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LINC00240 sponges miR-4465 to promote proliferation, migration, and invasion of hepatocellular carcinoma cells *via* HGF/c-MET signaling pathway

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Abstract. – OBJECTIVE: LINC00240, as a novel long non-coding RNAs (lncRNAs), has never been studied in hepatocellular carcinoma (HCC). This research reported the expression and function of LINC00240 in HCC.

PATIENTS AND METHODS: LINC00240 expression in 180 HCC patients was downloaded from the Cancer Genome Atlas (TCGA) database. HCC patients' survival was analyzed *via* Kaplan-Meier analysis. The expression of LINC00240, miR-4465 and HGF in Hep3B and Huh7 cells were regulated by transfection. Cell viability was determined by MTT assay. Transwell experiment was used for the detection of cells migration and invasion abilities. The interaction between LINC00240, miR-4465 and HGF was reflected by Luciferase reporter assay. LINC00240, miR-4465, HGF and p-c-MET expression in HCC cells were researched by real-time quantitative polymerase chain reaction (RT-qPCR) and Western blot.

RESULTS: TCGA data showed that high LINC00240 expression was markedly associated with lower survival of HCC patients (p = 0.036). LINC00240 expression was aberrantly up-regulated in HCC cells. Silencing of LINC00240 significantly reduced HCC cells viability, migration and invasion. miR-4465 was a target gene of LINC00240. Silencing of LINC00240 reduced HCC cells viability, migration and invasion *via* directly promoting miR-4465 expression. HGF was target gene of miR-4465. miR-4465 upregulation obviously suppressed HGF and *p*-c-MET expression. According to rescue experiment, LINC00240 silencing inhibited HCC cells viability, migration and invasion by suppressing HGF/c-MET signaling pathway *via* targeting miR-4465.

CONCLUSIONS: LINC00240 sponges miR-4465 to promote HCC cells proliferation, migration and invasion *via* HGF/c-MET signaling pathway.

Key Words:
HCC, LINC00240, MiR-4465, HGF/c-MET signaling, Progression.

Introduction

Hepatocellular carcinoma (HCC) is a ubiquitous tumor, which is the third leading cause of cancer-related death in the world[1]. The development of HCC is mainly related to the genetic and environmental factors, and more than 700,000 cases die annually from HCC[2]. Despite the improvement in diagnosis and treatment strategies, several important issues such as frequent recurrence and high incidence of metastasis still remain[3]. A better understanding of the underlying pathogenesis and development mechanisms of HCC is one of the important tasks in identifying effective targets for HCC treatment.

Long chain non-coding RNAs (lncRNAs) are well known for its length of more than 200 nucleotides and the lack of protein-coding capabilities[4]. They were involved in multiple biological processes, such as cell differentiation, proliferation, and apoptosis. The relevant mechanism was that lncRNAs participated in the regulation of gene expression through epigenetic alteration, transcriptional control and post-transcriptional modification[5,6]. Aberrant expression of lncRNAs is an important regulator of multiple human malignancies, including HCC[7,8]. LINC00240 has recently been revealed to be dysregulated in the esophageal squamous cell carcinoma[9]. It was also found to be prominently overexpressed in cervical cancer, which exacerbated the growth, migration and invasion of cervical cancer cells *via*

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microRNA-124-3p/STAT3/MICA axis[10]. More data on the function of LINC00240 in human tumors are not yet available. Through preliminary research of data from The Cancer Genome Atlas (TCGA) database, we noticed that high expression of LINC00240 was associated with low survival rate in HCC patients. Therefore, in this article, the function of LINC00240 in HCC was researched for the first time.

LncRNAs play a vital role in the tumorigenesis and progression with acting as sponges for miRNAs as one of the main ways, thereby hindering the availability of miRNAs to bind to target mRNAs[11]. MiR-4465 has been found to inhibit the proliferation and metastasis of non-small cell lung cancer[12]. However, the role of miR-4465 in HCC has not been reported. Furthermore, hepatocyte growth factor (HGF) and its receptor tyrosine kinase (c-MET) have been found to participate in the progression of tumors[13]. Thus, with LINC00240/miR-4465/HGF/c-MET signaling pathway as the axis, this study initially explored the possible underlying mechanism of LINC00240 regulating HCC development. The findings of this paper would provide new insights into the pathogenesis and target treatment of HCC.

Patients and Methods

The Cancer Genome Atlas (TCGA) Analysis

Data of LINC00240 expression in 180 HCC cases were downloaded from TCGA database. According to LINC00240 level, patients were divided into high expression (n = 90) group and low expression group (n = 90). The survival analysis of HCC patients in the two groups was explored *via* Kaplan-Meier survival analysis.

Cell Culture

Human normal liver cell line (THLE-3) and HCC cell lines (Hep3B, Huh7 and HCCLM3) (the Cell Bank of Chinese Academy of Sciences, Shanghai, China) were incubated at 37°C, 5% CO_2 by Dulbecco's Modified Eagle's Medium (DMEM; Corning Life Sciences, MA, USA) containing 10% fetal bovine serum (FBS, Gibco, Rockville, MD,USA). Cells were passaged every three days.

