



LINC00511 promotes cervical cancer progression by regulating the miR-497-5p/MAPK1 axis

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Abstract

Background Long non-coding RNA (lncRNA) exhibits a crucial role in multiple human malignancies. The expression of lncRNA LINC00511, reportedly, is aberrantly up-regulated in several types of tumors. Our research was aimed at deciphering the role and mechanism of LINC00511 in the progression of cervical cancer (CC).

Method Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to quantify the expression levels of LINC00511, miR-497-5p and MAPK1 mRNA in CC tissues and cell lines. Cell counting kit-8 (CCK-8), 5-bromo-2'-deoxyuridine (BrdU) and Transwell assays were conducted for detecting the proliferation, migration and invasion of CC cells. Dual-luciferase reporter gene experiments were performed to verify the targeting relationships amongst LINC00511, miR-497-5p and MAPK1. Besides, MAPK1 expression in CC cells was detected via Western blot after LINC00511 and miR-497-5p were selectively regulated.

Results Up-regulation of LINC00511 expression in CC tissues and cell lines was observed, which was in association with tumor size, clinical stage and lymph node metastasis of the patients. LINC00511 overexpression facilitated the proliferation, migration and invasion of CC cells, while opposite effects were observed after knockdown of LINC00511. Mechanistically, LINC00511 was capable of targeting miR-497-5p and up-regulating MAPK1 expression.

Conclusion LINC00511/miR-497-5p/MAPK1 axis regulates CC progression.

Keywords Cervical cancer · LINC00511 · miR-497-5p · Mitogen-activated protein kinase 1

Introduction

Cervical cancer (CC) is the most common gynecological malignancies among women worldwide, which poses a serious threat to females' health, with approximately 570,000 CC new cases diagnosed and more than 275,000 deaths reported annually [1–4]. It is imperative to further clarify

the molecular mechanism underlying CC tumorigenesis, so as to improve the therapeutics of CC.

It is well documented that long non-coding RNAs (lncRNAs) are regulators of the malignant phenotypes of cancer cells [5–8]. For example, the significant augmentation of ZEB2-AS1 expression in non-small cell lung cancer is reported, and its high expression markedly promotes the migration, invasion and epithelial-mesenchymal transition (EMT) of tumor cells [9]. LncRNA MIR4435-2HG potentiates the migration and invasion of prostate cancer cells via up-regulation of TGF- β 1 expression [10]. Moreover, LINC00511 exhibits cancer-promoting effects in multiple types of cancers. For example, LINC00511 facilitates the growth of breast cancer by accelerating the transition of G1/S phase and repressing apoptosis [11]. LINC00511, whose expression is also markedly up-regulated in CC tissues and cell lines, expedites the proliferation of CC cells and suppresses autophagy and apoptosis by modulating PLD1 [12]. Nevertheless, the expression characteristics, biological

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functions and regulatory mechanisms of LINC00511 in CC warrant further clarification.

MicroRNAs (miRNAs) are capable of binding to 3' untranslated region (3'-UTR) of mRNA, further inducing target mRNA degradation or translational inhibition and hence regulating gene expression at the post-transcriptional level [13]. Previous studies report that miRNAs play crucial roles in cancer progression [14–18]. For example, miR-335 impedes the proliferation of lung cancer cells by targeting Tra2 β [15]. Bone metastasis of prostate cancer cells is associated with the down-regulation of miR-141-3p expression [16]. In CC, miR-497-5p, a well-known tumor suppressor in cancer biology, induces cell cycle arrest and represses cancer cell proliferation by targeting CBX4 [17, 18]. Nonetheless, the molecular mechanism of miR-497-5p dysfunction in CC requires further delineation.

In the present research, the expression pattern, biological function, clinical implication and underlying mechanism of LINC00511 in CC were investigated. Functional experiments substantiated that LINC00511, whose expression was up-regulated in CC, promoted the proliferation, migration and invasion of CC cells. In terms of mechanism, LINC00511 was validated to accelerate the progression of CC by regulating miR-497-5p/mitogen-activated protein kinase 1 (MAPK1) axis.

Materials and methods

Tissue samples

CC tissues and para-cancerous tissues were collected from the Guizhou Medical University. Specimens were obtained with signed informed consent. This research, endorsed by the Ethics Review Board of the Guizhou Medical University, followed all principles of the Declaration of Helsinki.

Cell cultivation and transfection

Non-malignant cervical epithelial cell line (End1/E6E7) and CC cell lines (SiHa, HeLa, C4-1 and HT-3) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Hyclone, Logan, UT, USA) which was added with 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), in an incubator with saturated humidity containing 5% CO₂ at 37 °C. pcDNA empty vector (NC), pcDNA-LINC00511 (LINC00511), siRNA normal control (si-NC), siRNAs against LINC00511 (si-LINC00511), negative control of miR-497-5p mimic (miR-NC), negative control of miR-497-5p inhibitors (miR-in), miR-497-5p mimic and miR-497-5p inhibitors were

purchased from GenePharma Co., Ltd. (Shanghai, China). Transfection was performed employing Lipofectamine™ 2000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from tissues and cells employing TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. cDNA synthesis was conducted by reverse transcription utilizing a PrimeScript™ RT reagent Kit with gRNA Eraser (Invitrogen, Carlsbad, CA, USA). qRT-PCR was conducted employing a SYBR Premix Ex Taq II kit (Takara Bio, Inc., Otsu, Japan). β -actin and U6 served as the endogenous controls, and the quantification of relative expression was conducted employing 2^{- $\Delta\Delta$ Ct} method. The sequences of primers were shown as follows: LINC00511: forward: 5'-CGCAAGGACCCTCTGT-TAGG-3'; reverse: 5'-GAAGGCGGATCGTCTCTCAG-3'. MAPK1: forward: 5'-TCTGCACCGTGACCTCAA-3'; reverse: 5'-GCCAGGCCAAAGTCACAG-3'. miR-497-5p: forward: 5'-CCTTCAGCAGCACACTGTGG-3'; reverse: 5'-CAGTGCAGGGTCCGAGGTAT-3'. U6: forward: 5'-CTCGCTTCGGCAGCACA-3'; reverse: 5'-TGGTGTCTGGAGTCG-3'. β -actin: forward: 5'-TCACCAACTGGGACGACATG-3'; reverse: 5'-GTCACCGGAGTCCATCAGAT-3'.

