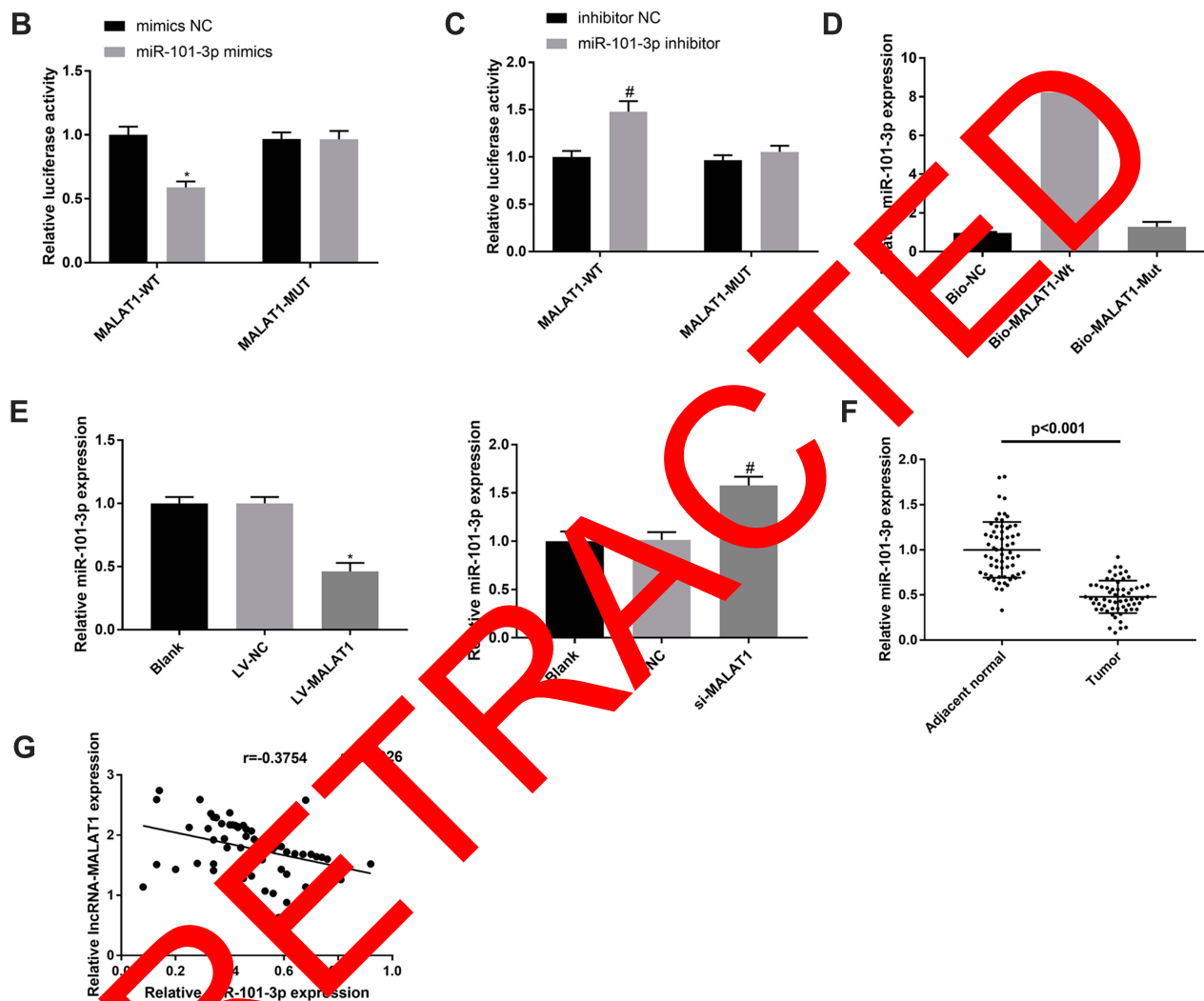
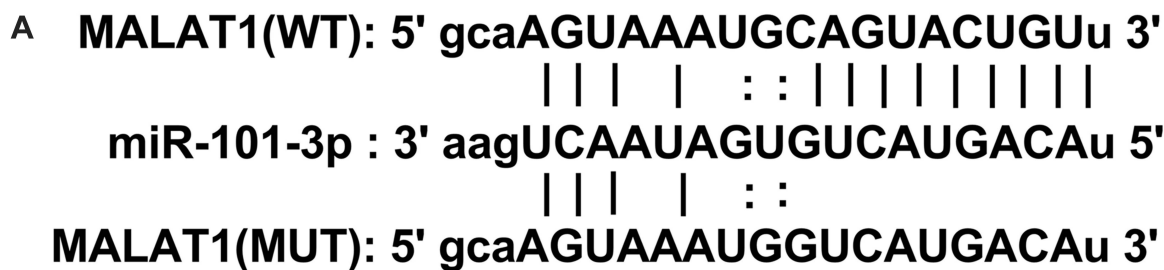


