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Increased long noncoding RNA LINC00511 is correlated with poor prognosis and contributes to cell proliferation and metastasis by modulating miR-424 in hepatocellular carcinoma

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Abstract. – OBJECTIVE: Long noncoding RNAs (lncRNAs) have been reported to be abnormally expressed in human hepatocellular carcinoma (HCC) and associated with the progression of HCC. LncRNA LINC00511 (LINC00511) has been confirmed to act as a tumor promoter in several tumors. However, as a novel lncRNA, the specific effect of LINC00511 is still largely obscure. In this study, we aimed to evaluate the effect of LINC00511 in HCC behaviors and to elucidate the mechanism by which this occurs.

PATIENTS AND METHODS: The expressions of LINC00511 in HCC tissues and cell lines were evaluated by qRT-PCR. The correlations between LINC00511 expression and the clinicopathological parameters and prognosis of HCC patients were determined using several statistical methods. CCK-8 assay, colony formation assay, flow cytometry cell cycle, apoptosis assay, EdU assay, wound healing assay, and transwell assay were used to investigate the role of LINC00511 on the malignant phenotypes *in vitro*. Insights into the potential mechanisms of ceRNAs were determined by bioinformatics analysis, dual-luciferase reporter assays and RT-PCR.

RESULTS: LINC00511 expression was significantly up-regulated in HCC tissues and cell lines, and its high expression was distinctly associated with nodal metastasis, vascular invasion, and clinical stage. Furthermore, statistical assays revealed that HCC patients with higher LINC00511 expression levels had worse overall survival rates. Importantly, the multivariate analysis confirmed that LINC00511 expression was an independent prognostic factor of the overall survival in patients with HCC. Functionally, the inhibition of LINC00511 significantly suppressed the capability of proliferation, migration, and invasion in HCC cell lines. Bioinformatic tools predicted that miR-424 both targeted the 3'-UTR of LINC00511, which was confirmed using the luciferase reporter assay and RT-PCR.

CONCLUSIONS: LINC00511 plays an important role in the malignant progression of HCC via modulation of miR-424.

Key Words:
Long non-coding RNA, LINC00511, MiR-424, Prognosis, Metastasis, Hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC), which occurs in the setting of chronic liver inflammation, is currently the fifth most commonly diagnosed cancer and ranks as the third most devastating malignancy, accounting for more than 9% of total cancer deaths[1,2]. Up to date, the incidence is still rising not only in China but also in the world. Since the majority of cases of HCC are not sensitive to radiotherapy or chemotherapy, functional surgery may be the only effective therapeutic tool[3,4]. Although significant progress has been made in the diagnosis and treatment of HCC, the long-term post-treatment prognosis remains poor due to the high rate of recurrence and metastasis[5,6]. Despite the important effects of genetic abnormalities and environmental factors in HCC have been explored and analyzed extensively, the detailed molecular mechanisms involved in the progression and metastasis of HCC remain largely unclear.

Long non-coding RNAs (lncRNAs) are RNA molecules that are longer than 200 nt in length and lack an open reading frame[7]. Although lncRNAs are lack protein-coding potential, new evidence suggests lncRNAs as the core of epigenetic regulation. Growing data suggest that lncRNAs participate in multiple biological processes, including imprin-

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ting, embryonic development, RNA decay alternative splicing, and cell cycle contr[ol8-10]. Recently, the important regulatory effects of lncRNAs attract increasing attention and it is reported that lncRNAs are capable of performing as oncogenes or tumor suppressors through the modulation of tumor-related genes or cell signal pathway[11-13]. MicroRNAs (miRNAs) are non-coding RNAs that are 18-23 nucleotides in length and it is also confirmed that they act as tumor promoters or suppressors by targeting the tumor-associated genes[14,15]. Notably, lncRNA is found to interact with miRNAs, eventually leading to the derepression of miRNA targets at the post-transcriptional level16,17. For instance, lncRNA FTX suppressed HCC proliferation and metastasis by targeting miR-374a18. LncRNA PVT1 acted as a tumor promoter in the HCC progression through regulating miR-21419.

Long intergenic non-coding RNA 00511 (LINC00511), located at 17q24.3, is a new tumor-related lncRNA, identified first by Oh et al[20]. Recently, the overexpression of LINC00511 was found in various tumors, such as pancreatic ductal adenocarcinoma[21], tongue squamous cell carcinoma[22], and breast cancer[23]. Also, previous studies confirmed the tumor-promotive roles of LINC00511 in various tumors, suggesting that LINC00511 may be a candidate for the treatment of the tumor. However, the expression and function of LINC00511 in HCC have not been reported.

For the first time in this study, we provided evidence analyzing clinical data and performing in vitro experiments that LINC00511 was frequently up-regulated in HCC and may act as a candidate indicator for prognosis and diagnosis of HCC patients, as well as a potential therapeutic target.

Patients and Methods

Patients and Tissue Samples

HCC specimens and matched normal tissue samples from 127 patients undergoing surgery at the Affiliated Hospital of Qingdao University from May 2008 to October 2011. All patients had a histological diagnosis of primary HCC according to the clinicopathological criteria of the International Union for Cancer Control. None of the patients had received any anti-tumor therapy before surgery. All tissues were stored in liquid nitrogen until use. Written informed consents were received from all patients, and the process was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University. The clinical information of patients was shown in Table I.