Cell counting kit-8 (CCK-8) assay

HT-3 and C4-1 cells (2 \times 10³/well) were seeded into a 96-well plate and cultured overnight. Subsequently, the cells in each well were incubated with 10 μ L of CCK-8 reagent (Dojindo, Kumamoto, Japan) for 2 h, and then a microplate reader was used to detect the optical density (OD) values of the cells at 450 nm wavelength. With the same method, the OD values were detected for three consecutive days.

5-bromo-2'-deoxyuridine (BrdU) assay

After CC cells were seeded in 96-well plates at a density of 2 \times 10³ cells/well and cultured for 24 h, the BrdU kit (BD Pharmingen, San Diego, CA, USA) was added into each well (final concentration: 10 μ M), and the culture was continued for 8 h. Subsequently, the medium was removed, and cells were fixed with 4% paraformaldehyde for 30 min and then incubated with anti-BrdU antibody (Beyotime, Shanghai, China) for 1 h at room temperature. After the cells were rinsed with phosphate buffer saline (PBS) for 3 times, Hoechst staining solution (Beyotime, Shanghai, China) was used for marking cell nuclei. Three fields were

randomly selected under a fluorescence microscope. Cell proliferation rate = BrdU positive cells / Hoechst positive cells \times 100%.

Transwell assay

Cell suspensions (2×10^5 cells/mL) of CC cells were prepared utilizing serum-free medium. A total of 0.2 mL cell suspension was added to the upper compartment of the Transwell system (Nest, Wuxi, China) while the lower compartment was filled with the medium containing 10% FBS. After the cells were placed in the incubator for 12 h, the migrated and invaded cells were fixed with 4% paraformaldehyde and then stained with 0.5% crystal violet solution. Under a microscope, five visual fields were selected in each group for counting the cell number. For the invasion assay, the bottom of the Transwell system was coated with a layer of Matrigel (30 μ g/well; BD, San Jose, CA, USA) before the inoculation of the cells.

Western blot

The extraction of total protein from cells was performed employing RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, USA). After the samples were mixed with 5 \times loading buffer and denatured in boiling water for 5 min, the separation of the proteins was completed via SDS-PAGE, followed by subsequent transfer of separated proteins onto polyvinylidene difluoride (PVDF) membranes (Beyotime, Shanghai, China). After that, the membranes were blocked in 5% skimmed milk for 2 h at room temperature and then incubated with primary antibodies against MAPK1 (1:5000; ab257525; Abcam, Shanghai, China) and β -actin (1:2000; ab5694; Abcam, Shanghai, China) overnight at 4 $^{\circ}$ C. Next, the membranes were incubated with horseradish peroxidase-labeled secondary antibodies (1:1000, Beyotime, Shanghai, China) at room temperature for 2 h. The development of protein bands was conducted employing an enhanced chemiluminescent kit (Biozym, Hessisch Oldendorf, Germany).

Dual-luciferase reporter gene assay

The amplification of wild-type (WT) LINC00511 sequence and MAPK1 3'UTR sequence was completed by PCR, followed by the selective mutation of the sequences. Next, mutant (MUT) LINC00511 sequence, MUT MAPK1 sequence, WT LINC00511-WT sequence and WT MAPK1 sequence were inserted into pmirGLO luciferase reporter vectors (Promega, Madison, WI, USA). Subsequently, pmirGLO-LINC00511-MUT, pmirGLO-LINC00511-MUT, mirGLO-MAPK1-MUT and pmirGLO-MAPK1-WT were

transfected into HT-3 and C4-1 cells, respectively, together with the transfection of miR-497-5p mimics or miR-NC. After 24 h, the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) with a GloMax 20/20 Luminometer (Promega, Madison, WI, USA) were used to detect the luciferase activity of the cells in each group in line with manufacturer's protocol.

Statistical analysis

All experiments were performed in triplicate, and all experimental data expressed by mean \pm standard deviation were analyzed utilizing GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA). Kaplan-Meier method with log-rank test was performed to evaluate the relationship between LINC00511 expression and progression-free survival (PFS) and overall survival (OS) of CC patients. The comparison of data was performed using student's *t*-test (between two groups) and one-way ANOVA (among multiple groups), with $P < 0.05$ statistically indicating a significant difference.

Results

LINC00511 was highly expressed in CC tissues and cells

First of all, the up-regulation of LINC00511 expression in CC samples was observed through the analysis of Gene Expression Profiling Interactive Analysis (GEPIA) database (gepia.cancer-pku.cn) (Fig. 1A). qRT-PCR was performed to quantify LINC00511 expression in cancer tissues and adjacent tissues collected from 53 CC patients, the results of which indicated a significant up-regulation of LINC00511 expression in CC tissue (Fig. 1B). Besides, qRT-PCR suggested that LINC00511 expression in CC cells (SiHa, HeLa, C4-1 and HT-3 cells) was remarkably higher than that in non-malignant cervical endometrial epithelial cell line End1/E6E7 (Fig. 1C). Additionally, to analyze the correlation between LINC00511 expression and clinicopathological features, 53 CC patients were divided into high expression group ($n=27$) and low expression group ($n=26$) with the median value of LINC00511 expression as the cutoff value. The results showed a remarkable correlation between LINC00511 expression and tumor size, clinical stage as well as lymph node metastasis, implying a cancer-promoting effect of LINC00511 in the development of CC (Table 1). In addition, we also analyzed the relationship between the expression of LINC00511 and the survival time of CC patients. Survival analysis showed that patients with high LINC00511 expression had significantly lower