Figure 4 MALAT1 targets *miR-101-3p*. (A) The putative binding site between MALAT1 and *miR-101-3p* was predicted by the StarBase database. (B and C) Dual-luciferase reporter assay in HCT116 and HT29 cells. *P < 0.05 vs mimics NC; #P < 0.05 vs inhibitor NC. (D) RNA pull-down assay in HCT116 cells. *P < 0.05 vs Bio-NC. (E) Relative expression of *miR-101-3p* in HCT116 cells transfected with LV-MALAT1 or LV-NC, and in HT29 cells transfected with si-MALAT1-I or si-NC. *P < 0.05 vs LV-NC; #P < 0.05 vs si-NC. (F) Relative expression of *miR-101-3p* in CC tissues and adjacent normal tissues, P < 0.001. (G) Correlation analysis of MALAT1 and *miR-101-3p* expression in CC tissues, r = -0.3754, P = 0.0026. Each experiment was performed in three replicates.

with distant metastasis, functioning as an oncogene.³⁰ The expression of MALAT1 in cancerous tissues of CRC is 2.26 times higher than that in noncancerous tissues, which serves as a negative prognostic marker for CRC patients in stage II/III.³¹ Consistently, the expression of MALAT1

expression is up-regulated in CRC tissues,³² and is considered as a poor prognostic factor of CRC.¹⁸ Analogously, we observed that MALAT1 expression was up-regulated in CC tissues and HCT116 and HT29 cells. Additionally, the expression of MALAT1 was elevated in

