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*Original Article*

# Long noncoding RNA LINC00665 is a diagnostic biomarker that enhances cell proliferation and migration in hepatocellular carcinoma

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**Abstract:** Background: This study aimed to evaluate the relationship between LINC00665 expression levels and the risk of hepatocellular carcinoma (HCC) in Chinese Han nationality patients and to explore the influence of LINC00665 dysregulation on the proliferation and migration potential of HCC cells. Patients and methods: We investigated the expression of LINC00665 in The Cancer Genome Atlas (TCGA) database. Then, we confirmed the expression of LINC00665 in 54 pairs of surgical tissues from HCC patients and in liver cancer cell lines by quantitative real-time polymerase chain reaction. Furthermore, we manipulated the expression level of LINC00665 and examined the cell proliferation and migration abilities of HCC cells. Results: In the TCGA cohort, a high level of LINC00665 in patients with HCC was significantly associated with tumor stage, tumor differentiation grade, and overall survival. In our HCC patient cohort, overexpression of LINC00665 in patients showed positive correlations with alpha-fetoprotein level, Barcelona Clinic Liver Cancer stage, and tumor differentiation grade. In addition, LINC00665 was upregulated in HCC cells, especially in cells with rapid growth rates and high migration abilities. A new LINC00665 isoform with a length of 1,371 nucleotides was identified in MHCC-97H cells. Interfering with LINC00665 expression weakened the proliferation and migration abilities of HCC cells. In contrast, LINC00665 overexpression enhanced proliferation and migration abilities. Conclusion: LINC00665 was upregulated in HCC tissues and cells and might be used to predict a poor prognosis of HCC patients. In addition, LINC00665 may promote the malignant progression of HCC by enhancing proliferation and migration capacities.

**Keywords:** Hepatocellular carcinoma (HCC), LINC00665, cell proliferation, cell migration

## Introduction

Liver malignancy represents China's second leading cause of cancer-related mortality, with an estimated 0.39 million deaths in 2020 [1]. Hepatocellular carcinoma (HCC) is the main form of liver cancer, comprising 75%-85% of incidence cases [2]. The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin-contaminated foods, alcohol abuse, smoking, non-alcohol fatty liver disease, obesity, and diabetes [3-5]. In general, HCC patients can be found by imaging diagnosis techniques such as Contrast-enhanced ultrasound (CEUS), Contrast-enhanced computed tomography (CECT), and magnetic resonance imaging (MRI) [6]. Furthermore, The GALAD score based on patient gender, age, and serum levels of α-fetoprotein (AFP), AFP isoform L3 (AFP-L3), and des-gamma-carboxy prothrombin (DCP) are recommended to determine the risk of HCC [7]. Circulating cell-free DNAs and RNAs also serve as promising biomarkers for early HCC [8]. For patients with early-stage HCC, liver resection, ablation, and orthotopic liver transplantation (OLT) are curative-intent options. Resection, transplantation, transarterial chemoembolization, and systemic treatment are for more progressed HCC patients [9]. The improvements mentioned above have collectively contributed to a significantly prolonged patient survival. Currently, the expected survival attains > 5, > 2.5, and > 2 years for patients with early, inter-

mediate, and advanced HCC, respectively [10]. In order to improve HCC progrnosis, understanding the molecular features of HCC, identifying more diagnostic biomarkers and therapeutic targets is urgently needed.

Recently, Mai et al. reported that long noncoding RNAs, transcripts larger than 200 nucleotides with little or no protein-coding potential, might be a new class of biomarkers and a therapeutic target for HCC [11]. Long intergenic non-protein coding RNA 665 (LINC00665), on chromosome 19q13.12, is a novel lncRNA dysregulated in different human cancers [12], such as gastric cancer [13, 14], breast cancer [15], ovarian cancer [16], colorectal cancer [17, 18], melanoma [19], glioma [20], and HCC [21-23]. LINC00665 plays an oncogenic role in cancer cell proliferation, migration, and invasion through various molecular mechanisms [12, 21, 24-27]. Several studies found that LINC00665 was highly expressed in HCC tissues compared to adjacent normal tissues. High LINC00665 expression was associated with advanced tumor size, TNM stage, Barcelona Clinic Liver Cancer (BCLC) stage, and poor prognosis in HCC patients [21, 23, 25]. However, the function and mechanism of LINC00665 in HCC are not yet clear.

In this study, we analyzed LINC00665 expression in liver hepatocellular carcinoma (LIHC) tissues from The Cancer Genome Atlas (TCGA) cohort and presented the correlation of LINC00665 expression with clinicopathologic factors. Then, we examined the RNA level and clinical significance of LINC00665 in surgical specimens from HCC patients at Peking University Shenzhen Hospital. Finally, we looked at the proliferation and cell migration abilities of HCC cells upon disturbing the LINC00665 RNA level. This study aimed to investigate the expression characteristics of LINC00665 in HCC tissues and to explore the influence of dysregulation of LINC00665 on the biological behavior of HCC cells.