Transfection

Hep3B and Huh7 cells were harvested at 80% confluence. DMEM (without FBS) (Corning Life Sciences, MA, USA) was used to disperse cells into a single cell suspension (1×10^5 cells/mL). Cell suspension was added into 6-well plates with a volume of 1 mL. LINC00240 shRNA (shLINC00240 group) and scrambled negative control (NC) (shNC group), miR-4465 mimics (miR-4465 mimics group) and its negative control (miR-NC group), and miR-4465 inhibitor and its negative control (NC inhibitor) were respectively transfected into Hep3B and Huh7 cells. LINC00240 shRNA and miR-4465 inhibitor were used simultaneously to transfect Hep3B and Huh7 cells (shLINC00240 + miR-4465 inhibitor group). HGF overexpression vector and LINC00240 shRNA or miR-4465 mimics were simultaneously used to cotransfect Hep3B and Huh7 cells (shLINC00240+ HGF group, miR-4465 mimics+ HGF group). Transfection was performed using Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA). All RNA interference sequences and vectors were designed and synthesized by Gene Pharma (Shanghai, China). After 8 h of transfection, the residual liquid per well was replaced with fresh DMEM (with 10% FBS) (Corning Life Sciences, MA, USA). Cells were cultured at 37°C, 5% CO_2 for 24 h. The transfection efficiency was measured through real-time quantitative polymerase chain reaction (RT-qPCR).

MTT Assay

Hep3B and Huh7 cells at 80% confluence were collected. Cells were dispersed in DMEM (with 10% FBS) (Corning Life Sciences, MA, USA) to a density of 1×10^5 cells/mL. Totally 100 µL cell suspension was added into 96-well plates with five duplicate wells. At 37°C, 5% CO_2, cells in the plates were incubated for 24, 48 and 72 h respectively. MTT solution (5 mg/ml, 10 µL; Bioswamp, Wuhan, China) was then added into each well and cells were continuously cultured at 37°C for 4 h. Subsequently, the residual liquid in each well was replaced by 150 µL dimethyl sulfoxide (DMSO; Bio-Swamp, Wuhan, China). Plates were gently shaken on a shaker for 10 min. The optical density (OD) of each well was analyzed using an automatic microplate reader (Molecular Devices Corporation, Palo Alto, CA, USA) at 495 nm.

Transwell Experiment

Hep3B and Huh7 cells at 80% confluence were prepared as single cell suspension using DMEM (without FBS) (2×10^4 cells/mL) (Corning Life Sciences, Tewksbury, MA, USA). Matrigel (0.2 µg/µL, 100 µL; BD Biosciences, Bedford, MA, USA) was spread on the upper chamber of transwell in-

serts. The transwell inserts were placed in 24-well plates containing 600 μL DMEM (with 10% FBS) (Corning Life Sciences, Tewksbury, MA, USA). Cell suspension (500 μL) was then added onto the upper chamber of the transwell inserts. Cells were cultured for 24 h 37°C, 5% CO_2. A cotton swab was used to gently scrape off cells that failed to pass through the filter membrane. Cells that entered the lower surface of the filter membrane were fixed with 4% formaldehyde for 10 min and stained with 0.1% crystal violet for 15 min at room temperature. The number of invading cells that passed through the filter membrane was counted under a microscope in 5 random non-overlapping fields. Furthermore, the number of migrating cells was also obtained according to the method described above. The only difference was that the upper layer of the filter was not spread with Matrigel (BD Biosciences, Bedford, MA, USA).

Luciferase Reporter Assay

Using 293T cells, Luciferase reporter assay was carried out to investigate the relationship between LINC00240 and miR-4465 or between miR-4465 and HGF. Briefly, 293T cells were cultured with DMEM (with 10% FBS) (Corning Life Sciences, Tewksbury, MA, USA) at 37°C, 5% CO_2. At 80% confluence, 293T cells were collected and dispersed in DMEM (without 10% FBS) (Corning Life Sciences, Tewksbury, MA, USA). MiR-4465 mimics and NC duplex was transfected into 293T cells respectively using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). Wild type and mutant type fragments of LINC00240 and HGF were synthesized by GenePharma (Shanghai, China). The fragments contained the predicted miR-4465 targeting regions. Based on the manual, the fragments were inserted into the Luciferase reporters respectively. These luciferase reporters were then used to cotransfect 293T cells which were transfected by miR-4465 mimics and NC duplex previously. All 293T cells were cultured for 48 h at 37°C, 5% CO_2. The Luciferase activity of 293T cells was determined by the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Real Time-Quantitative Polymerase Chain Reaction (RT-qPCR)

LINC00240, miR-4465 and HGF expression in Hep3B and Huh7 cells were explored *via* RT-qPCR. Cells were harvested and washed three times with phosphate-buffered saline (PBS) (Gibco, Gaithersburg, MD, USA). TRIzoL reagent (Solarbio, Beijing, China) was added into cells to extract total RNA. cDNA was obtained by reverse transcription reaction with Primescript RT Reagent (TaKaRa, Otsu, Shiga, Japan). RT-qPCR was performed by ABI 7500 Fast Real-Time PCR System (Life Tech, Carlsbad, CA, USA) with the following conditions: 95°C for 40 s, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. With $2^{-\Delta\Delta Ct}$ method, relative LINC00240 and miR-4465 expression were calculated with U6 as control, and relative HGF mRNA expression was calculated with GAPDH as control.