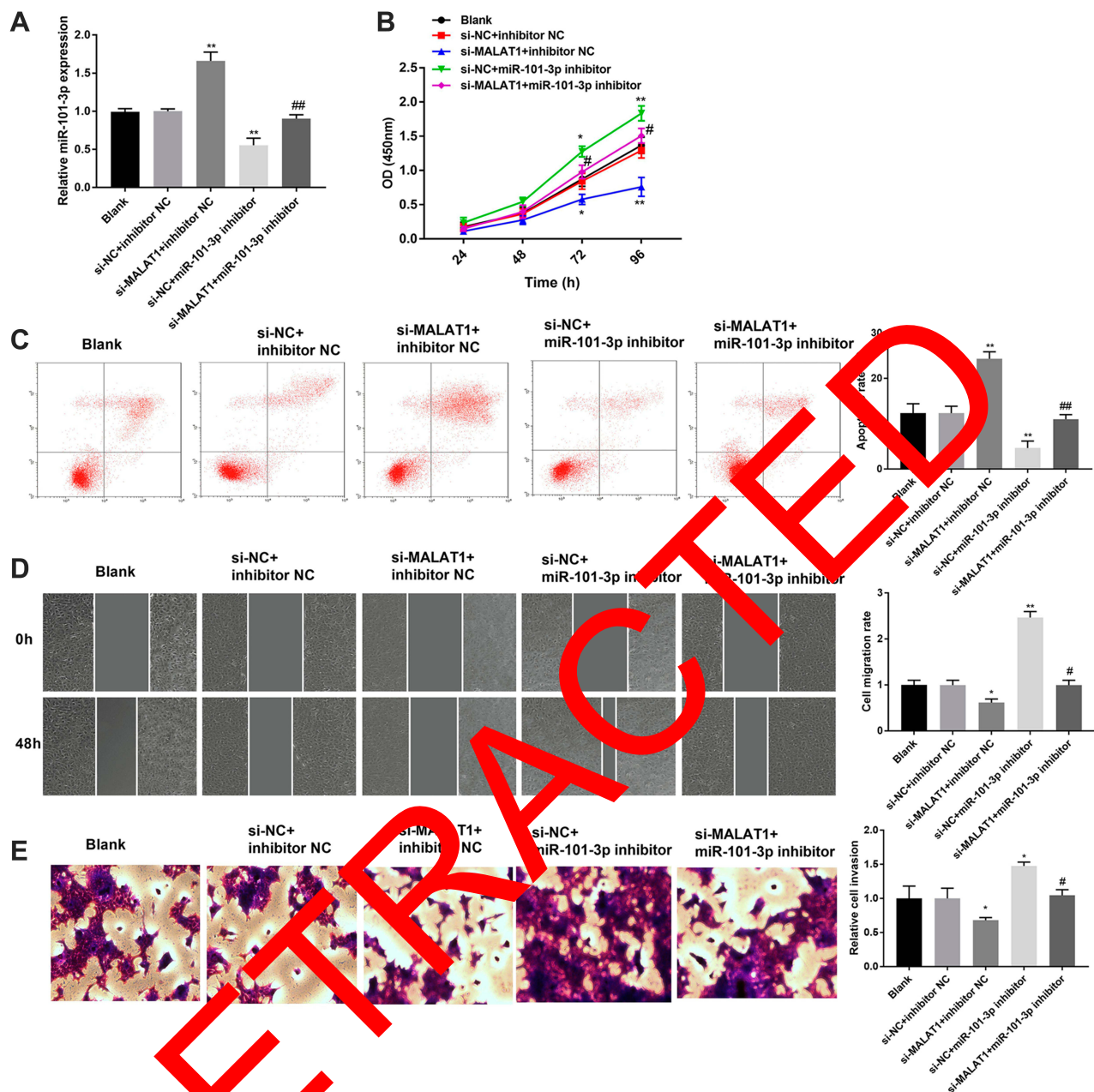


Figure 5 Silencing of miR-101-3p reverses the anti-tumor effect of MALAT1 knockdown on CC progression. **(A)** Relative expression of miR-101-3p in transfected HCT116 cells. **(B)** Cell proliferation of transfected HCT116 cells. **(C)** The apoptosis of transfected HCT116 cells. **(D)** The migration of transfected HCT116 cells. **(E)** The invasion of transfected HCT116 cells. ** $P < 0.01$ vs si-NC + inhibitor NC; * $P < 0.05$, ### $P < 0.01$ vs si-MALAT1 + inhibitor NC. Each experiment was performed in three replicates.

CC tissues with LN metastasis, invasive depth (T3 + T4), and/or TNM stage (III + IV). These findings indicated a high potential of MALAT1 for predicting the poor prognosis of CC patients.

MALAT1 affects cell proliferation and apoptosis in a variety of cancers, including CC.³³ Wu et al have found that MALAT1 knockdown markedly promotes the colony formation and induces the apoptosis of CC

cells.¹⁹ Zhang et al have found that MALAT1 knockdown inhibits the apoptosis and promotes the proliferation of CC cells.³⁴ Likewise, we found that overexpression of MALAT1 enhanced the proliferation and restrained the apoptosis of HCT116 cells, and the silencing of MALAT1 exhibited opposite results in HT29 cells. These results indicated a tumor-promoting role of MALAT1 in CC.