## Materials and methods

### *Cell lines, culture conditions, and plasmids*

The HCC cell lines MHCC-97H, MHCC-97L, Bel-7404, HuH-7, hepatoma cell lines HepG2 and Hep3B, and human fetal hepatocyte cell line L-02 were used. All cell lines were purchased from China's national collection of authenticated cell cultures (Chinese Academy of Medical Science, Beijing, China) and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C in a humidified atmosphere with 5% $CO_2$. The LINC00665 overexpression plasmid pcDNA3.1-LINC00665-1,371 nt was constructed by a biotechnology company (RiboBio, Guangzhou, Guangdong, China) according to the identified sequence.

### *Bioinformatic analysis of LINC00665*

Transcriptome data associated with 371 LIHC samples and relevant clinical information were obtained from the TCGA database (https://portal.gdc.cancer.gov). The transcriptome and clinical information, including survival data, age, sex, grade, and stage, were processed.

### *HCC tissues and ethical statement*

The 54 pairs of HCC tumor tissues and matched adjacent nontumor liver tissues were used to analyze the RNA levels and clinical significance of LINC00665. In our institution, these specimens were obtained from patients who underwent hepatectomy for HCC from September 2017 to August 2021. Tissue specimens were immediately frozen in liquid nitrogen and stored at -80°C for further investigation. All participants in this study provided informed consent, and the study received ethical approval from the Peking University Shenzhen Hospital institutional review board.

### *RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)*

Total RNA was extracted with TRIzol reagent (Invitrogen, Waltham, MA, USA). Cultured cells were lysed by adding TRIzol directly. For surgical tissue specimens, approximately 100 mg of tissue was alternatively frozen in liquid nitrogen and thawed to room temperature three times. Then, the tissues were broken down with a hammer and lysed by adding 1 mL of TRIzol reagent. When RNAs were purified, first-strand cDNA was synthesized with the PrimeScript RT reagent Kit (TaKaRa, Shiga, Japan) using 500 ng of RNA. LightCycler 480 System (Roche

**Table 1.** Primers for real-time PCR and RACE

| PCR primers | | |
|---|---|---|
| Genes | Forward (5'-3') | Reverse (5'-3') |
| LINC00665 | GTCCACGGGTGGGAAATTGG | TCCGGTGGACGGATGAGAAA |
| MALAT1 | CGCCATTTTAGCAACGCAGA | CCCAAGGACTCTGGGAAACC |
| 18S rRNA | TACCTGGTTGATCCTGC | GACCAAAGGAACCATAACTG |
| GAPDH | TGCACCACCAACTGCTTAG | GACGCAGGGATGATGTTC |
| RACE primers | | |
| 5' RACE primer | 5'-GATTACGCCAAGCTTGGCACCCACATGGTAGTCGATCCG-3' | |
| 3' RACE primer | 5'-GATTACGCCAAGCTTACGACATTTGGAGGCCCCAGCGAGAAAC-3' | |
| Universal primer | 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3 | |

Diagnostics, Basel, Switzerland) was used for detection. Samples were quantified using the SYBR Premix Ex Taq reagent (TaKaRa, Shiga, Japan) with primer final concentration at 0.2 μM in a 20 μL reaction system. A two-step PCR amplification procedure was applied according to kit instructions. The primer sequences have been listed in **Table 1**. LINC00665, MALAT1, and ribosomal 18S rRNA were normalized to the mean expression level of GAPDH. The $2^{-\Delta\Delta Ct}$ method was used for the analysis of quantitative changes in gene expression.

*3'- and 5'-rapid amplification of cDNA ends (RACE)*

The LINC00665 isoform in the HCC cell line was identified according to the instructions of the 3'- and 5'-RACE kits (Clontech, TaKaRa, Shiga, Japan). RNAs were purified from MHCC-97H cells and converted into RACE-Ready first-strand cDNA. The 5' RACE primer and 3' RACE primer were used to pair with universal primers provided by the kit to amplify 5'-end and 3'-end sequences by PCR. The primer sequences have been listed in **Table 1**. PCR products were segregated by agarose gel electrophoresis. Target bands were cut down and purified. Purified DNA fragments were ligated into sequencing vectors and sent to the sequence. This way, the 5'-end and 3'-end sequences of the LINC00665 isoform were identified. The total length of LINC00665 was determined by splicing the overlapping 5'-end and 3'-end sequences.

*Nuclear and cytoplasmic RNA extraction*

Nuclear and cytoplasmic extractions of MHCC-97H and MHCC-97L cells were separated and prepared by introducing NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Waltham, MA, USA). Approximately 1 mL of TRIzol reagent was added to the nuclear and cytoplasmic extraction to purify RNAs. Then, the RNAs were used to synthesize cDNAs, which were templates for examining LINC00665, MALAT1, and GAPDH cellular location among HCC cells by quantitative real-time PCR.