Western Blot

Hep3B and Huh7 cells were washed with precooled PBS and then were added to cell lysate to extract total proteins. The total protein concentration was detected using bicinchoninic acid assay (BCA) protein assay kit (Pierce, Rockford, IL, USA). Proteins were added into 5 × loading buffer for 10 min incubation in a boiling water bath at 100°C. Equal amounts (50 μg) of each total protein sample were undergone sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). After being transferred to polyvinylidene difluoride (PVDF; Thermo Fisher Scientific, Waltham, MA, USA) membrane, proteins were blocked with 5% skimmed milk for 1 h. The PVDF membrane was probed with anti-HGF antibody (1:1000, Abcam, Cambridge, MA, USA), anti-pc-MET antibody (1:1000, Abcam, Cambridge, MA, USA) and anti-GAPDH antibody (1:3000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibody overnight at 4°C. Horseradish peroxidase (HRP)-conjugated secondary antibody (1:2000, Boster, Wuhan, China) was added onto the PVDF (Thermo Fisher Scientific, Waltham, MA, USA) membrane for 2 h incubation at room temperature. The blots were visualized by enhanced chemiluminescence. The immunoblot signal was quantified by Quantity One software (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

All experiments were repeated three times independently. SPSS 19.0 software (SPSS, IBM, Armonk, NY, USA) was served to analyze the data. Data were presented as mean ± standard deviation (SD). The differences between two groups were compared by the Student's *t*-test, while at least three groups were analyzed *via* one-way analysis of variance (ANOVA). The threshold of statistical significance was defined as $p < 0.05$.

Results

Silencing of LINC00240 Reduced HCC Cells Viability, Migration and Invasion

According to TCGA analysis, HCC patients with high LINC00240 expression (n = 90) had markedly lower survival than those with low LINC00240 expression (n = 90) (p = 0.036) (Figure 1A). Based on this result, we further detected the expression of LINC00240 in HCC cells. As shown in Figure 1B, LINC00240 expression was significantly increased in HCC cell lines (Hep3B, Huh7 and HCCLM3) than that in human normal liver cell line (THLE-3). Among the three HCC cell lines, Hep3B and Huh7 cell lines exhibited higher LINC00240 expression than HCCLM3 cell line. Thus, Hep3B and Huh7 cell lines were used for subsequent functional studies.

The transfection efficiency was assessed by RT-qPCR after Hep3B and Huh7 cell lines being transfected by LINC00240 shRNA and scrambled NC. Notably, relative to Hep3B and Huh7 cells in shNC group, those in shLINC00240 group showed prominently lower LINC00240 expression (Figure 1C). Therefore, LINC00240 expression in Hep3B and Huh7 cells was effec-