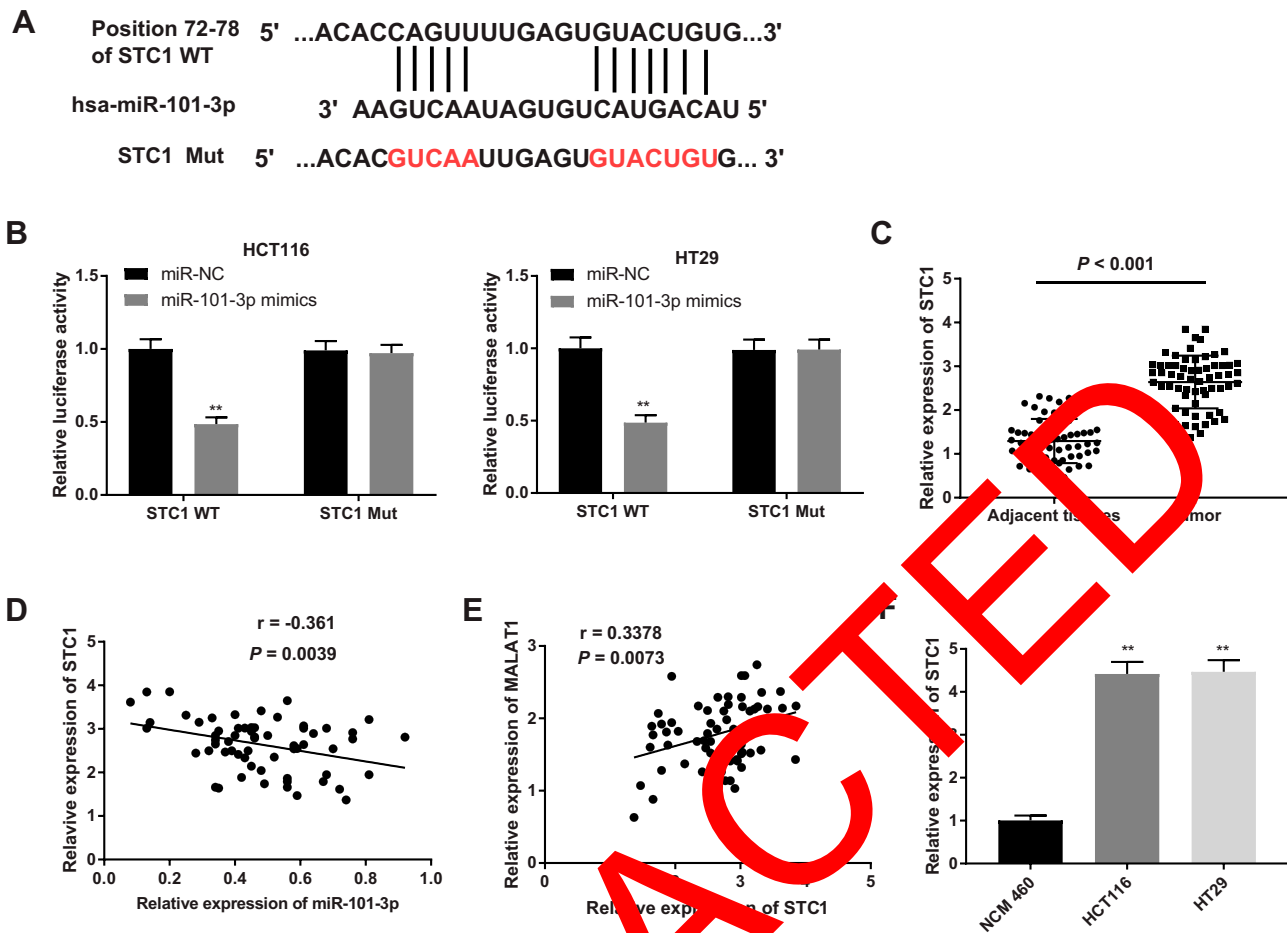


Figure 6 *MiR-101-3p* targets *STC1*. (A) The putative binding site between *MiR-101-3p* and *STC1* was predicted by the StarBase database. (B) Dual-luciferase reporter assay in HCT116 and HT29 cells. ** $P < 0.01$ vs miR-NC. (C) Relative expression of *STC1* in CC tissues and adjacent normal tissues, $P < 0.001$. (D) Correlation analysis of *STC1* and *miR-101-3p* expression in CC tissues, $r = -0.361$, $P = 0.0039$. (E) Correlation analysis of *STC1* and *MALAT1* expression in CC tissues, $r = 0.3378$, $P = 0.0073$. (F) Relative expression of *STC1* in HCT116 and HT29 cells. ** $P < 0.01$ vs NCM 460. Each experiment was performed in three replicates.