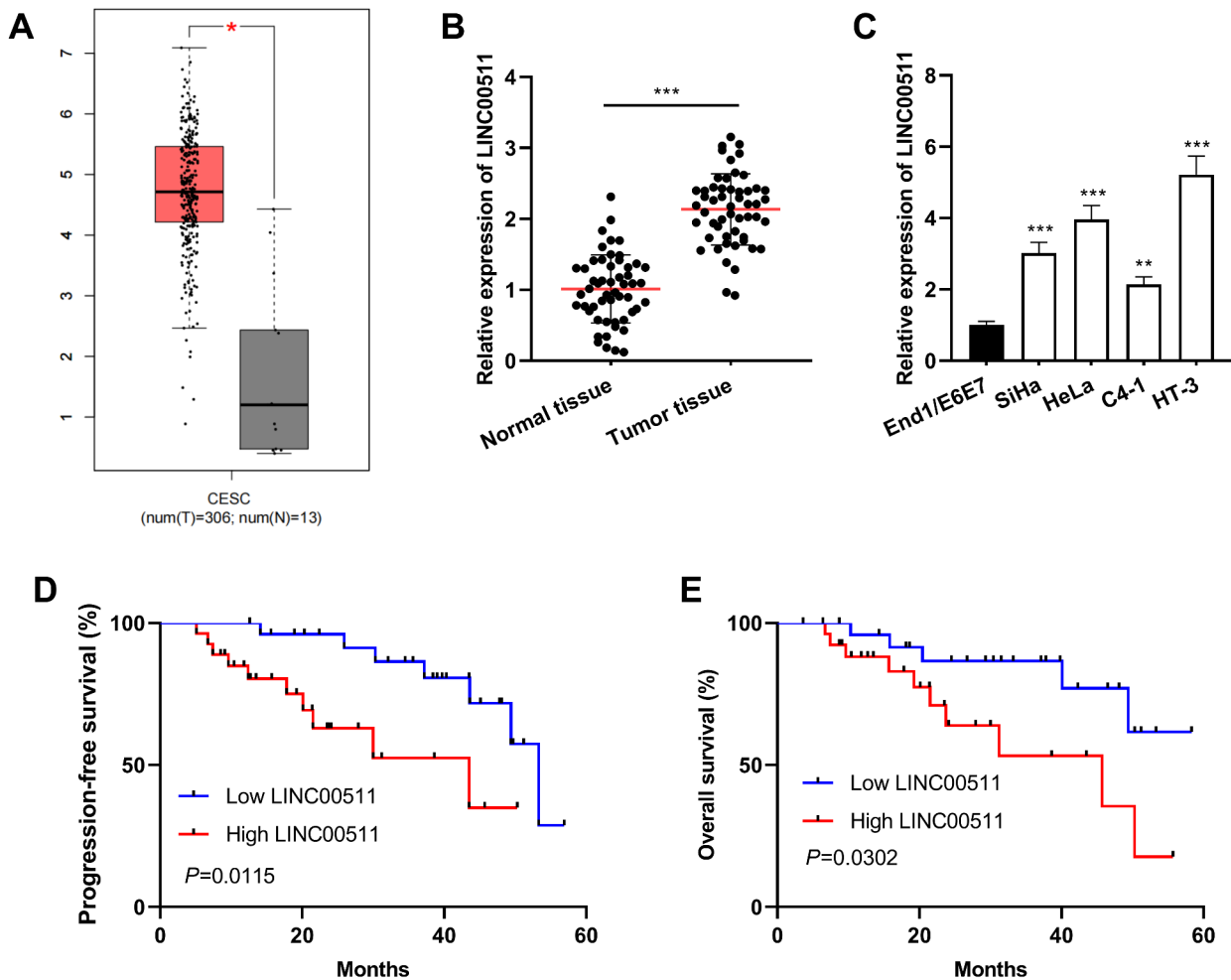


Fig. 1 LINC00511 was highly expressed in CC tissues and cells. **A**. LINC00511 expression in cancer tissues and normal cervical tissues was analyzed utilizing GEPIA database. Red represents tumor tissues and grey represents normal tissues. CESC: cervical squamous cell carcinoma and endocervical adenocarcinoma, T (tumor): 306 cases, N (normal): 13 cases; $|\text{Log2FC}|$ cutoff: 1; p -value cutoff: 0.01. LINC00511 expression in 53 pairs of CC tissues and adjacent tissues was quantified by qRT-PCR. qRT-PCR was employed to detect LINC00511 expression in normal cervical endometrial epithelial cells End1/E6E7 cells and CC cells (SiHa, HeLa, C4-1 and HT-3 cells). **D–E**. Kaplan-Meier curve was used to analyze the relationship between LINC00511 expression and progression-free survival and overall survival in CC patients. All experiments were performed in triplicate. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

PFS and OS than the patients with low LINC00511 expression (Fig. 1D–E).

LINC00511 expedited proliferation, migration and invasion of CC cells

Among the CC cell lines, LINC00511 expression was the lowest in C4-1 cells and the highest in HT-3 cells (Fig. 1 C). Accordingly, LINC00511 overexpression plasmid and empty plasmid were transfected into C4-1 cells, si-LINC00511#1, si-LINC00511#2 and si-NC were transfected into HT-3 cells, and the gain-of-function and loss-of-function models were established (Fig. 2 A). Following

that, CCK-8 and BrdU assays were implemented, which indicated overexpression of LINC00511 significantly promoted the proliferation of CC cells (Fig. 2B–D). Transwell experiments were also performed, and it was revealed that overexpression of LINC00511 promoted the metastatic potential of C4-1 cells (Fig. 2E–F). Conversely, compared with the control group, LINC00511 silencing with siRNAs inhibited the proliferation, migration and invasion of HT-3 cells (Fig. 2B–F). These data suggested that LINC00511 positively regulated the malignant phenotypes of CC cells.