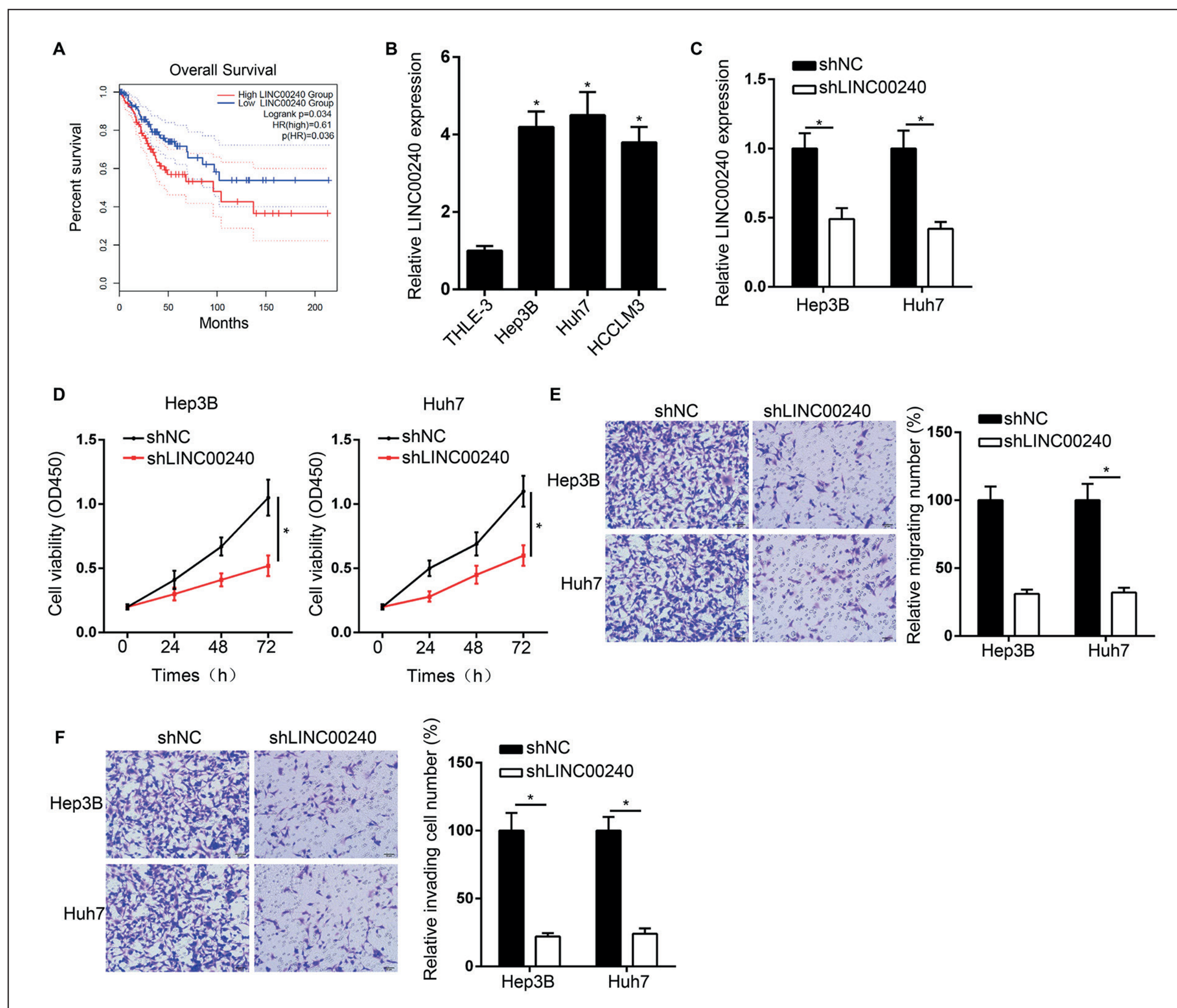

Figure 1. Silencing of LINC00240 reduced HCC cells viability, migration and invasion. **A,** TCGA analysis revealed that high LINC00240 expression was associated with low survival of HCC patients. **B,** LINC00240 expression was significantly increased in HCC cell lines (Hep3B, Huh7, HCCLM3) compared with that in human normal liver cell line (THLE-3). **C,** RT-qPCR indicated that LINC00240 expression in Hep3B and Huh7 cells was effectively regulated by transfection. **D,** Silencing of LINC00240 reduced HCC cells viability according to MTT assay. **E, F,** Transwell experiment illustrated that silencing of LINC00240 weakened HCC cells migration and invasion (magnification: 200×). * $p < 0.05$.

tively regulated by transfection. MTT assay exhibited much lower OD450 value of Hep3B and Huh7 cells in shLINC00240 group than that in shNC group (Figure 1D). Transwell experiment was performed to research Hep3B and Huh7 cells migration and invasion abilities. As a result, dramatically reduced number of migrating and invading cells was found in shLINC00240 group when compared with shNC group (Figure 1E and F). Thus, LINC00240 expression was up-regulated in HCC, but silencing of LINC00240 reduced HCC cell viability, migration and invasion.

Silencing of LINC00240 Reduced HCC Cells Viability, Migration and I nvasion Via Directly Promoting MiR-4465 Expression

TargetScan and miRanda were applied to predict miRNA targets of circRNAs. The mutant LINC00240 and wildtype LINC00240 fragments containing the binding site for miR-4465 was listed in Figure 2A. Hep3B and Huh7 cells of miR-4465 mimics group showed remarkably higher miR-4465 expression than miR-NC group (Figure 2B), indicating that Hep3B and Huh7 cells were successfully transfected. Luciferase reporter assay was then conducted to explore the relationship between LINC00240 and miR-4465. As shown in Figure 2C, compared with miR-NC group, 293T cells of miR-4465 mimics group had much lower Luciferase activity of wildtype LINC00240 Luciferase reporter. However, no significant statistical differences were found in the Luciferase activity of mutant LINC00240 Luciferase reporter between the two groups. Thus, miR-4465 was confirmed as a target gene of LINC00240 and miR-4465 expression was directly inhibited by LINC00240.

Subsequently, rescue experiments were performed to examine the effects of LINC00240 and miR-4465 on the phenotype of HCC cells. From Figure 2D, Hep3B and Huh7 cells of shLINC00240 group showed much higher miR-4465 expression than shNC group. However, significantly lower miR-4465 expression was observed in Hep3B and Huh7 cells of shLINC00240 + miR-4465 inhibitor group compared to shLINC00240 group. Functional studies exhibited that, relative to shNC group, Hep3B and Huh7 cells of shLINC00240 group had significantly lower OD450 value and number of migrating and invading cells. However, when compared with shLINC00240 group, Hep3B and Huh7 cells of shLINC00240 + miR-4465 inhibitor group showed markedly higher OD450 value and number of migrating and invading cells (Figure 2E-G). Therefore, silencing of LINC00240 reduced HCC cell viability, migration and invasion *via* directly promoting miR-4465 expression.