MALAT1 serves as a metastasis promoter in CRC, neuroblastoma, osteosarcoma, and lung cancer, or metastasis suppressor in glioma, CRC, breast cancer, and glioma.³⁵ Ji et al have verified that *MALAT1* overexpression promotes the proliferation and migration of CRC in vitro and in vivo via binding to SFPQ and releasing oncogene PTBP2 from SFPQ/PTBP2 complex.³⁶ However, similar results were observed. The up-regulation of *MALAT1* promoted the migration and invasion of HCT116 cells. Conversely, the silencing of *MALAT1* inhibited the migration and invasion of HT29 cells. All these results indicated a pro-migration and invasion role of *MALAT1* in CC.

lncRNAs act as competing for endogenous RNAs (ceRNAs) to sponge miRNAs to participate in the occurrence and development of gastric cancer, liver cancer, and CC.^{37,38} *MALAT1* is involved in the development of CC by interacting with various miRNAs. Xu et al have proved that the silencing of *MALAT1* suppresses the viability and metastasis of CRC

cells through sponging *miR-145*.¹⁷ Wu et al have shown that *MALAT1-miR203-DCP1A* axis is associated with the malignancy of CC.³⁹ Sun et al have demonstrated that *MALAT1* enhances the proliferation and inhibits the apoptosis of OC cells via sponging *miR-503-5p*.⁴⁰ Herein, *miR-101-3p* was predicted to be a target gene of *MALAT1* by StarBase, and this target relationship was validated by dual-luciferase reporter assay and RNA pull-down assay in CC cells. *MiR-101-3p* plays a tumor suppressor role in diverse human cancers, such as OC,⁴¹ gastric cancer,⁴² lung cancer⁴³ and cervical cancer.⁴⁴ In this study, we found that the expression of *miR-101-3p* was significantly down-regulated in CC tissues, and was inversely correlated with *MALAT1* expression in CC tissues. Thus, we deduced that *miR-101-3p* also exerts a tumor suppressor role in CC. Our following functional analysis revealed that the silencing of *miR-101-3p* evidently enhanced the proliferation, migration, invasion, and restrained the apoptosis of HT29 cells. These results illustrated the tumor suppressor role of