Table I. Association between LINC00511 expression and different clinicopathological features of 127 human HCC.

		LINC00511 expression		
Variable	Number	Low	High	*p*-value
Age (years)				0.250
< 50	67	30	37	
≥ 50	60	33	27	
Sex				0.232
Male	76	41	35	
Female	51	22	29	
Tumor number				0.654
Single	61	29	32	
Multiple	66	34	32	
Tumor size (cm)				0.167
< 5 cm	77	42	35	
≥ 5 cm	50	21	29	
Histologic grade				0.236
High and moderate	74	40	34	
Poor	53	23	30	
Nodal metastasis				0.046
Present	43	16	27	
Absent	84	47	37	
Vascular invasion				0.010
Present	42	14	28	
Absent	85	49	36	
Clinical stage				0.026
I-II	79	45	34	
III-IV	49	18	31	

Cell Lines and Cell Culture

LO2 (normal liver cells) and liver cancer cell lines (Hep3B, HepG2, SMMC-7721, MHCC97H, Huh7, and HCCLM3) were purchased from Suer Biological Technology Co., Ltd. (Jiading, Shanghai, China). Cells were all cultured using Roswell Park Memorial Institute-1640 (RPMI-1640) medium (KeyGen, Nanjing, Jiangsu, China) at 37°C in an incubator with 5% of CO_2. In addition, the culture medium was added with 10% of fetal bovine serum (FBS, Life Technologies, Carlsbad, CA, USA) and penicillin-streptomycin antibiotics (1%).

Cell Transfection

The small interfering RNA (siRNA) targeting LINC00511 (siRNA#1 and siRNA#2), control siRNAs (si-control), miR-424 mimics, and control mimics were all purchased from Shanghai Sangong Biotechnology Co., Ltd. (Songjiang, Shanghai, China). The LINC00511-overexpressing plasmid, pcDNA3.1-LINC00511, was constructed by BioRun Biotechnology Co., Ltd. (Wuhan, Hubei, China). Cell transfection was carried out using an Entranster-H4000 or R4000 transfection reagent (Engreen Biosystem, Tongzhou, Beijing, China). In brief, the cells were plated in 6-well plates and cultured at 70% confluence. Then, indicated siRNAs, miRNA mimics or plasmids were mixed with the transfection reagent for about 20 min. Subsequently, the mixture was added into the cells and the cells were cultured for 5 h. After changing the fresh medium, the cells were used to conduct other experiments.

RNA Purification and Real-Time Quantitative RT-PCR (qRT-PCR)

We used TRIzol reagent (DarFun, Changsha, Hunan, China) to extract total RNA from HCC tissues and cells. For the LINC00511 detection, total RNA (2 μg) was first reversely transcribed into cDNA using Invitrogen M-MLV Reverse Transcriptase mix kit (MaiBio, Xuhui, Shanghai, China). Then, the qRT-PCR analysis was carried out by a Biomiga SYBR Green qPCR mix kit (Biodee, Haidian, Beijing, China). The reaction was performed on a CFX96 Touch qPCR detection system (Bio-Rad, Pudong, Shanghai, China). For miR-424 measurement, an HG SYBR Green miRNA detection kit (HaiGene, Haerbin, Heilongjiang, China) was utilized according to the protocols provided in the kit. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control for lncRNA or miRNA, respectively. The relative expression was analyzed using the $2^{-\Delta\Delta}Ct$ method. Primers used in this study were synthesized by Sangong Biotechnology (Songjiang, Shanghai, China) and the sequences were listed in Table II.

Cell Counting Kit-8 (CCK-8) Assay

We used a CCK-8 assay kit (ACMEC, Fengxian, Shanghai, China) to assess the cell viability of HCC cells after LINC00511 was depressed. In short, after planting the treated cells in 96-well plates (3000 cells per well), the CCK-8 solution (10 μl per well) was added into the cells, and the plates were incubated at 37°C with 5% of CO_2 for 2 h. Then, the cellular viability was measured by detecting the absorbance of 450 nm wavelength.

Colony Formation Assay

The HepG2 and Huh7 cells transfected with LINC00511 siRNAs or si-control were seeded into 6-well plates (500-600 cells per well). After culturing for about 15 days at 37°C with 5% of CO2, the cell colonies were visible. Phosphate buffer saline (PBS) was utilized to wash the cell colonies and the crystal violet solution (0.1%) was applied to stain the colonies. Finally, the cell colonies were observed and photographed using a DMI6000B microscope (Leica, Heppenheim, Germany).

EdU Assay

The cell proliferation was also evaluated by EdU (5-Ethynyl-2'-deoxyuridine) immunofluo-

Table II. Primers sequence for qRT-PCR assay in this study.