*Cell migration assay*

Cell migration was determined using Transwell cell culture inserts with a pore size of 8 μm membranes (Corning, NY, USA). MHCC-97H cells were transfected with an intelligent silencer (a mixture of ASO (antisense oligonucleotides) and siRNA (small interfering RNA)) targeting LINC00665. Approximately 48 h after transfection, $8.0\times10^4$ MHCC-97H cells were suspended in 100 μL of serum-free DMEM and seeded on the upper inserts of a 24-well Transwell culture chamber. Then, 650 μL of DMEM containing 10% fetal bovine serum was added to the lower wells. For pcDNA-3.1-LINC00665 transfected MHCC-97L cells, $2.0\times10^5$ cells were seeded on the upper Transwell inserts. After 12-18 h, cells migrated to the membrane's lower side, were fixed with methanol, and stained with 2% crystal violet dissolved in methanol. The number of migrated cells was quantified in 5 random fields using a light microscope (DMi8, Leica, Wetzlar, Germany).

*Colony formation assay*

LINC00665-overexpressing HepG2 and MHCC-97L cells and LINC00665-silenced Hep3B and MHCC-97H cells were digested by trypsin and suspended by culture medium at a density of

500 cells/100 μL after 48 h transfection. Then, 400 μL suspension was added to every well of the 6-well plates, replenishing to 2 mL. Cells grow to naked-eye visible colonies containing approximately 50 cells viewed by a microscope. The cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Solarbio, Beijing, China) for 15 min at room temperature.

*CCK-8 assay*

LINC00665-overexpressing HepG2 and MHCC-97L cells and LINC00665-silenced Hep3B and MHCC-97H cells were suspended at a density of 500 cells/100 μL and cultured for 12 h after transfection. 200 μL of suspension were added to every well of the 96-well plates. CCK-8 (Beyotime, Shanghai, China) was added at 10 μL/well at different time points and incubated for 1 hour. The OD value at 450 nm absorbance was measured.

*Statistical analysis*

GraphPad Prism 7.0 was used for all analyses. All experiments were independently performed in triplicate, and data are presented as the mean ± standard deviation (SD). Student's t-test, one or two-way ANOVA with the Tukey-Kramer test were used for statistical data analyses according to the features of the data. Statistical significance was set at $P < 0.05$.

## Results

*LINC00665 upregulation in LIHC tissues is associated with tumor stage, histologic grade, and overall survival of patients in the TCGA cohort*

We analyzed LINC00665 expression in 371 tumor tissues and 50 adjacent normal tissues from the TCGA-LIHC cohort. We found that the LINC00665 level in tumor tissues was higher than that in nontumor tissues (**Figure 1A**). Among the TCGA-LIHC cohort, 50 pairs of tumor tissues were matched with adjacent normal tissues. The LINC00665 expression level in tumor tissues was also higher than that in matched nontumor tissues (**Figure 1B**). To explore the relationship between the LINC00665 expression level and the clinical features of patients, we collected the clinical information of 371 patients in the TCGA-LIHC cohort. We found that the RNA level of LINC00665 was positively correlated with the tumor stage. The LINC00665 expression level in stage I was significantly lower than in stage III (**Figure 1C**). This phenomenon was similar to the LINC00665 expression profile in the tumor T stage. The LINC00665 expression level in stage T1 was significantly lower than that in stage T3 (**Figure 1D**). The LINC00665 expression level was positively correlated with the tumor histologic grade of LIHC patients. LINC00665 expression levels in high tumor-grade patients were higher than those in low-grade patients (**Figure 1E**). Based on the LINC00665 expression characteristics observed above, we divided LIHC patients into high and low clusters according to the LINC00665 expression level and then graphed survival curves. We found that the high LINC00665 expression cluster had relatively shorter overall survival than the low LINC00665 expression cluster (**Figure 1F**). These results suggested that LINC00665 was upregulated in LIHC patients and positively related to tumor stage and grade. The LINC00665 expression level also influenced the overall survival time of LIHC patients.

*LINC00665 is frequently upregulated in hepatocellular carcinoma tissues and related to clinicopathologic factors: AFP level, BCLC stage, and tumor grade of HCC patients*

We examined LINC00665 expression in 54 pairs of HCC tumor tissues and matched adjacent nontumor tissues from surgical specimens in our hospital. We found that the LINC00665 RNA level in HCC tumor tissues was higher than that in nontumor tissues (**Figure 2A**). There were 50% of patients in whom the LINC00665 RNA level in tumor tissues was more than two-fold-fold higher than that in nontumor tissues ($\log_2$ fold change (tumor/non-T) > 1) (**Figure 2B**). Furthermore, we grouped patients by BCLC stage. We found that the LINC00665 expression level in tumor tissues was higher than that in nontumor tissues only in HCC patients with a tumor stage of BCLC-C (**Figure 2C**). Moreover, the percentages of patients with $\log_2$-fold change (Tumor/non-T) > 1 were 27.8% (5/18), 50.0% (9/18), and 72.2% (13/18) in BCLC-A, BCLC-B, and BCLC-C, respectively (**Figure 2D**). We then analyzed the correlation between the patient's clinical features and LINC00665 expression. We found that LINC00665 RNA