Table 1 Correlation between LINC00511 expression and multiple clinicopathological characteristics in CC patients. (n = 53)

Characteristics	Number	LINC00511 expression		χ^2	P value
		High	Low		
Age (years)					
< 50	25	15	10	1.5531	0.2127
≥ 50	28	12	16		
Gender					
Male	30	13	17	1.6020	0.2056
Female	23	14	9		
Tumor size (cm)					
≤ 5	22	6	16	8.4328	0.0037
> 5	31	21	10		
Clinical stage					
I–IIA	21	4	17	14.1588	0.0001
IIB–IV	32	23	9		
Distant metastasis					
Absent	28	14	14	0.021	0.884
Present	25	13	12		
Histological type					
Adenocarcinoma	15	7	8	0.1531	0.6956
Squamous cell carcinoma	38	20	18		
Lymph node metastasis					
Absent	29	8	21	13.9804	0.0001
Present	24	19	5		

LINC00511 directly targeted miR-497-5p

Next, bioinformatics analysis with ENCORI database (<http://starbase.sysu.edu.cn/>) was utilized for predicting the downstream miRNAs of LINC00511. The results indicated a potential binding site between LINC00511 and miR-497-5p (Fig. 3 A). Subsequently, dual-luciferase reporter assay was conducted, and as shown, overexpression of miR-497-5p led to the reduction of the luciferase activity of wild-type LINC00511 reporter whereas miR-497-5p transfection did not affect the luciferase activity of mutant LINC00511 reporter (Fig. 3B). Additionally, the data from qRT-PCR assays indicated a negative correlation between miR-497-5p expression and LINC00511 expression in the CC samples (Fig. 3 C). Additionally, overexpression of LINC00511 remarkably impeded miR-497-5p expression in C4-1 cells while knockdown of LINC00511 markedly increased miR-497-5p expression in HT-3 cells (Fig. 3D). These results implied that miR-497-5p was a direct target of LINC00511 and its expression could be negatively regulated by LINC00511.

MiR-497-5p exhibited a tumor-suppressive role in CC

For further clarifying the role of miR-497-5p in CC, expression characteristics of miR-497-5p in CC tissues and cell lines were detected by qRT-PCR, showing a significantly lower expression of miR-497-5p in CC tissues and cells than that in adjacent tissues and End1/E6E7 cell line (Fig. 4 A–B). Subsequently, the transfection of miR-497-5p mimics into C4-1 cells and the transfection of miR-497-5p inhibitors into HT-3 cells were performed (Fig. 4 C). Following that, CCK-8, BrdU and Transwell assay were implemented, and the results suggested that the up-regulation of miR-497-5p expression repressed the proliferation, migration and invasion of C4-1 cell, and the inhibition of miR-497-5p expression promoted these malignant phenotypes of HT-3 cells, suggesting that miR-497-5p was a tumor suppressor in CC (Fig. 4D–H).

MiR-497-5p counteracted the biological effects of LINC00511 in CC

To further pinpoint the underlying mechanism and specific function of LINC00511/miR-497-5p axis in CC cells, miR-497-5p mimics and miR-497-5p inhibitors were co-transfected into C4-1 cells with LINC00511 overexpression and HT-3 cells with LINC00511 knockdown, respectively (Fig. 5 A). Functional experiments revealed that overexpression of miR-497-5p partially weakened the promotive effects of proliferation, migration and invasion of C4-1 cells ascribed to overexpression of LINC00511 while miR-497-5p inhibitors partially counteracted the inhibitory effects of knockdown of LINC00511 on proliferation, migration and invasion of HT-3 cells (Fig. 5B–F). These data revealed that LINC00511 regulated CC cell proliferation, migration and invasion by modulating miR-497-5p.

LINC00511/miR-497-5p axis regulated MAPK1 expression

ENCORI database suggested a probable binding site between miR-497-5p and 3'UTR of MAPK1, which possessed cancer-promoting effects in CC [19] (Fig. 6 A). In dual-luciferase reporter assay, miR-497-5p mimics were observed to suppress the luciferase activity of wild type MAPK1 reporter whereas no significant effect of miR-497-5p on the luciferase activity of mutant MAPK1 reporter was observed (Fig. 6B). Furthermore, overexpression of miR-497-5p or knockdown of LINC00511 triggered a reduction of MAPK1 protein and mRNA expressions in C4-1 and HT-3 cells while the transfection of miR-497-5p inhibitors or LINC00511 overexpression plasmid promoted the expression of MAPK1