MiR-4465 Suppressed HGF/c-MET Signaling Pathway Via Directly Inhibiting HGF

TargetScan was used to predict mRNA targets of miR-4465. The mutant HGF and wildtype HGF fragments containing the binding site for miR-4465 was shown in Figure 3A. According to Luciferase reporter assay, much lower Luciferase activity of wildtype HGF Luciferase reporter was found in 293T cells of miR-4465 mimics group when compared with miR-NC group. There was no significant difference in the Luciferase activity of the mutant HGF Luciferase reporter between the two groups (Figure 3B). Therefore, HGF was identified as a target gene of miR-4465. The expression of HGF was directly suppressed by miR-4465. In addition, Hep3B cells of miR-4465 mimics group had dramatically higher miR-4465 expression and lower HGF mRNA expression than miR-NC group (Figure 3C). Western blot showed markedly lower HGF and p-c-MET proteins expression in Hep3B cells of miR-4465 mimics group when relative to miR-NC group (Figure 3D). Thus, miR-4465 suppressed HGF/c-MET signaling pathway *via* directly inhibiting HGF.

LINC00240 Silencing Inhibited HCC Cells Viability, Migration and Invasion by Suppressing HGF/c-MET Signaling Pathway Via Targeting MiR-4465

RT-qPCR indicated that relative to sh-NC group, Hep3B and Huh7 cells of shLINC00240 group, shLINC00240 + HGF group, miR-4465 mimics group and miR-4465 mimics + HGF group had prominently lower HGF mRNA expression. Much higher HGF mRNA expression was observed in Hep3B and Huh7 cells of shLINC00240 + HGF group compared with shLINC00240 group. Meanwhile, Hep3B and Huh7 cells of miR-4465 mimics + HGF group showed markedly higher HGF mRNA expression than miR-4465 mimics group (Figure 4A). Thus, Hep3B and Huh7 cells were successfully transfected. Function assay showed that, compared with sh-NC group, much lower OD450 value and number of migrating and invading cells was observed in the other four groups. Relative