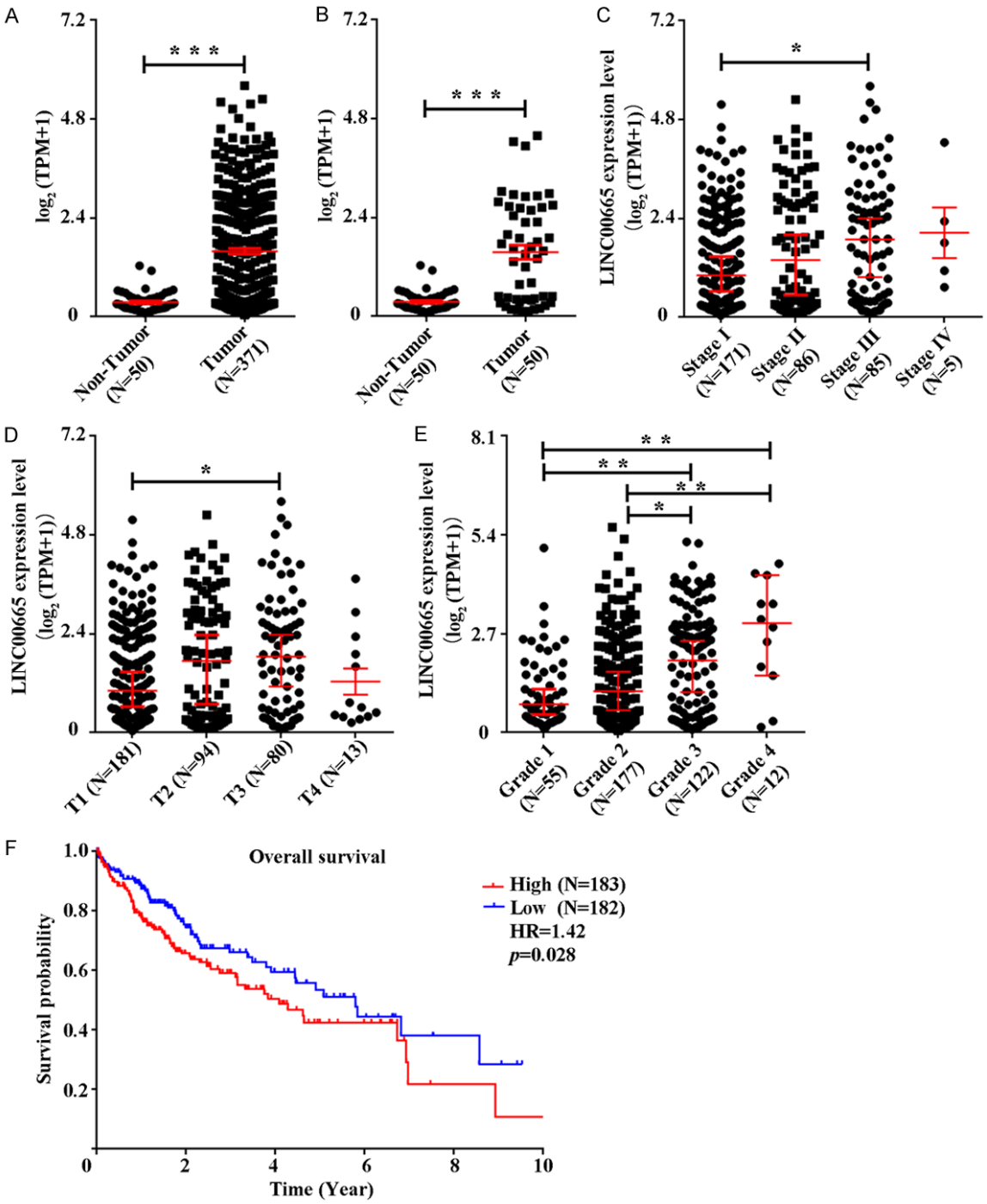


**Figure 1.** LINC00665 is frequently upregulated in tumor tissues and correlated with poor prognosis of patients from the TCGA-LIHC cohort. A. The RNA levels of LINC00665 in tumor tissues of 371 patients and adjacent normal tissues of 50 patients were derived from the TCGA database LIHC (liver hepatocellular carcinoma) cohort. *** means P < 0.001 in the Mann-Whitney test. B. The RNA levels of LINC00665 in 50 pairs of matched tumor tissues and adjacent normal tissues were derived from the TCGA database. *** means P < 0.001 in the Wilcoxon matched-pairs signed rank test. C. The expression of LINC00665 in tumor number stages of LIHC was analyzed. * means P < 0.05 in a one-way ANOVA test. D. The expression of LINC00665 in tumor T stages of LIHC was analyzed. * means P < 0.05 in a one-way ANOVA test. E. The expression of LINC00665 in tumor grades of LIHC was analyzed. * means P < 0.05 and ** means P < 0.01 in a one-way ANOVA test. F. The correlation between LINC00665 RNA levels and overall survival in 365 HCC patients. The significance of the difference in the survival curve was tested by the Gehan-Breslow-Wilcoxon test. HR, hazard ratio.