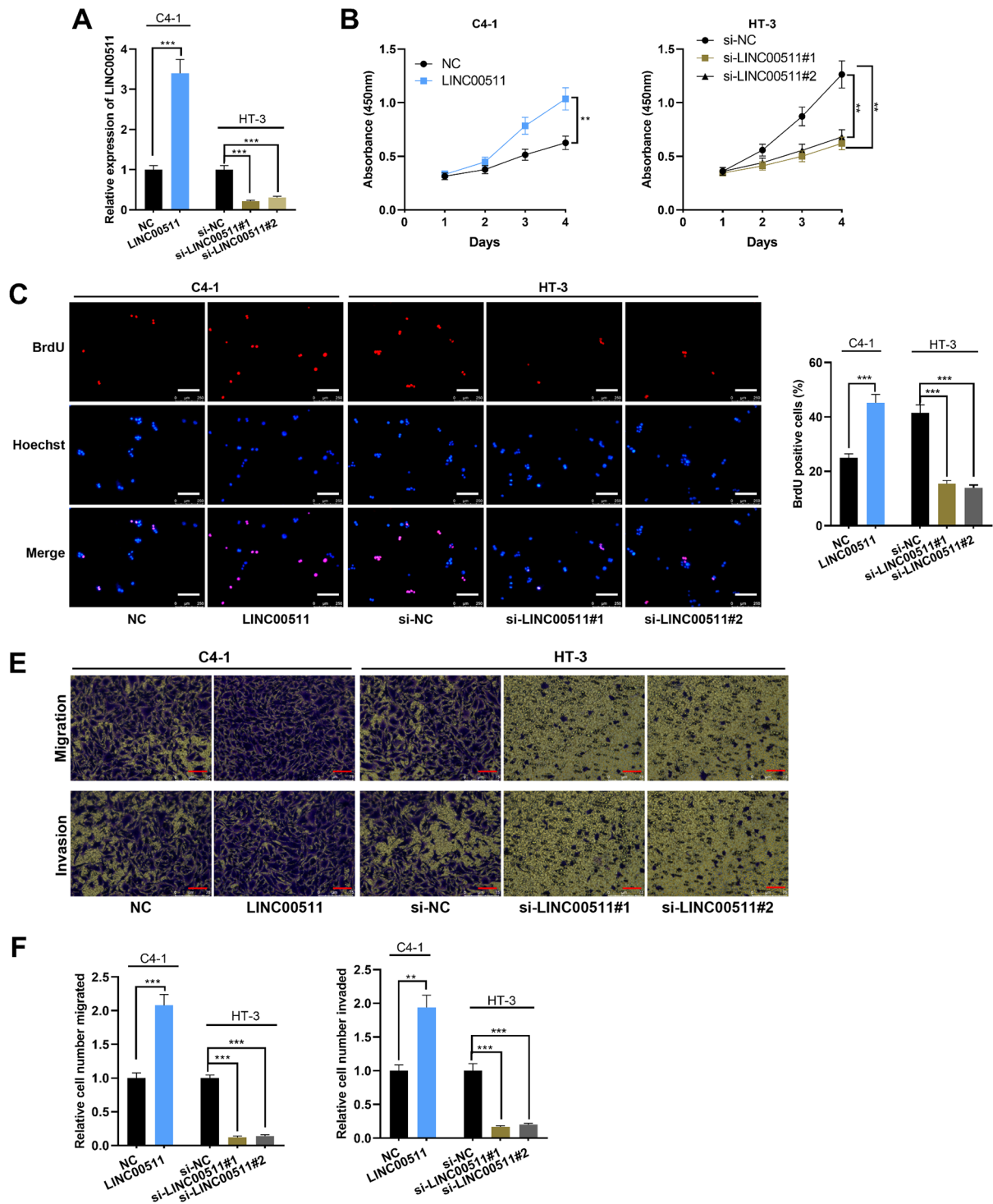


Fig. 2 The effects of LINC00511 on proliferation, migration and invasion of CC cells. **A**. LINC00511 expression in C4-1 cells transfected with LINC00511 overexpression plasmid and HT-3 cells transfected with LINC00511 siRNAs was quantified employing qRT-PCR. **B-D**. CCK-8 method (**B**) and BrdU assay (**C-D**) were performed to detect the proliferation of CC cells after overexpression or knockdown of LINC00511. Scale bar, 250 μ m. **E-F**. Transwell assay was utilized for monitoring migration and invasion of CC cells. Scale bar, 75 μ m. All experiments were performed in triplicate. ** $P < 0.01$ and *** $P < 0.001$

(Fig. 6 C-D). Meanwhile, miR-497-5p overexpression partially weakened the up-regulation of MAPK1 expression

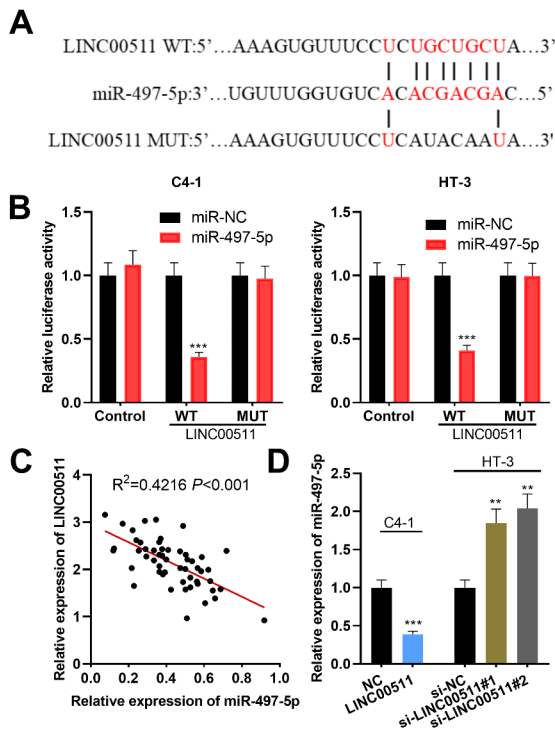


Fig. 3 MiR-497-5p was the target of LINC00511 in CC cells. **A**. Bioinformatics was utilized for the prediction of binding site between LINC00511 and miR-497-5p. **B**. Dual-luciferase reporter gene experiment was employed for verifying the binding relationship between miR-497-5p and LINC00511. **C**. The correlation between LINC00511 and miR-497-5p expressions in CC tissues. **D**. MiR-497-5p expression in CC cells with LINC00511 overexpression or knockdown was assessed employing qRT-PCR. All experiments were performed in triplicate. ** $P < 0.01$ and *** $P < 0.001$

resulted from LINC00511 overexpression, while the inhibition of miR-497-5p expression partially counteracted the reduction of MAPK1 expression induced by LINC00511 knockdown (Fig. 6 C-D). Furthermore, qRT-PCR results showed an augmentation of MAPK1 mRNA expression in CC tissues and cells (Fig. 6E-F), and a negative correlation between miR-497-5p expression and MAPK1 mRNA expression and a positive correlation between LINC00511 expression and MAPK1 mRNA expression were found in CC tissues (Fig. 6E-H). These experimental findings suggested that MAPK1 was a downstream target of miR-497-5p, and LINC00511 could indirectly up-regulate MAPK1 expression by modulating miR-497-5p expression.

Discussion

Currently, surgery, chemotherapy, radiotherapy, targeted therapy and immunotherapy are the main treatment strategies for cancers [20–22]. However, these methods still couldn't cure most of the human malignancies, especially the metastatic diseases. In recent years, non-coding RNA therapeutics attracted a lot of attention, which are promising to improve the prognosis of the patients [22]. LncRNAs possess multiple functions including serving as a host gene of miRNA, preventing RNA and protein from binding to targets, or acting as a molecular scaffold to guide proteins to their targets [23]. LncRNAs exhibit a pivotal regulatory role in modulating the proliferation, migration and invasion of CC cells. For example, lnc-UICC is highly expressed in CC and accelerates the growth and metastasis of CC cells in vivo and in vitro [24]; the up-regulation of lncRNA HOXD-AS1 in CC regulates the proliferation of CC cells by activating Ras/ERK signaling pathway [25]. Reportedly, LINC00511 knockdown weakens the proliferation, migration and invasion of HeLa and C33A cells [26]. In another research, LINC00511 expression is increased in CC tissue, and LINC00511 overexpression significantly facilitates the proliferation and represses the apoptosis of HeLa cells [27]. In the current research, we verified that LINC00511 was highly expressed in CC tissues and cells, and its promoting effects on the proliferation, migration and invasion of CC cells were further validated, which was consistent with previous reports [12, 26, 27].