Genes	Sequences (5'-3')
LINC00511: forward	CTAACAAGAGGGTAAGTGTCAG
LINC00511: reverse	AAGTCGACAACCCCATCGTTAC
miR-424: forward	CAGCAGCAATTCATGTGTTTTGAA
miR-424: reverse	ACCTGGCCGTCAGGCAGCTC
GAPDH: forward	CATCACCATCTTCCAGGAGCG
GAPDH: reverse	TGACCTTGCCCACAGCCTT

rescence staining using an EdU assay kit (KeyGen, Nanjing, Jiangsu, China). In brief, cells were transfected with indicated siRNAs and re-plated into 48-well plates. Then, 100 μl EdU reagent (50 μM) was added into each well and incubated for 2 h. Subsequently, Apollo Buffer and DAPI were added into the cells. Finally, a DMI6000B microscope (Leica, Heppenheim, Germany) was applied to take pictures of the stained cells.

Flow Cytometry Analysis

Cell cycle and apoptosis of HepG2 and Huh7 cells, after the indicated siRNAs transfection, were evaluated by flow cytometry. In short, the cells were transfected with LINC00511 siRNAs as described above. Then, the cells were collected, and resuspended in 1 × binding buffer (500 μl) supplemented with propidium iodide (5 μl) alone (for cell cycle determination), or both Annexin V-FITC reagent (5 μl; YaoYun, Minhang, Shanghai, China) and 5 μl of propidium iodide (for cell apoptosis detection). After incubation for 20 min away from light, the cells were washed using ice-cold PBS and subsequently analyzed by a MACS QuanR Analyzer flow cytometer (Miltenyi, Changning, Shanghai, China).

WoundHealing Assay

HepG2 and Huh7 cells were transfected with indicated siRNAs and then collected. After that, the cells (1×106 cells per well) were added into 12-well plates and cultured at about 100% confluence. Then, the cells were scratched with a pipette tip (200 μl). After washing with PBS three times, the wound closure was photographed by a DMI6000B microscope (Leica, Heppenheim, Germany) at 0 and 48 h.

Transwell Assay

Transwell assays were performed to evaluate the cell invasion capacity. In brief, 1×105 cells after treatment were suspended in a medium (without serum) and then the cell suspension was added into the upper chambers of a transwell insert (8 μm pore size; pre-coated with Matrigel). Then, the medium (with 15% of fetal bovine serum) was added into the lower chambers. After 24 h, the cells on the bottom of the insert chambers were stained using crystal violet solution (0.1%) and observed by a DMI6000B microscope (Leica, Heppenheim, Germany). The Corning transwell inserts were purchased from Biodee Biotechnology Co., Ltd. (Haidian, Beijing, China).

RNA Immunoprecipitation (RIP) Assay

A Millipore Magna-RIP assay kit (Nobel Ryder, Haidian, Beijing, China) was utilized to conduct the RIP assays. In brief, the cells were separately transfected with LINC00511 overexpressing plasmids: pcDNA3.1-LINC00511 or miR-424 mimics as described above. Then, the cells were collected and lysed using the lysis buffer provided in the assay kit, and subsequently the cell lysates were incubated with the RIP buffer containing the Ago2 antibody (Abcam, Pudong, Shanghai, China) or negative control antibody (provided by the assay kit) conjugated with magnetic beads. The RNA was isolated according to the protocols and the relative fold changes of LINC00511 and miR-424 were measured using qPCR as the above methods described.

Dual-Luciferase Reporter Assay

The LINC00511 wild-type reporter plasmids (LINC00511 wt) and LINC00511 mutant reporter plasmids (LINC00511 mut) was constructed by BioRun Biotechnology Co., Ltd. (Wuhan, Hubei, China). In brief, the reporter plasmids and miRNA mimics (LINC00511 wt plasmid and miR-424 mimics; LINC00511 wt plasmid and control mimics; LINC00511 mut plasmid and miR-424 mimics; LINC00511 mut plasmid and control mimics) were co-transfected into HepG2 and Huh7 cells as described above. After culturing for 48 h, the luciferase activity was measured by a Dual-Luciferase Assay kit (Promega, Dongcheng, Beijing, China).

Statistical Analysis

All statistical analyses in this study were conducted by the use of SPSS version 19.0 software (SPSS Inc., Chicago, IL, USA). Statistical significance between groups was made by unpaired Student's t-test or one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. For overall survival analysis, we applied the log-rank test and the Kaplan-Meier method. Univariate and multivariate analysis were used to evaluate the influence of each clinical covariate on patient survival. A p-value of less than 0.05 was considered statistically significant.

Results

LINC00511 Expression in HCC Tissues and Cell Lines

To determine the role of LINC00511 in HCC, the expression levels of LINC00511 were deter-

mined in 127 pairs of human HCC samples and adjacent normal tissues using RTqPCR. As shown in Figure 1A, we found that LINC00511 expression in HCC tissues was significantly higher than that in non-tumor tissues ($p < 0.01$). Besides, we also found that tumor tissues from a patient with advanced clinical stages showed a higher expression (Figure 1B). Moreover, the expression pattern of LINC00511 in normal liver cells (LO2) and in several human HCC cell lines (Hep3B, HepG2, SMMC-7721, MHCC97H, Huh7, and HCCLM3) was detected with qRT-PCR. As shown in Figure 1C, the results demonstrated that LINC00511 expression was significantly increased in all the HCC cell lines compared with LO2. These results imply that LINC00511 might be involved in the progression of HCC.