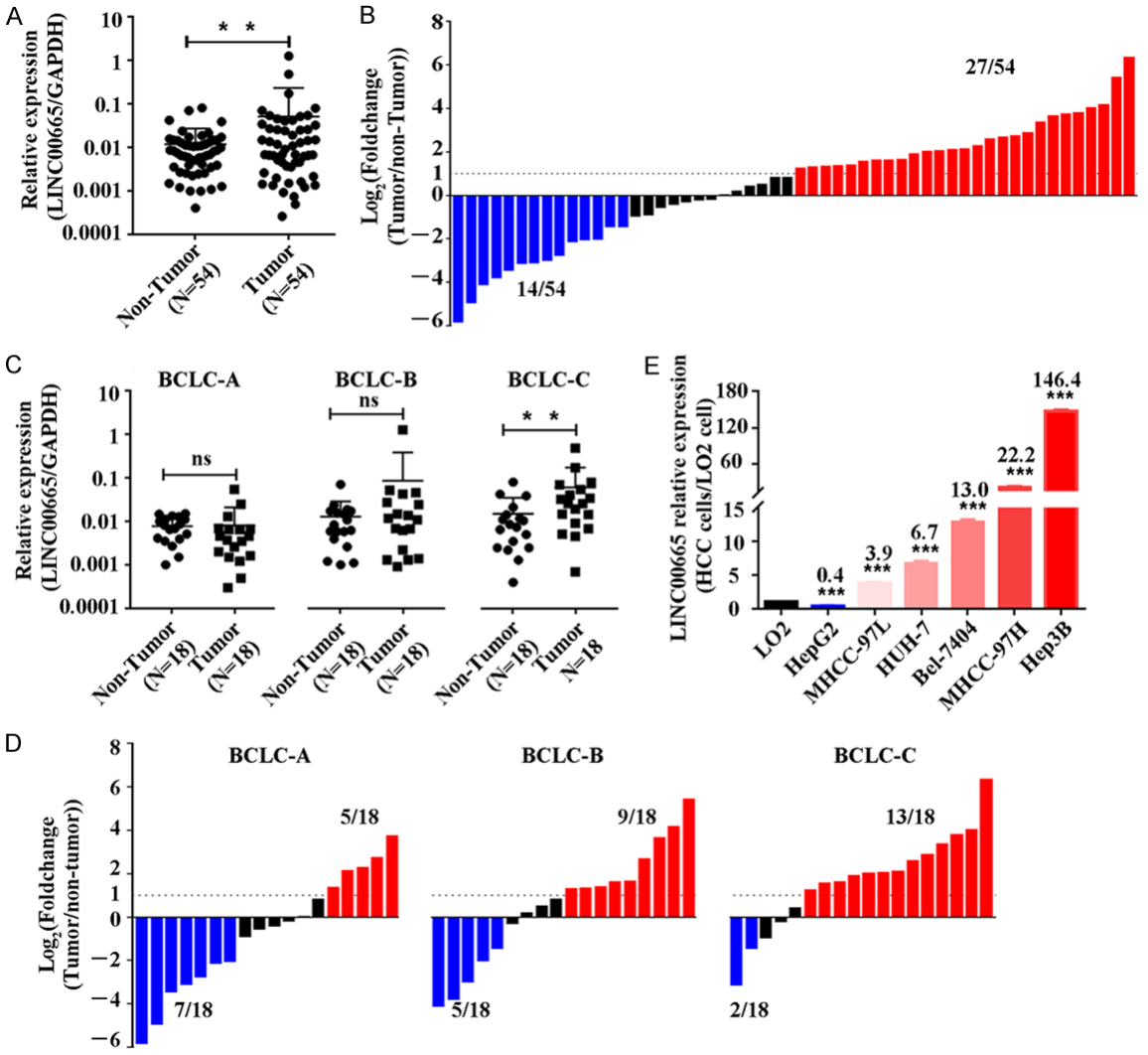


**Figure 2.** LINC00665 is upregulated in HCC patients with later BCLC stage and high migration potential immortalized liver cancer cells. A. The RNA levels of LINC00665 were quantified in 54 pairs of HCC tissues and adjacent normal tissues using qRT-PCR. ** means $P < 0.01$ in the Wilcoxon matched-pairs signed rank test. B. Fold changes in LINC00665 expression in 54 paired tissues. Downregulated expression, blue; no difference, black; upregulated expression, red. C. The RNA levels of LINC00665 in HCC tissues and adjacent normal tissues grouped by BCLC stage. ** means $P < 0.01$ in the Wilcoxon matched-pairs signed rank test. D. LINC00665 expression fold changes in paired tissues grouped by BCLC stage. Downregulated expression, blue; no difference, black; upregulated expression, red. E. The RNA levels of LINC00665 were quantified in immortalized liver cancer cell lines. *** means $P < 0.001$ in a one-way ANOVA test. qRT-PCR, quantitative real-time polymerase chain reaction; BCLC, Barcelona Clinic Liver Cancer; ANOVA, analysis of variance; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

levels were positively correlated with alpha-fetoprotein (AFP) levels, BCLC stage, and tumor grade in HCC patients (**Table 2**). For immortal liver cancer cell lines to maintain the characteristics of the original tissues, we examined the LINC00665 RNA level in liver cancer cell lines. We found that LINC00665 expression levels in cells with high migration potential were significantly higher than those in cells with low migration potential and normal liver cells (L02) (**Figure 2E**). These results indicated that a high level of LINC00665 might be an essential factor in HCC progression and the prognosis of HCC patients.