MiRNA exhibits a crucial role in CC progression. For instance, miR-218, whose expression is down-regulated in CC tissues and cells, represses the viability and induces the apoptosis of CC cells [28]; miR-501 expression is up-regulated in CC, and miR-501 expedites the proliferation, migration and invasion of CC cells by targeting CYLD [29]. Besides, miR-497-5p exhibits a cancer-inhibiting effect in lot of tumors. In hepatocellular carcinoma, the proliferation, migration and invasion of cancer cells were inhibited by miR-497-5p [30]; in non-small cell lung cancer, miR-497-5p suppresses the proliferation and invasion of cancer cells by targeting FGF2 [31]. In CC, miR-497-5p also functions as a tumor suppressor [18]. Herein, the inhibitory impacts of miR-497-5p on the malignant phenotypes of CC cells were further validated.

LncRNAs serve as competitive endogenous RNAs (ceRNA, or molecular sponge) for miRNAs, thereupon reducing the binding between miRNAs and mRNAs, and thereby modulating gene expression. LINC00511 functions as a molecular sponge in several types of cancers. For example, in gastric cancer, LINC00511, serving as the ceRNA of miR-124-3p, regulates PDK4 expression, hence promoting the growth of tumors [32]. Besides, it promotes

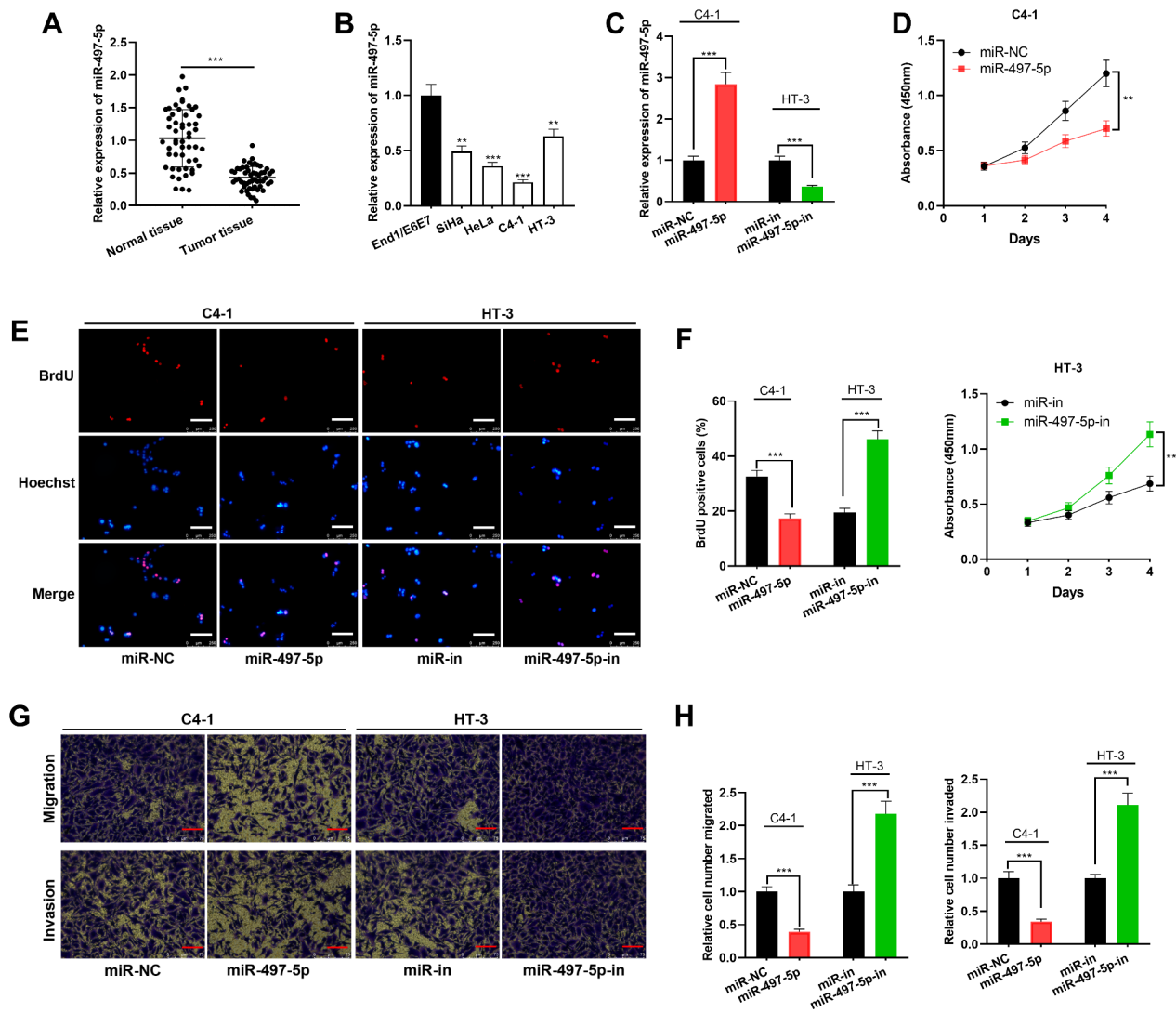


Fig. 4 The effects of miR-497-5p on proliferation, migration and invasion of CC cells. **A**. MiR-497-5p expression in 53 pairs of CC tissues and adjacent tissues was quantified employing qRT-PCR. **B**. MiR-497-5p expression in End1/E6E7 cells and CC cell lines was detected employing qRT-PCR. **C**. qRT-PCR was utilized for quantifying miR-497-5p expression in CC cells transfected with miR-497-5p mimics or inhibitors. **D-F**. CCK-8 assay (**D**) and BrdU assay (**E-F**) were implemented for detecting the proliferation of CC cells. Scale bar, 250 μ m. **G-H**. Transwell assay was utilized for monitoring the migration and invasion of CC cells. Scale bar, 75 μ m. All experiments were performed in triplicate. ** $P < 0.01$ and *** $P < 0.001$

the proliferation and metastasis of osteosarcoma cells by sponging miR-618 and up-regulating MAEL expression [33]. Herein, for the first time, our study reported that LINC00511 directly targeted miR-497-5p, and miR-497-5p partially counteracted the functions of LINC00511 on the proliferation, migration and invasion of CC cells. It was concluded that LINC00511 acted as a ceRNA for miR-497-5p, and our demonstrations provided a novel mechanism by which LINC00511 regulated CC progression.