Association Between LINC00511 Expression and Prognosis in HCC Patients

According to the median expression level of LINC00511, we categorized the patients into low and high expression groups to better study the associations between LINC00511 expression and clinicopathological factors in HCC patients. As shown in Table I, LINC00511 overexpression in HCC patients had a significant association with nodal metastasis ($p = 0.046$), vascular invasion ($p = 0.010$), and clinical stage ($p = 0.026$). However, no significant difference was observed between LINC00511 expression and other clinical features such as patients' age, sex, and tumor number (all $p > 0.05$). To further evaluate the prognostic value of LINC00511 expression in HCC, survival curves were constructed by the Kaplan-Meier method and compared by the log-rank test. As shown in Figure 1D, we observed that patients with higher LINC00511 expression levels displayed lower overall survival rates than patients with low LINC00511expression levels ($p = 0.0339$). Univariate and multivariate analyses of factors related to the prognosis of HCC patients were shown in Table III. In univariate analyses, nodal metastasis, vascular invasion, clinical stage, and LINC00511 expression were confirmed to be associated with the overall survival of HCC patients. Further, in multivariate analyses, the results confirmed that LINC00511 (RR = 3.016, 95% CI: 1.216-3.889, p = 0.008) was independently associated with the overall survival, suggesting that this lncRNA may serve as a novel prognostic indicator for HCC.

Knockdown of LINC00511 Inhibited HCC Cell Growth and Promoted Cell Apoptosis

To investigate the functional roles of LINC00511 in HCC, loss-of-function studies using siRNAs against LINC00511 (siRNA#1 and siRNA#2) were conducted in HepG2 and Huh7 cells. The results of the qRT-PCR analysis revealed that LINC00511 was markedly decreased in HepG2 and Huh7 cells following transfection with LINC00511 siRNAs (Figure 2A). As examined by CCK-8 assays, the depletion of LINC00511 resulted in a remarkable decline in the cellular growth of HepG2 and Huh7 cells at 48, 72, and 96 h (Figure 2B). Subsequently, we carried out cell colony formation assays to investigate the impact of LINC00511 on colony formation capacity of HCC cells. The results suggested that LINC00511 knockdown caused lower colony formation rates in HepG2 and Huh7 cells (Figure 2C). Besides, we also performed EdU assays to evaluate the proliferation of LINC00511-silenced HepG2 and Huh7 cells. The data demonstrated that down-regulation of LINC00511 notably suppressed the cellular proliferation, which was consistent with the data of CCK-8 and colony formation assays (Figure 2D). Additionally, we conducted flow

Table III. Prognostic factors in Cox proportional hazards model.

	Univariate analysis			Multivariate analysis		
Variable	RR	95% CI	p	RR	95% CI	p
Age	1.452	0.662-2.244	0.167	-	-	-
Sex	1.557	0.472-1.775	0.452	-	-	-
Tumor number	1.372	0.674-2.223	0.138	-	-	-
Tumor size	1.864	0.894-2.544	0.114	-	-	-
Histologic grade	2.123	1.132-2.667	0.089	-	-	-
Nodal metastasis	2.776	1.342-3.887	0.033	2.442	1.217-3.542	0.041
Vascular invasion	3.125	1.372-4.266	0.004	2.895	1.218-3.997	0.014
Clinical stage	3.342	1.457-4.663	0.001	2.894	1.238-4.172	0.005
LINC00511 expression	3.234	1.326-4.213	0.002	3.016	1.216-3.889	0.008