*Characteristics of LINC00665 transcripts in HCC cells*

Several LINC00665 isoforms have a common fragment with a length of 241 nucleotides. We

**Table 2.** Relationships between LINC00665 expression and clinicopathologic factors of HCC patients

| Data | No. of patients | High LINC00665 (No. of cases) | Low LINC00665 (No. of cases) | *p*-value |
|---|---|---|---|---|
| Gender | | | | |
| Female | 9 | 5 | 4 | |
| Male | 45 | 22 | 23 | > 0.999 |
| Age (Year) | | | | |
| ≤ 60 | 37 | 20 | 17 | |
| > 60 | 17 | 7 | 10 | 0.559 |
| Liver cirrhosis | | | | |
| Yes | 35 | 20 | 15 | |
| No | 19 | 7 | 12 | 0.254 |
| Hepatitis | | | | |
| Yes | 51 | 26 | 25 | |
| No | 3 | 1 | 2 | > 0.999 |
| Tumor size | | | | |
| ≤ 5 cm | 30 | 14 | 16 | |
| > 5 cm | 24 | 13 | 11 | 0.785 |
| Cancer Embolus | | | | |
| Yes | 21 | 12 | 9 | |
| No | 33 | 15 | 18 | 0.577 |
| Tumor capsular | | | | |
| Yes | 35 | 15 | 20 | |
| No | 19 | 12 | 7 | 0.254 |
| Tumorous No. | | | | |
| ≤ 1 | 40 | 17 | 23 | |
| > 1 | 14 | 10 | 4 | 0.119 |
| AFP | | | | |
| ≤ 20 ng/mL | 23 | 5 | 18 | |
| > 20 ng/mL | 27 | 20 | 7 | |
| NA | 4 | 2 | 2 | 0.001 |
| PIVKA-II | | | | |
| ≤ 40 mAU/mL | 9 | 3 | 6 | |
| > 40 mAU/mL | 35 | 20 | 15 | |
| NA | 10 | 4 | 6 | 0.374 |
| BCLC stage | | | | |
| A | 18 | 5 | 13 | |
| B | 18 | 9 | 9 | |
| C | 18 | 13 | 5 | 0.029 |
| Differentiation Grade | | | | |
| High | 10 | 0 | 10 | |
| Intermediate | 27 | 11 | 16 | |
| Low | 17 | 16 | 1 | < 0.001 |

Notes: High, $\log_2$ Fold change (T/N) ≥ 1; Low, $\log_2$ Fold change (T/N) < 1. Abbreviations: AFP, alpha-fetoprotein; BCLC, the Barcelona clinic liver cancer; PIVKA-II, protein induced by vitamin K absence; No., number of; NA, unavailable.

designed primers to amplify the 5' and 3' ends of the LINC00665 isoform in HCC cells according to the common sequence (**Figure 3A**). We amplified 5' and 3' end fragments from the MHCC-97H cDNA template by RACE assay. These fragments were purified, cloned, and inserted into vectors for sequencing. In this way, we identified the 5' and 3' end sequences of the LINC00665 isoform in HCC cells (**Figure 3B**). A new LINC00665 isoform, LINC00665-1371 nt, was determined by splicing the overlapping 5' and 3' end sequences of LINC00665 (**Figure 3C**). LINC00665-1371 contained three exons and was different from other LINC00665 isoforms (**Figure 3D**). Thus, we identified a new LINC00665-1371 nt transcript in HCC cells.

*LINC00665 promotes HCC progression by enhancing the migration potential of HCC cells*

We observed that a high LINC00665 expression level was associated with the clinicopathologic profiles of HCC. To explore the potential mechanisms by which LINC00665 induced HCC progression, we first examined the LINC00665 cellular distribution and found that LINC00665 was located in both the nucleus and cytoplasm. The LINC00665 distribution ratio in MHCC-97H cells was opposite to that of MHCC-97L cells (**Figure 4A**). Then, we downregulated LINC00665 expression by RNA silencing (**Figure 4B**). Conversely, we upregulated LINC00665 expression by transfecting the pcDNA-LINC00665 plasmid into cells with low LINC00665 expression (**Figure 4C**). High potential proliferation and migration abilities are features of malignant cells. We interfered