Mitogen-activated protein kinase 1, encoded by human MAPK1 gene and termed as extracellular signal-regulated

kinase (ERK), is a member of MAPK family, which is a crucial protein kinase in signal transduction, with serine/threonine kinase activity, and is indispensable for multiple biological processes [34–36]. For instance, the up-regulation of MAPK1 expression contributes to the augmentation of survivin expression, thereupon enhancing the resistance of multiple myeloma cells to vincristine [37]. Another study reports that inhibiting MAPK1 expression can attenuate YAP protein expression and then modulate Hippo pathway, thus constraining the migration and invasion of non-small cell lung cancer cells [38]. Importantly, MAPK1 is a

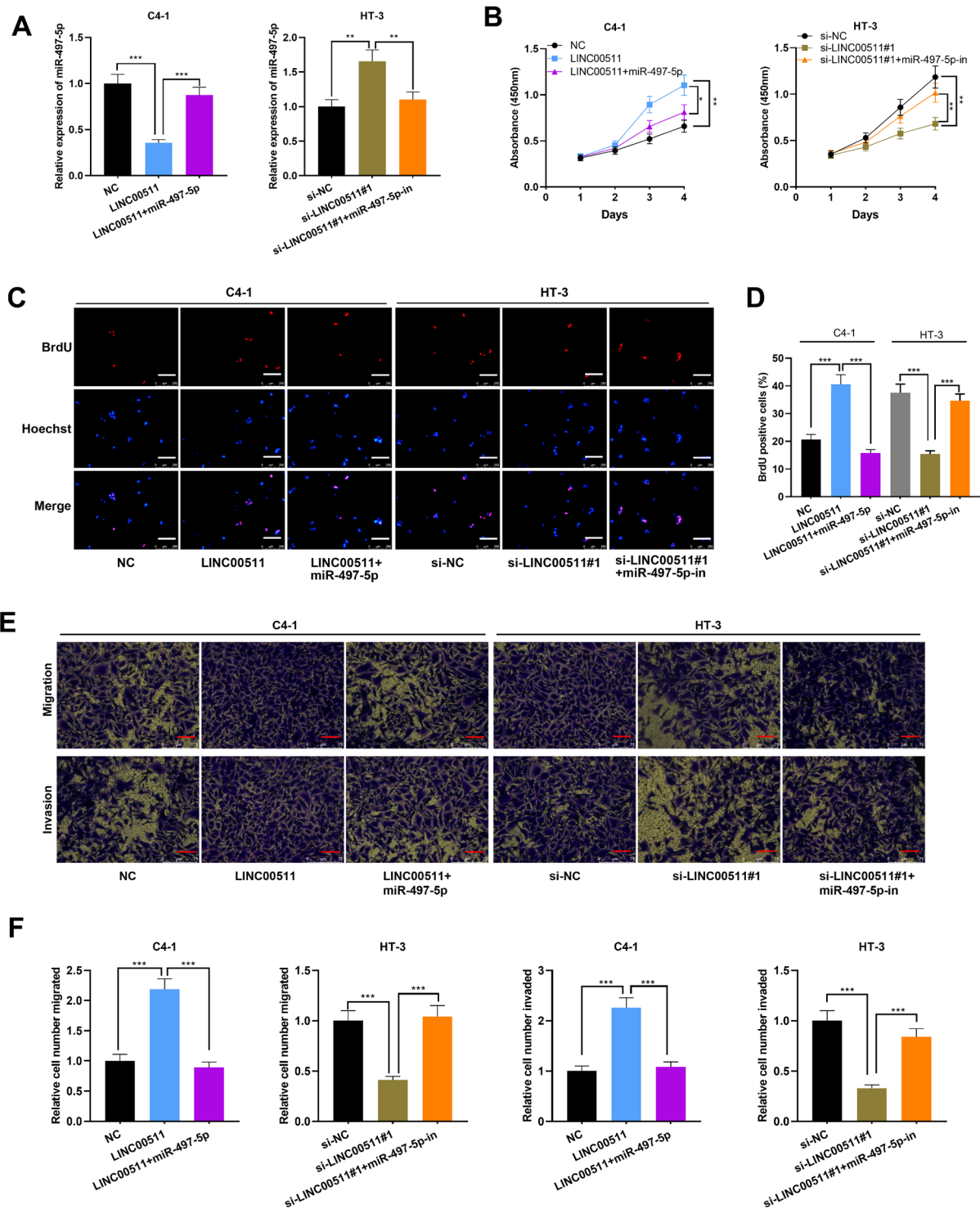


Fig. 5 MiR-497-5p counteracted LINC00511's effects on the proliferation, migration and invasion of CC cells. MiR-497-5p mimics and LINC00511 overexpression plasmids were co-transfected into C4-1 cells, and miR-497-5p inhibitors and LINC00511 siRNA (si-LINC00511#1) were co-transfected into HT-3 cells. The relative expression of miR-497-5p in C4-1 and HT-3 cells after transfection was detected by qRT-PCR. B-D. Cell proliferation was assessed utilizing CCK-8 assay (B) and BrdU assay (C-D). Scale bar, 250 μ m. E-F. The migration and invasion of CC cells were monitored employing Transwell assay. Scale bar, 75 μ m. All experiments were performed in triplicate. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