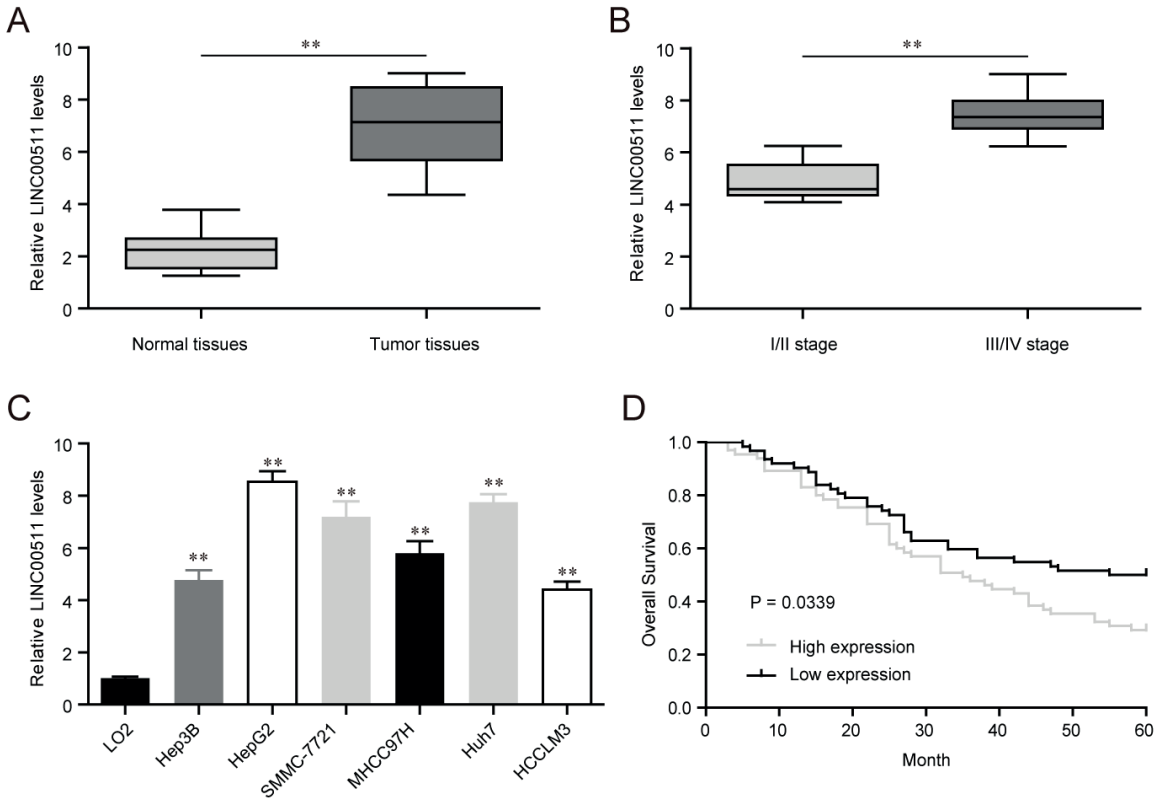

Figure 1. Expression levels of LINC00511 in HCC and its clinical significance. ***A,*** 127 paired of HCC and matched normal tissues were subjected to qRT-PCR for LINC00511 expression. Quantitative data revealed that LINC00511 expression was increased in HCC tissue compared with tumor-adjacent tissues. ***B,*** LINC00511 expression was significantly higher in HCC patients with advanced stages. ***C,*** Relative expression of LINC00511 in six HCC cell lines (Hep3B, HepG2, SMMC-7721, MHCC97H, Huh7, and HCCLM3) and normal liver cells (LO2) was determined by qRT-PCR. ***D,*** Patients with high LINC00511 expression had a significantly shorter 5-year survival overall rate than those with low LINC00511 expression. $^{*}p < 0.05$, $^{**}p < 0.01$.

cytometry to further elucidate whether LINC00511 depletion inhibited HCC cell proliferation by modulating the cell cycle. The data revealed that the knockdown of LINC00511 resulted in an evident change in cell cycle distribution of HepG2 and Huh7 cells: the percentage of cells in G1 phase increased and cells in S phase decreased, which indicated that LINC00511 deficiency induced the G0/G1 phase arrest (Figure 2E). Moreover, flow cytometry was also performed to assess the apoptosis of LINC00511-depleted HepG2 and Huh7 cells, and the data demonstrated that suppressing the expression of LINC00511 promoted cell apoptosis (Figure 2F). Taken together, our data suggested that LINC00511 acted as oncogenic roles in modulating the cellular growth of HCC.

Depletion of LINC00511 Impaired the Migration and Invasion of HCC Cells

After confirming that LINC00511 was implicated in modulating HCC cellular growth, we sought to explore whether a reduction of LINC00511 expression was capable to impact the metastasis of HCC. Hence, we separately conducted Matrigel-coated transwell assays and wound healing assays using HepG2 and Huh7 cells when they were transfected with LINC00511 siRNAs or si-control. According to the results of transwell assays, after transfection with LINC00511 siRNAs, the number of invasive HepG2 or Huh7 cells was significantly decreased, which indicated that the silence of LINC00511 was able to impede the invasion ability of HCC cells (Figure 3A). Furthermore, we carried out wound healing assays to evaluate the alteration of migration in HepG2 and Huh7 cells after transfection with LINC00511 siRNAs or si-control. As the data presented in Figure 3B and C, HepG2 and Huh7 cells with LINC00511 deficiency showed notably slower wound closure than the controls, which implied that down-regulation of LINC00511 could repress the migratory capacity of HCC cells. Therefore, these data suggested that LINC00511

played crucial roles in regulating the metastatic potentials of HCC.

MiR-424 Directly Targeted LINC00511 in HCC Cells

To uncover the underlying molecular mechanism by which LINC00511 acted as a promoter of HCC tumorigenesis, we next conducted mechanical studies. Since accumulating evidence had indicated that lncRNAs might exert their functions mainly via working as "sponge" of miRNAs, we applied a bioinformatic tool: starBase, to predict the potential target miRNAs of LINC00511. We found that miR-424, which was certified to be a tumor suppressor in various cancer types, might be a potential target of LINC00511 (Figure 4A). Indeed, as a determination by qRT-PCR assays, we found that the expression of miR-424 was down-regulated in HCC tissue samples (Figure 4B). Therefore, we next aimed to certify whether