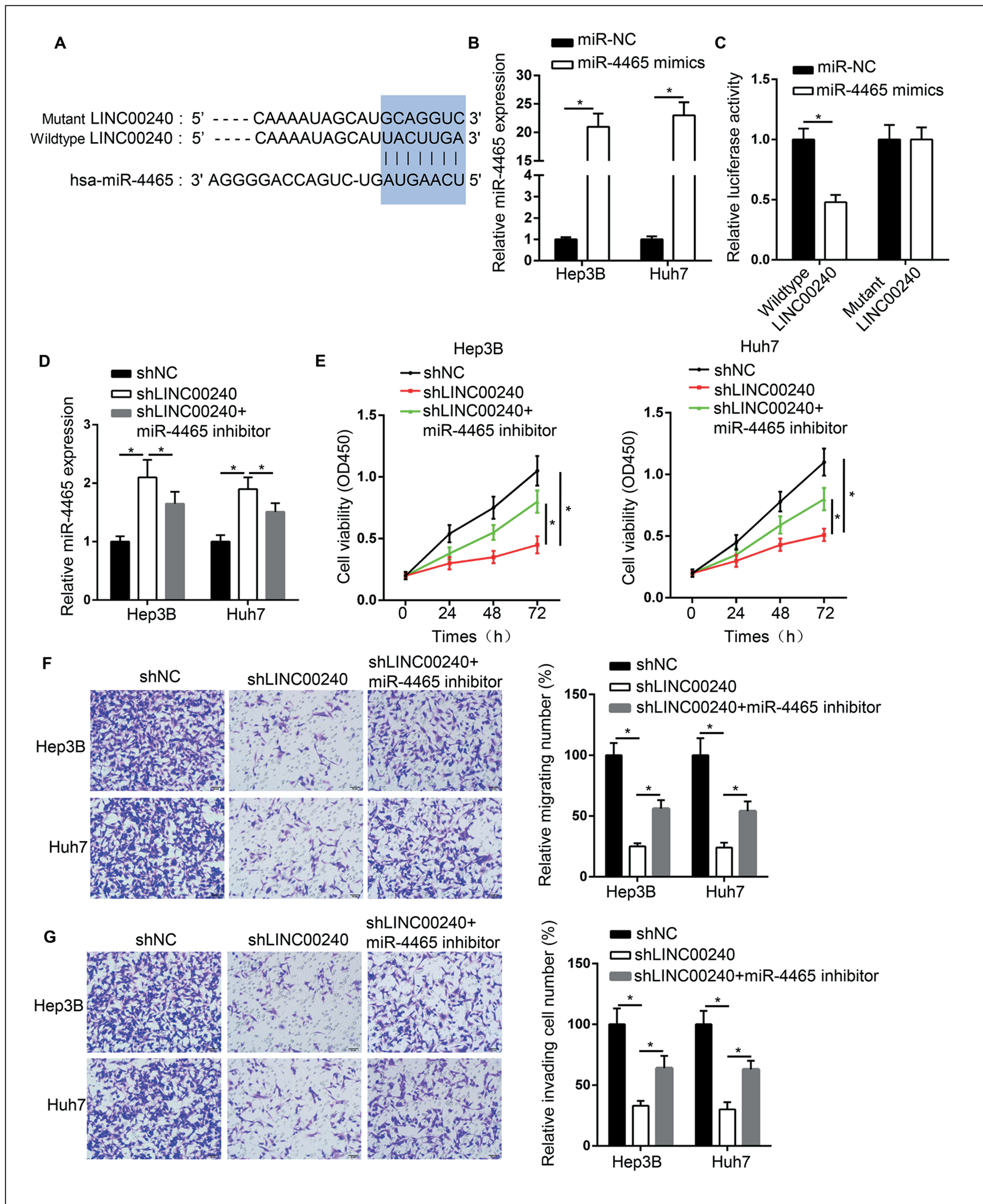

Figure 2. Silencing of LINC00240 reduced HCC cells viability, migration and invasion via directly promoting miR-4465 expression. **A,** The mutant LINC00240 and wildtype LINC00240 fragments containing the binding site for miR-4465 was listed. **B,** MiR-4465 expression in Hep3B and Huh7 cells were successfully regulated by transfection. **C,** MiR-4465 was confirmed as a target gene of LINC00240 according to Luciferase reporter assay. **D,** MiR-4465 expression in Hep3B and Huh7 cells were successfully regulated by cotransfection. **E,** Silencing of LINC00240 reduced HCC cells viability via directly promoting miR-4465 expression. **F, G,** Silencing of LINC00240 reduced HCC cells migration and invasion via directly promoting miR-4465 expression (magnification: 200×). * $p < 0.05$.

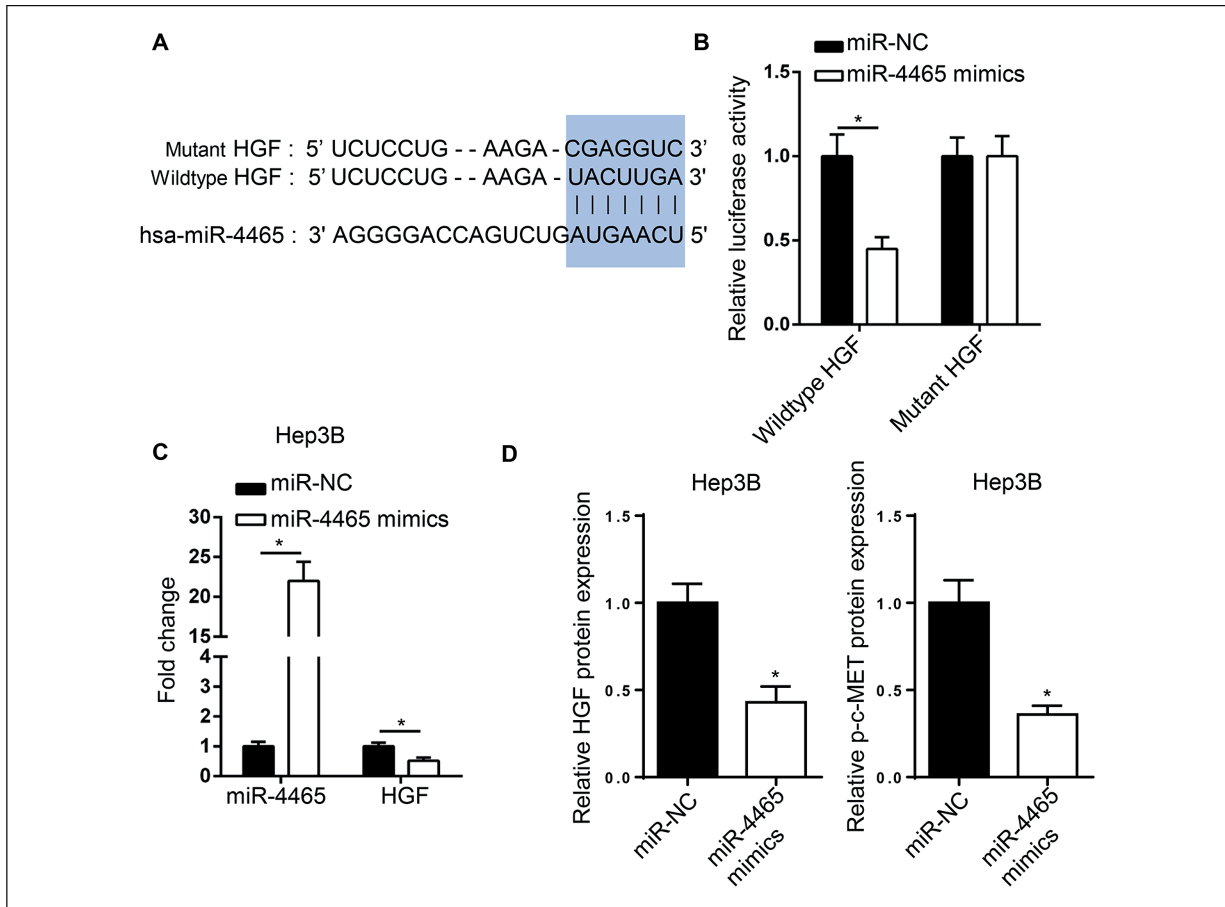

Figure 3. MiR-4465 suppressed HGF/c-MET signaling pathway via directly inhibiting HGF. **A,** The mutant HGF and wildtype HGF fragments containing the binding site for miR-4465 was shown. **B,** HGF was identified as a target gene of miR-4465 according to Luciferase reporter assay. **C,** MiR-4465 up-regulation in Hep3B cells reduced HGF mRNA expression. **D,** Western blot revealed that miR-4465 up-regulation suppressed HGF and p-c-MET proteins expression in Hep3B cells. * $p < 0.05$.