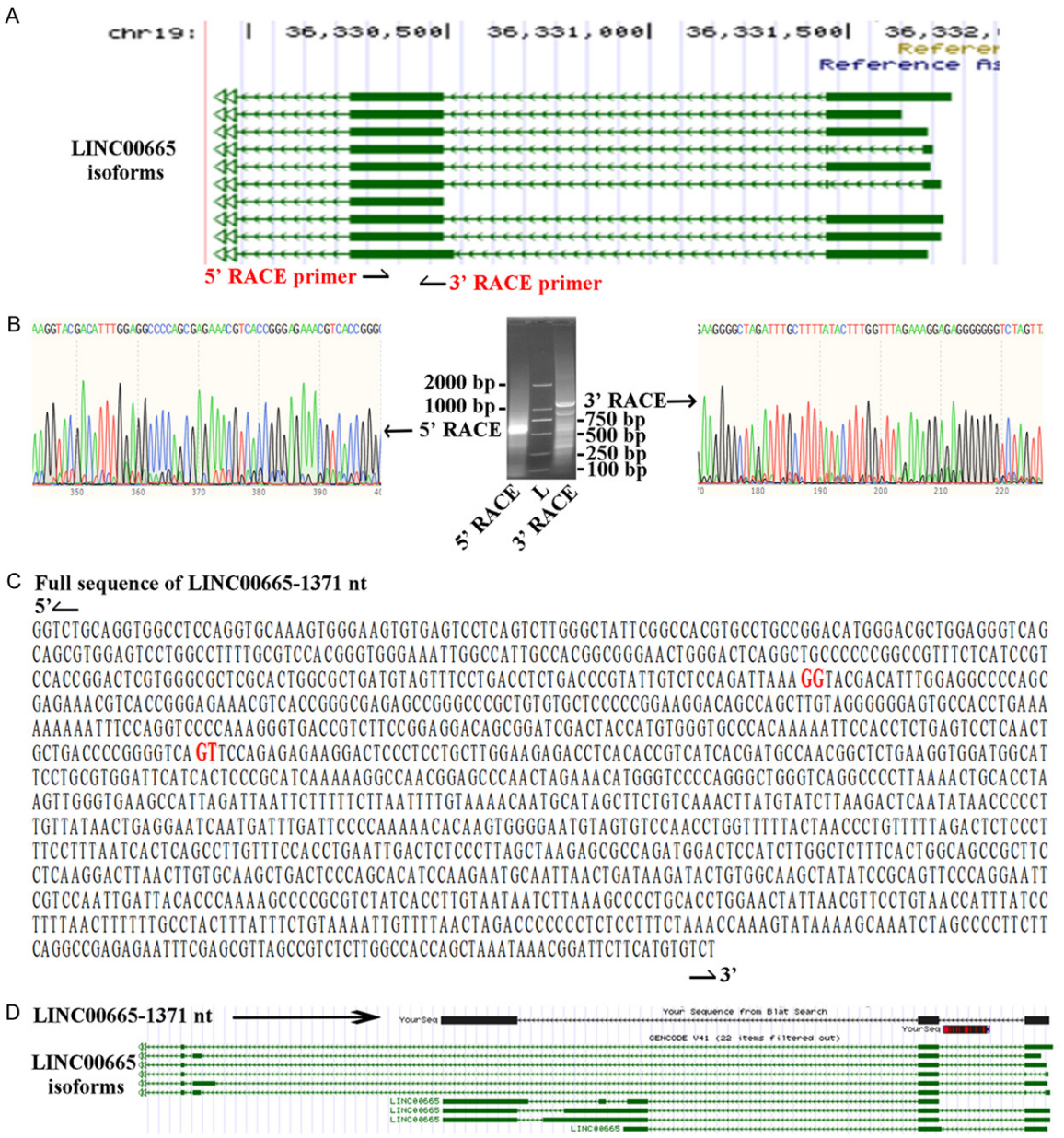


**Figure 3.** Identification of LINC00665 transcript by RACE assay in HCC cells. (A) Schematic diagram of the isoforms of LINC00665 available on the UCSC Genome Browser on the human website (https://genome.ucsc.edu/index.html). RACE primers for LINC00665 5' and 3' end amplification were designed within the indicated common region of LINC00665 isoforms. (B) DNA sequencing amplified, purified, and identified the 5' and 3' end fragments. (C) The full LINC00665-1371 nt isoform was obtained by splicing the overlapping 5' and 3' sequences in (B). The junction sites between exons are bold and in red. (D) Location of the LINC00665-1371 nt isoform in the human genome. RACE, Rapid amplification of the cDNA ends.

with LINC00665 expression in MHCC-97H and Hep3B cells. Then, we observed alterations in cell proliferation by colony formation (**Figure 4D**) and CCK-8 assays (**Figure 4G**, **4H**) and in MHCC-97H cell migration abilities (**Figure 5A**). We found that MHCC-97H and Hep3B cell proliferation was significantly inhibited upon LINC00665 downregulation (**Figure 4F-H**). The migration ability of MHCC-97H cells was weakened considerably (**Figure 5B**). In contrast, we observed changes in the proliferation of MHCC-97L and HepG2 cells (**Figure 4E**, **4I** and **4J**) and the migration of MHCC-97L cells (**Figure 5C**) after upregulating LINC00665 expression