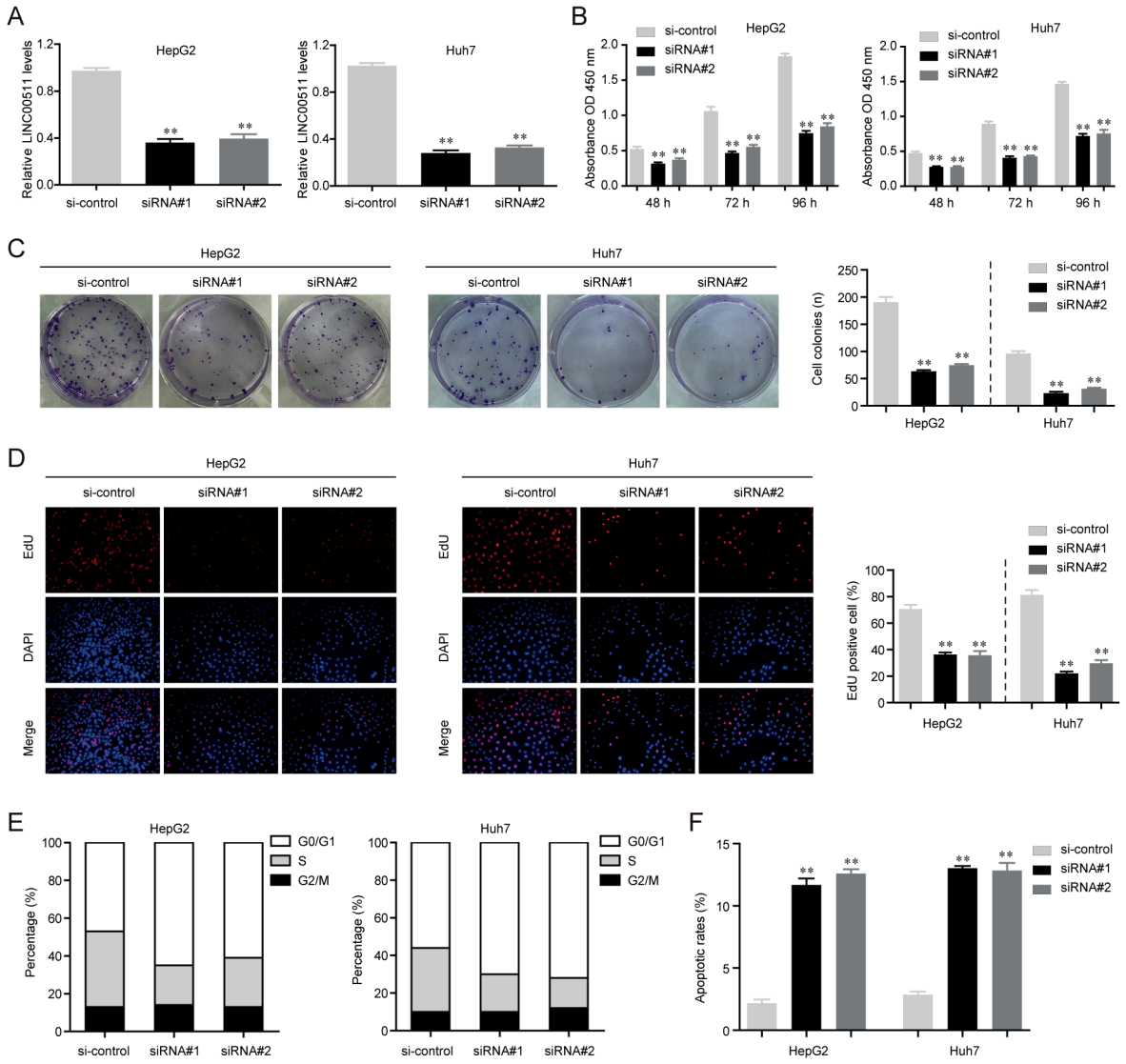

Figure 2. Effects of LINC00511 on the cellular growth and apoptosis of HepG2 and Huh7 cells. ***A,*** QRT-PCR analysis revealed the LINC00511 expression in HepG2 and Huh7 cells after transfection with LINC00511 siRNAs (siRNA#1, siRNA#2) and control siRNAs (si-control). ***B,*** CCK-8 assays evaluated the proliferative abilities in HepG2 and Huh7 cells of LINC00511 silencing or controls. ***C,*** Clone formation assay illustrated the clone number in HepG2 and Huh7 cells after their LINC00511 was knocked down (Magnification: 10×). ***D,*** Cell proliferation was evaluated using EdU immunofluorescence staining in HepG2 and Huh7 cells (Magnification: 100×). E, Flow cytometry detected the cell cycle. ***F,*** Cell apoptosis was determined by flow cytometry. $^{*}p < 0.05$, $^{**}p < 0.01$.