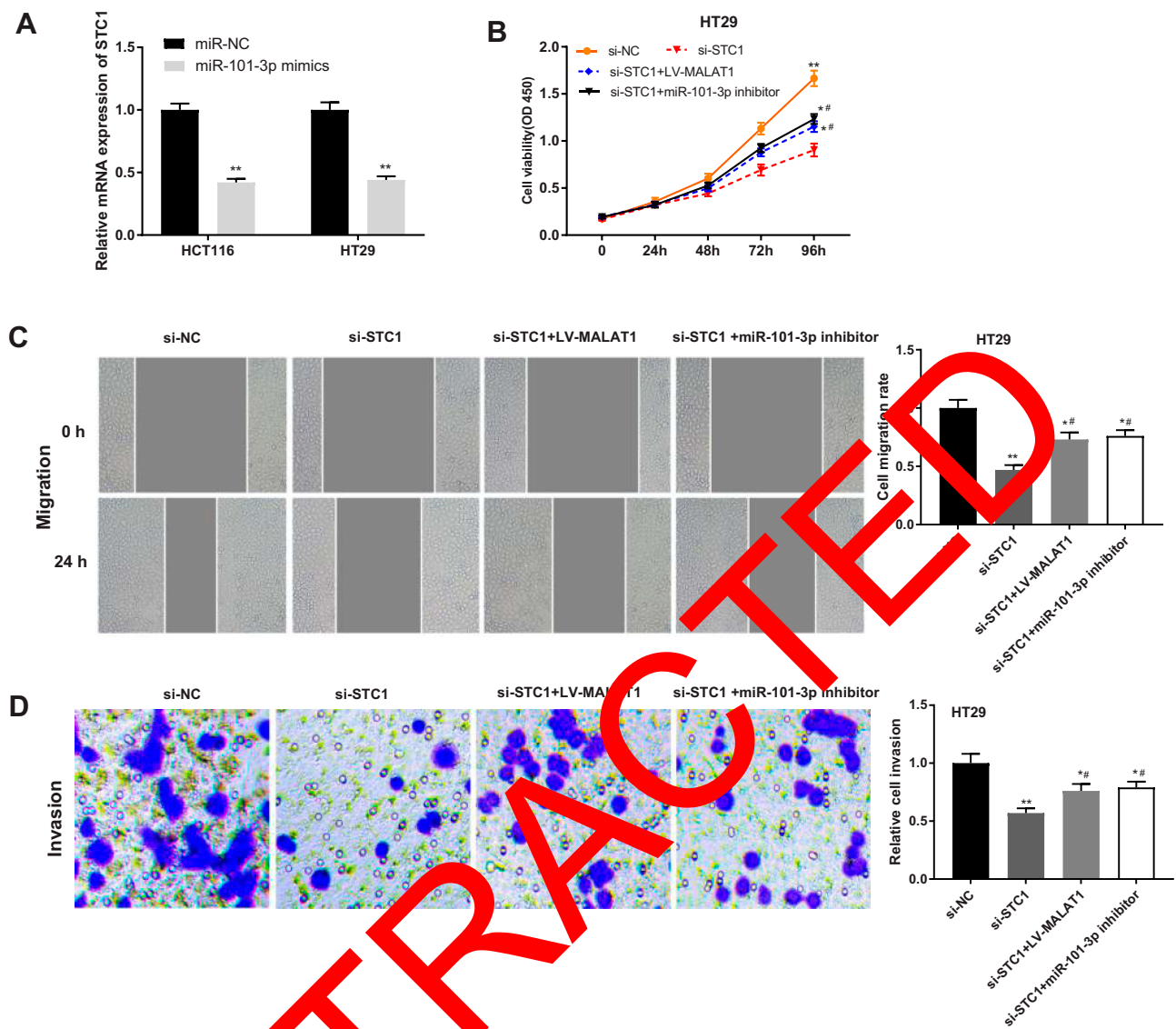


Figure 7 Silencing of STC1 reverses the tumor promoting effects of MALAT1 up-regulation and miR-101-3p down-regulation on CC progression. **(A)** Relative expression of STC1 in transfected HT29 cells. **(B)** The proliferation of transfected HT29 cells. **(C)** The migration of transfected HT29 cells. **(D)** The invasion of transfected HT29 cells. * $P < 0.05$, ** $P < 0.01$ vs si-NC; # $P < 0.05$ vs si-STC1. Each experiment was performed in three replicates.

miR-101-3p in CC. Rescue experiments were then performed to clarify the regulatory relationship between MALAT1 and *miR-101-3p* in CC. Our results showed that silencing of *miR-101-3p* reversed the anti-tumor effect of MALAT1 knock-down on CC cells. These findings indicated that silencing of MALAT1 may inhibit the development of CC through targeting *miR-101-3p*.

MiRNAs play key regulatory roles in the occurrence and development of CC by regulating specific genes. Zeng et al have proved that miR-7 inhibits the proliferation and migration of CC cells by targeting FAK.⁴⁵ Koo et al have found that miR-4779 induces the apoptosis and cell cycle arrest of CC cells through direct targeting PAK2 and

CCND3.⁴⁶ Chandramouli et al have shown that ectopic expression of miR-101 markedly inhibits the proliferation and motility of CC cells through targeting PGE2 receptor EP4.⁴⁷ In this study, STC1 was identified to be a target of *miR-101-3p*. STC1 is a glycoprotein hormone involved in calcium/phosphate homeostasis.⁴⁸ STC1 plays a key regulatory role in the occurrence and development of various cancers, such as breast cancer,⁴⁹ cervical cancer,⁵⁰ retinoblastoma,⁵¹ and prostate cancer.⁵² In this study, the expression of STC1 was up-regulated in CC tissues and cell lines, and inversely correlated with *miR-101-3p* expression in CC tissues. Silencing of STC1 significantly inhibited the proliferation, migration, invasion of HT29

cells. These results illustrated a tumor promoting role of STC1 in CC. Furthermore, rescue experiments showed that the tumor promoting effects of MALAT1 overexpression and *miR-101-3p* silencing on CC cells were reversed by STC1 silencing. These findings indicated that silencing of MALAT1 may inhibit the development of CC through targeting *miR-101-3p*/STC1 axis.

Conclusions

MALAT1 was upregulated in CC tissues and cells, functioning as an oncogene in CC development. MALAT1 expression was associated with lymph node metastasis, depth of invasion and TNM stage in patients with CC. MALAT1 promoted the proliferation, migration, and invasion, and inhibited the apoptosis of CC cells via targeting *miR-101-3p*/STC1 axis. The silencing of MALAT1 might be a hopeful therapeutic option for CC.

Ethics Approval and Consent Statement

This study was conducted after obtaining local ethical committee approval of Yidu Central Hospital of Weifang. Written informed consent was obtained from patients over the age of 18 years and parents of patients under the age of 18 years. This was conducted in accordance with the Declaration of Helsinki.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest to disclose.

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