to shLINC00240 group, Hep3B and Huh7 cells of shLINC00240 + HGF group had remarkably higher OD450 value and number of migrating and invading cells. At the same time, prominently higher OD450 value and number of migrating and invading cells occurred in Hep3B and Huh7 cells of miR-4465 mimics + HGF group when compared with miR-4465 mimics group (Figure 4B-D). Therefore, LINC00240 silencing inhibited HCC cell viability, migration and invasion by suppressing HGF/c-MET signaling pathway *via* targeting miR-4465.

Discussion

HCC is a highly aggressive, solid malignancy with insignificant clinical symptoms in the early stage[14]. Therefore, the identification of biomarkers and therapeutic targets for HCC is an important strategy to improve the prognosis. LncRNAs are participated in the tumorigenesis and progression, including HCC. LncRNA-MIAT acted as an oncogene in HCC and promoted the proliferation and invasion of HCC cells in vitro through sponging miR-214[15]. LncRNA SOX21-AS1 was observed to be up-regulated in HCC and predicted poor prognosis. It might be a therapeutic target for HCC because repression of SOX21-AS1 led to the attenuated HCC cell proliferation, invasion and induced apoptosis[16]. On the other hand, several lncRNAs are considered to be tumor suppressor genes of HCC. LINC00052 was reported to be down-regulated in HCC, which suppressed HCC cells malignant behavior *via* binding miR-452-5p[17]. The decreased lncWDR26 expression