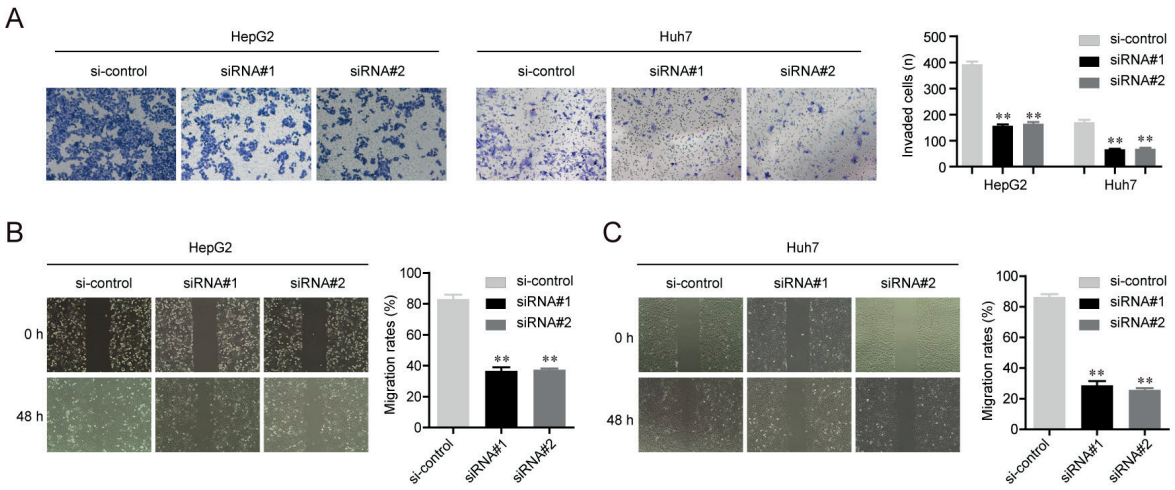

Figure 3. LINC00511 promoted the migration and invasion of HepG2 and Huh7 cells. ***A,*** Transwell assays showed markedly decreased invasion capabilities in both HepG2 and Huh7 cells following LINC00511 knockdown (Magnification: 40×). ***B-C,*** Wound healing assays showed significantly decreased migratory abilities in both HepG2 and Huh7 cells when LINC00511 was down-regulated (Magnification: 10×). $^{*}p < 0.05$, $^{**}p < 0.01$.

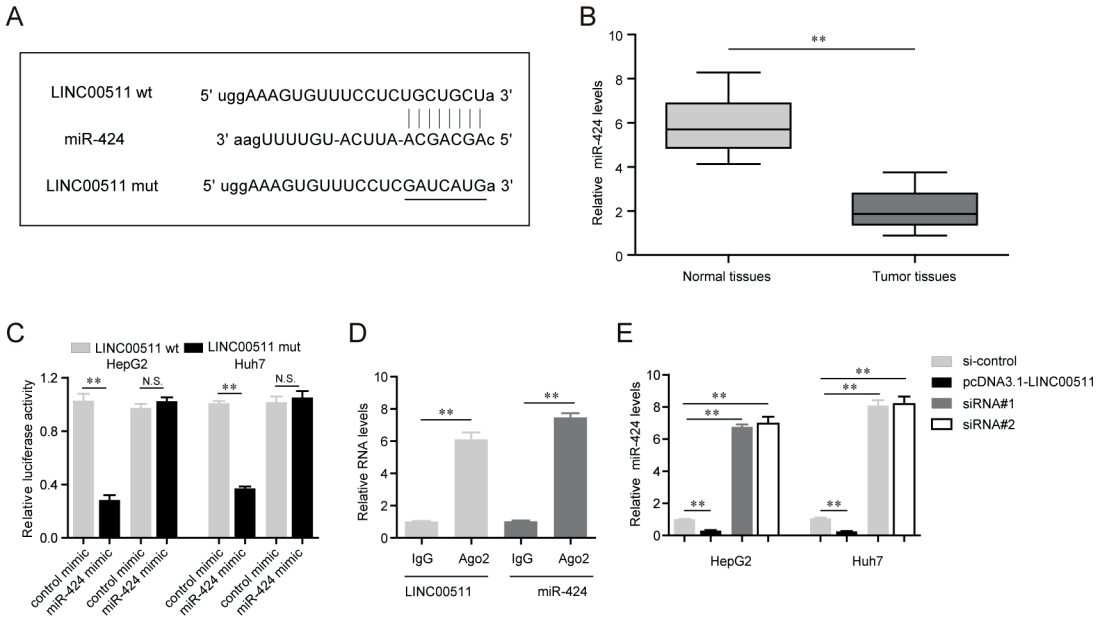

Figure 4. LINC00511 sponged miR-424 in HCC cells. ***A,*** Sketch for the predicted binding sites for LINC00511 and miR-424 using "starbase" (http://starbase.sysu.edu.cn/). ***B,*** The qRT-PCR analysis showed that miR-424 expression was down-regulated in hepatocellular carcinoma tissues. ***C,*** Dual luciferase reporter assay revealed that miR-424 overexpression inhibited the activities of LINC00511 wt reporter plasmid-transfected HepG2 and Huh7 cells. ***D,*** RIP assays evaluated the enrichment of LINC00511 and miR-424. ***E,*** QRT-PCR assays determined the relative expression of miR-424 in HepG2 and Huh7 cells when their LINC00511 was overexpression or knocked down. $^{*}p < 0.05$, $^{**}p < 0.01$.