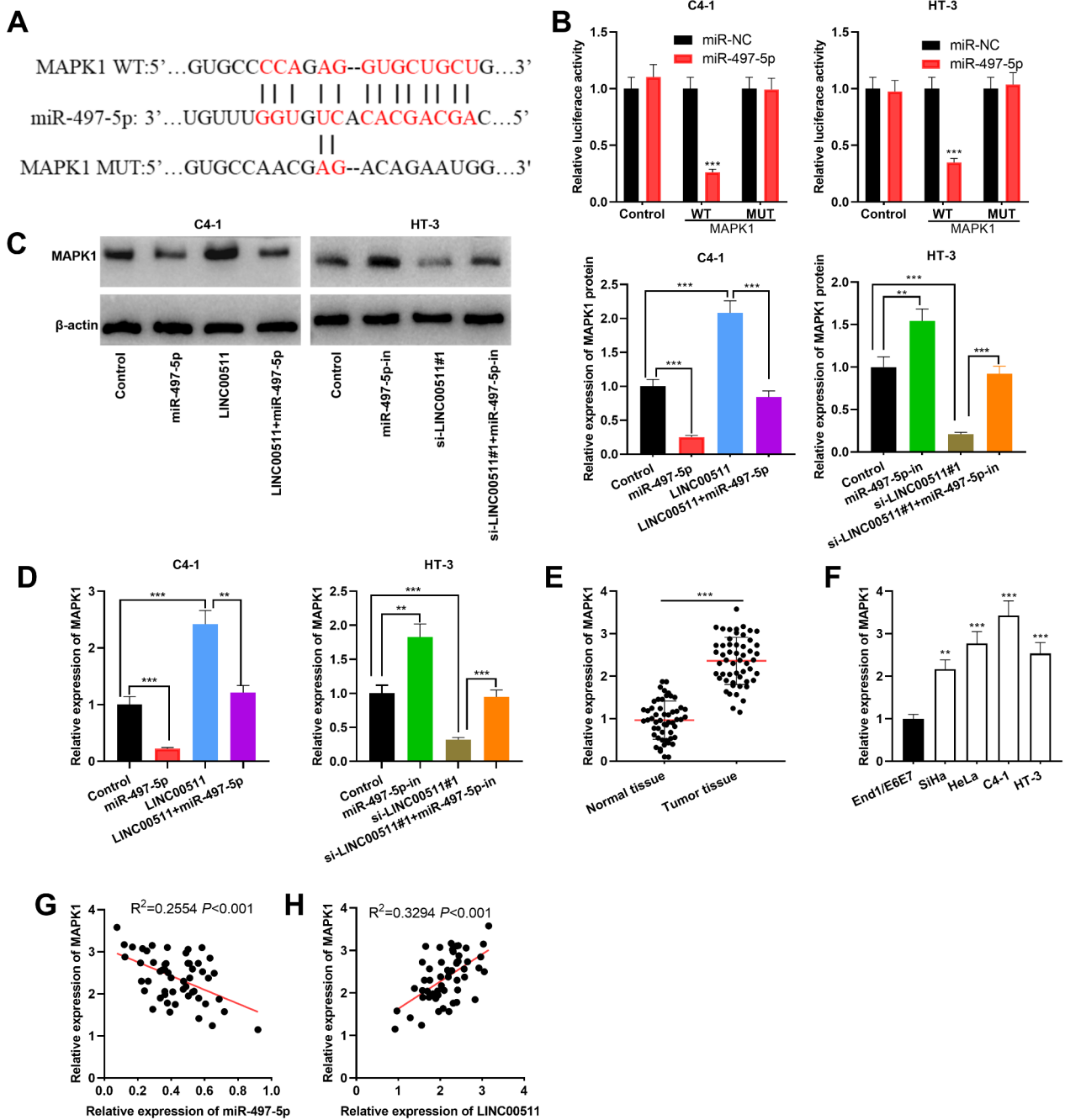


Fig. 6 MAPK1 was the target of miR-497-5p in CC cells. **A**. Bioinformatics was utilized for the prediction of the binding site between miR-497-5p and 3'UTR of MAPK1. **B**. Dual-luciferase reporter gene experiment was implemented for verifying the targeted relationship between miR-497-5p and 3'UTR of MAPK1. **C-D**. Western blot (**C**) and qRT-PCR (**D**) were employed for quantifying MAPK1 expression in CC cells after transfection. **E**. MAPK1 mRNA expression in CC tissues and adjacent tissues of 53 patients was detected employing qRT-PCR. **F**. MAPK1 mRNA expression in CC cells and End1/E6E7 cells was detected utilizing qRT-PCR. **G-H**. The correlations between MAPK1 mRNA and miR-497-5p (**G**), and MAPK1 mRNA and LINC00511 (**H**) in CC tissues. All experiments were performed in triplicate. ** $P < 0.01$ and *** $P < 0.001$

cancer-promoting factor in CC. MAPK1 expression in CC tissues is significantly higher than that in adjacent tissues, and MAPK1 knockdown results in the down-regulation of

MMP-2, MMP-9, Snail, TIMP-1 and TIMP-2 expressions, which remarkably suppresses the viability and invasion of HeLa cells [39]. In the current research, MAPK1 was

confirmed to be the downstream target of miR-497-5p in CC cells, and LINC00511 could up-regulate MAPK1 expression through sponging miR-497-5p. Our data further clarified the regulatory mechanism of MAPK1 in CC.

In summary, our research reports that LINC00511 expression is up-regulated in CC and associated with adverse pathological characteristics of the patients, and LINC00511 promotes the malignant phenotypes of CC cells. In terms of mechanism, LINC00511 exhibits a cancer-promoting effect via the regulation of miR-497-5p/MAPK1 axis. Our work provides a novel ceRNA network involved in CC progression, and LINC00511 may be a therapeutic target for this disease. Notably, we observed that miR-497-5p restoration only partly counteracted the biological effects of LINC00511 on CC cells, and this suggested that LINC00511 could probably modulate CC progression via other mechanisms. The other downstream targets / mechanisms of LINC00511 in CC remain to be deciphered in the following studies.

Authors' contributions MML, TTZ, JR and YFW designed the study, carried out and/or supervised the experiments and drafted the manuscript. MML, QG and TTZ performed the revision. All authors read and approved the final manuscript.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate This study was approved by the Ethics Review Board of the Guizhou Medical University, in accordance with all principles of the Declaration of Helsinki, and the written informed consent was obtained from each participant.

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

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