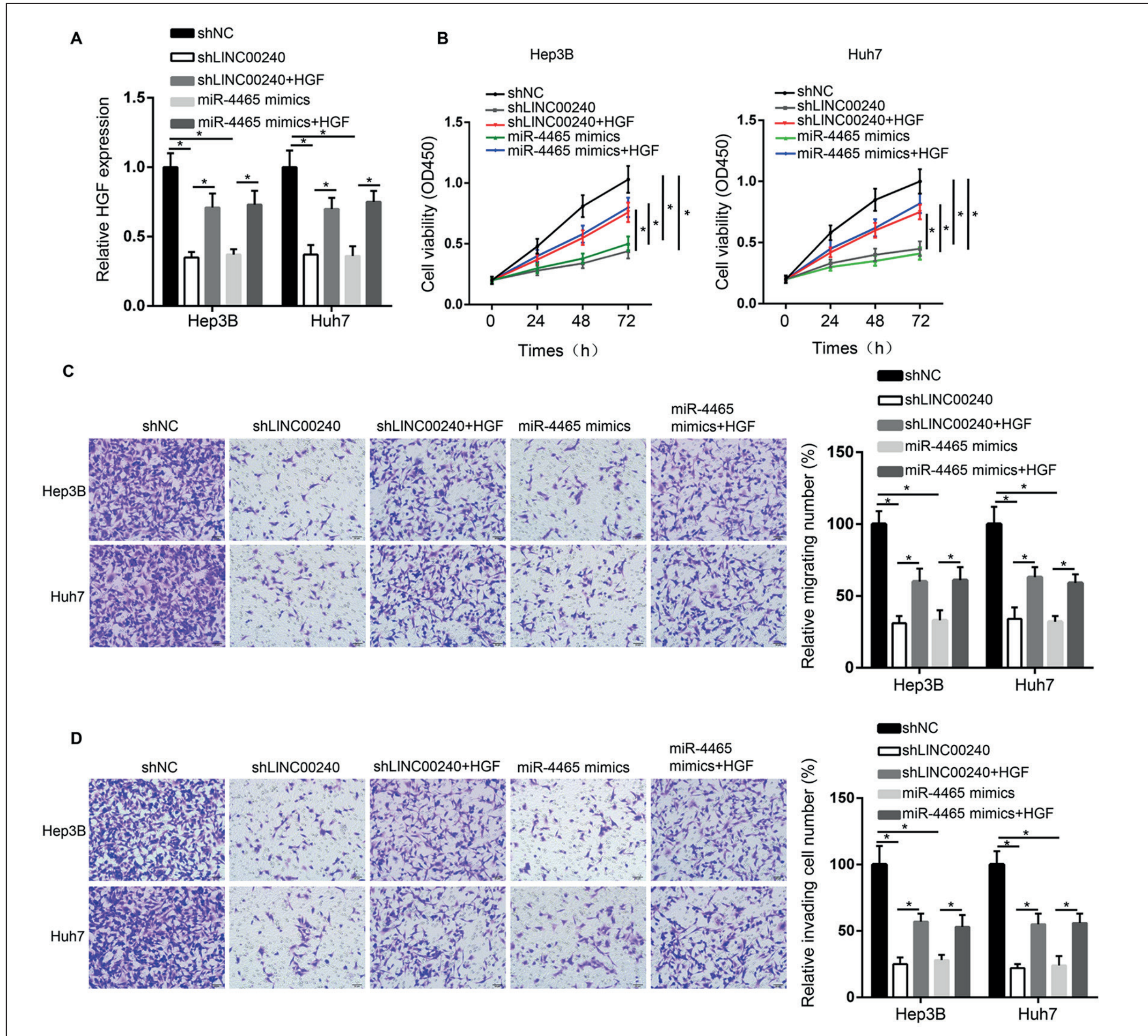

Figure 4. LINC00240 silencing inhibited HCC cells viability, migration and invasion by suppressing HGF/c-MET signaling pathway via targeting miR-4465. **A,** RT-qPCR indicated that Hep3B and Huh7 cells were successfully transfected. **B,** LINC00240 silencing inhibited HCC cells viability by suppressing HGF/c-MET signaling pathway via targeting miR-4465. **C, D,** LINC00240 silencing inhibited HCC cells migration and invasion by suppressing HGF/c-MET signaling pathway via targeting miR-4465 (magnification: 200×). * $p < 0.05$.

was also found in HCC, which was associated with poor prognosis such as high clinical stage, large tumor size and metastasis[18]. Many lncRNAs have been confirmed to participate in the progression of HCC, but the discovery of more other lncRNAs will provide a wider choice for the targeted treatment of HCC.

In the present study, we discovered that LINC00240 acted as an oncogene in HCC. The up-regulated LINC00240 expression in HCC indicated poor overall survival, whereas silencing of LINC00240 hindered HCC cells malignant behavior (such as viability, migration and invasion) *via* regulating miR-4465/HGF/c-MET axis. Currently, limited data are available on the research of LINC00240 in human diseases, especially in malignancies. It has only been reported to be dysregulated in the esophageal squamous cell carcinoma and cervical cancer, and to promote the malignant behavior of tumor cells *in vitro*[9,10]. As far as we know, this article was one of the few reports about the function of LINC00240 in human tumors, and also was the first research about the effect of LINC00240 on the progression

of HCC. LINC00240 could be served as a novel target for the targeted treatment of HCC.

In this research, the Luciferase reporter experiment showed that miR-4465 was a target gene of LINC00240 and was directly inhibited by LINC00240. In the previous study, miR-4465 was discovered to inhibit autophagy in cervical cancer cell line (hela) and neuroblastoma cell line (SH-SY5Y)[19]. In non-small cell lung cancer cells, miR-4465 could directly bind to the 3'-UTR region of EZH2 mRNA so as to inhibit the malignant proliferation and metastasis of tumor cells[12]. Researchers also noted that miR-4465 expression was reduced in ovarian clear cell carcinoma cells. LncRNA SNHG6 acted as a sponge for miR-4465 to facilitate the proliferation and metastasis of ovarian clear cell carcinoma[20]. Our rescue experiments still showed that the inhibition of miR-4465 reversed the inhibitory effect of LINC00240 knockout on the malignant behavior of HCC cells. Thus, similar to previous investigations, miR-4465 was acted as a tumor suppressor in HCC, which could be served as an important candidate for HCC targeted therapy.

HGF and its receptor c-MET have been thought to play a leading role in multiple types of malignancies[21]. High HGF/c-MET expression in tumors often associated with poor prognosis[22]. Meanwhile, the activation of c-MET can be induced by HGF, which further results in the stimulation of several important tumor-related downstream signaling pathways[23,24]. HGF can be derived from tumor cells and stromal cells in HCC[25]. The previous research[26] has demonstrated that the enhancement of HGF/c-MET signaling pathway could participate in the repression of HCC cells apoptosis. HGF/c-MET signaling pathway also discovered to facilitate HCC cells invasion and migration through enhancing the expression of Snail[27]. Recently, Zhao et al[28] illustrated that abnormal activation of HGF/c-MET signaling pathway was closely related to the metastasis of HCC. They further discovered that C7 peptide could inhibit the expression of HGF and c-MET as well as the phosphorylation of c-MET, thereby inhibiting the migration of HCC cells. In this study, we found that HGF was directly inhibited by miR-4465 *via* targeted binding in the 3'-UTR region. After transfected by miR-4465 mimics, HGF and p-c-MET expression in HCC cells were both declined. It might be that the reduced HGF expression induced by miR-4465 further suppressed the expression of p-c-MET. Rescue experiments revealed that HGF up-regulation reversed the inhibitory effect of LINC00240 silencing and miR-4465 overexpression on HCC cells malignant behavior.

Conclusions

In short, these results firstly reported the function of LINC00240 in HCC progression with miR-4465/HGF/c-MET signaling pathway as the axis. As a result, LINC00240 sponged miR-4465 to promote HCC cells proliferation, migration and invasion *via* HGF/c-MET signaling pathway. This was the first time that LINC00240 function has been studied in HCC, which would provide a novel candidate for the target treatment of HCC.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate
This study was approved by the Ethics Committee of The Second Affiliated Hospital of Nanjing University of Chinese Medicine.

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