LINC00511 directly interacted with LINC00511 in HCC cells. To achieve this purpose, we performed dual luciferase activity assays, and found that co-transfection with LINC00511 wt plasmids and miR-424 mimics significantly reduced the luciferase activity of HepG2 and Huh7 cells; whereas there was no change of luciferase activity in cells co-transfected with LINC00511 mut plasmids and miR-424 mimics, which indicated that miR-424 was directly binding with LINC00511 in HCC cells (Figure 4C). In addition, following RIP assays validated that LINC00511 and miR-

424 were both enriched in the immunoprecipitation (Figure 4D). Besides, the qRT-PCR analysis was performed to measure the expressing levels of miR-424 in HCC cells after various treatment. The results demonstrated that enhancing the expression of LINC00511 led to significant inhibition of miR-424 expressing levels, while the silence of LINC00511 remarkably promoted the miR-424 levels, which suggested that there was an inverse correlation between the expression of LINC00511 and miR-424 (Figure 4E). These data validated that LINC00511 acted as a miR-424 sponge in HCC.

Discussion

HCC represents one of the most common malignancies of the digestion system. More than a quarter of a million men will die of HCC every year all over the world24. Although the prognosis of HCC patients diagnosed at an early stage has been improved with the advancement of several treatment methods, confusion still lies in the problem of dealing with metastasis[25,26]. Recently, some functional lncRNAs, especially metastasis-associated lncRNAs, have provided a new understanding of cancer pathogenesis[27,28]. On the other hand, the great potential of lncRNAs as novel cancer biomarkers for screening, diagnosis, prognosis, and therapy response prediction has become a research hotspot[29-31]. Several important lncRNAs have been well studied in almost all types of tumors, such as lncRNA-ATB[32], lncRNA MALAT1[33], and lincRNA-ROR[34].

LINC00511 served as a tumor promoter in tumors and its function had been studied previously. For instance, a recent work from Yan et al[35] showed that LINC00511 was overexpressed in osteosarcoma cells and its ectopic expression promoted tumor cells growth, colony formation, and migration via sponging miR-765. Li et al[36] reported that LINC00511 was highly expressed in bladder cancer tissues and cells, and its suppression distinctly inhibited the proliferation and induced apoptosis of tumor cells via the modulation of Wnt/β-catenin signaling pathway. Further findings by Zhao et al[21] suggested that LINC00511 was significantly up-regulated in human pancreatic ductal adenocarcinoma tissues, which correlated with adverse clinical pathological characteristics and unfavorable prognosis. There results obtained online have provided evidence that LINC00511 acted as a tumor promoter in many tumors. However, the expression pattern and clinical significance of LINC00511 in HCC remains unknown. In this study, we first reported that LINC00511 expression was also up-regulated in both HCC tissues and cell lines. In addition, we also observed that a higher expression of LINC00511 was positively associated with nodal metastasis, vascular invasion, clinical stage, and shorter overall survival. Of note, the univariate and multivariate analysis showed that LINC00511 was an independent predictor for the prediction of overall survival of HCC patients. On the other hand, we also explored the potential function of LINC00511 in HCC cells via using si-LINC00511 to decrease the expression of LINC00511 in HepG2 and Huh7 cell lines. *In vitro* experiments indicated that the knockdown of LINC00511 distinctly suppressed the proliferation, migration, and invasion. Also, we explored the effects of LINC00511 on cells cycle and apoptosis, finding that inhibition of LINC00511 deficiency induced G0/G1 phase arrest and promoted apoptosis. Thus, our results provided clear evidence that LINC00511 served as a tumor promoter in the HCC progression. Recently, a novel regulatory mechanism of RNA, endogenous competing RNAs (ceRNAs) has been proposed[37]. A growing number of reports have indicated that lncRNAs act as 'sponges' to bind specific miRNAs and modulate their expression levels. For instance, lncRNA NEAT1 was reported to promote proliferation and invasion of lung cancer by acting as a miR-181a-5p sponge[38]. LncRNA-RMRP was shown to be highly expressed in gastric cancer and promote the proliferation and metastasis by targeting miR-206[39]. Also, LINC00511 has been reported to target several miRNAs in osteosarcoma[35], breast cancer[23], and tongue squamous cell carcinoma[22]. The ceRNA mechanisms for LINC00511 deregulation in HCC have not been thoroughly elucidated. In this study, we searched for candidate miRNAs and found that miR-424 may be involved in the LINC00511 mediated cell growth and invasion. Further results of RT-PCR revealed that only miR-424 expression was significantly increased upon the LINC00511 knockdown. Moreover, the luciferase reporter assay showed that LINC00511 combined with miR-424 at molecular bound, suggesting the integration between LINC00511 and miR-424. Previously, miR-424 had been reported to be dysregulated in various

tumors. However, the expression trend was not identical. In tongue squamous cell carcinoma and non-small cell lung cancer, miR-424 was highly expressed and served as a tumor promoter[40,41]. However, in glioma, colorectal cancer, and HCC, miR-424 was found to be lowly expressed and functioned as an oncogenic miRNA[42-44]. Thus, our findings evidenced that LINC00511 displayed its tumor-promotive roles via targeting miR-424.

Conclusions

We found that LINC00511 may play a crucial role during the HCC progression, and LINC00511 may interact with miR-424 to link miRNAs and the post-transcriptional network. LINC00511 is a potential prognostic and therapeutic target for the treatment of HCC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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