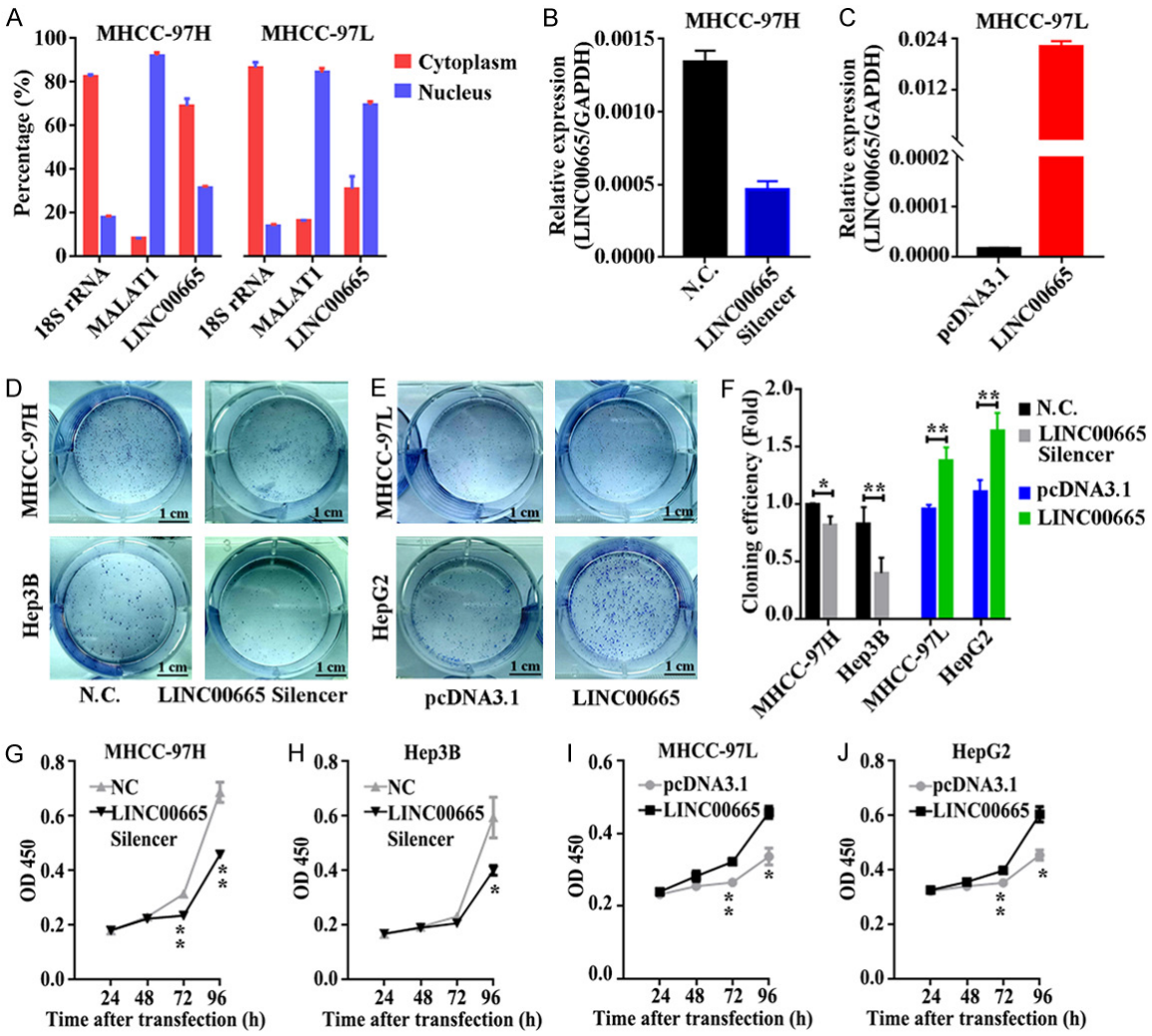


**Figure 4.** LINC00665 enhanced the proliferation potential of HCC cells. (A) The distributions of LINC00665 RNA in MHCC-97H and MHCC-97L cells. Ribosomal 18S rRNA was used as the endogenous cytoplasmic control. The long noncoding RNA MALAT1 was used as the endogenous nuclear control. (B) LINC00665 was knocked down by a silencer mixture that contained siRNA and ASO targeting LINC00665 in MHCC-97H cells. (C) LINC00665 was upregulated in MHCC-97L cells by transfecting pcDNA3.1-LINC00665 plasmids into cells. (D) Colony formation examination for LINC00665 expression interfered with MHCC-97H and Hep3B cells. Scale bar = 1 cm. (E) Colony formation examination of LINC00665-upregulated MHCC-97L and HepG2 cells. Scale bar = 1 cm. (F) Cloning efficiency for cells in (D) and (E). * means $P < 0.05$, and ** means $P < 0.01$ in the t-test. (G, H) CCK-8 assay for LINC00665 expression in MHCC-97H and Hep3B cells. * means $P < 0.05$, and ** means $P < 0.01$ in two-way ANOVA test. (I, J) CCK-8 assay for LINC00665-upregulated MHCC-97L and HepG2 cells. * means $P < 0.05$, and ** means $P < 0.01$ in two-way ANOVA test. MALAT1, metastasis-associated lung adenocarcinoma transcript 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

by pcDNA-LINC00665 plasmid transfection. We found that MHCC-97L and HepG2 cell proliferation potential was enhanced upon LINC00665 upregulation (**Figure 4F**, **4I** and **4J**). The migration ability of MHCC-97L cells was enhanced considerably (**Figure 5D**). These results indicated that LINC00665 plays a crucial role in HCC progression by enhancing the proliferation and migration abilities of HCC cells.

## Discussion

LINC00665 is a newly discovered lncRNA first reported in 2018 [23]. Studies have reported that LINC00665 is aberrantly upregulated in eighteen cancers and downregulated in two cancers [12]. The expression of LINC00665 can not only be used to distinguish tumors from adjacent tissues but is also closely related to patients' clinical characteristics and poor prog-

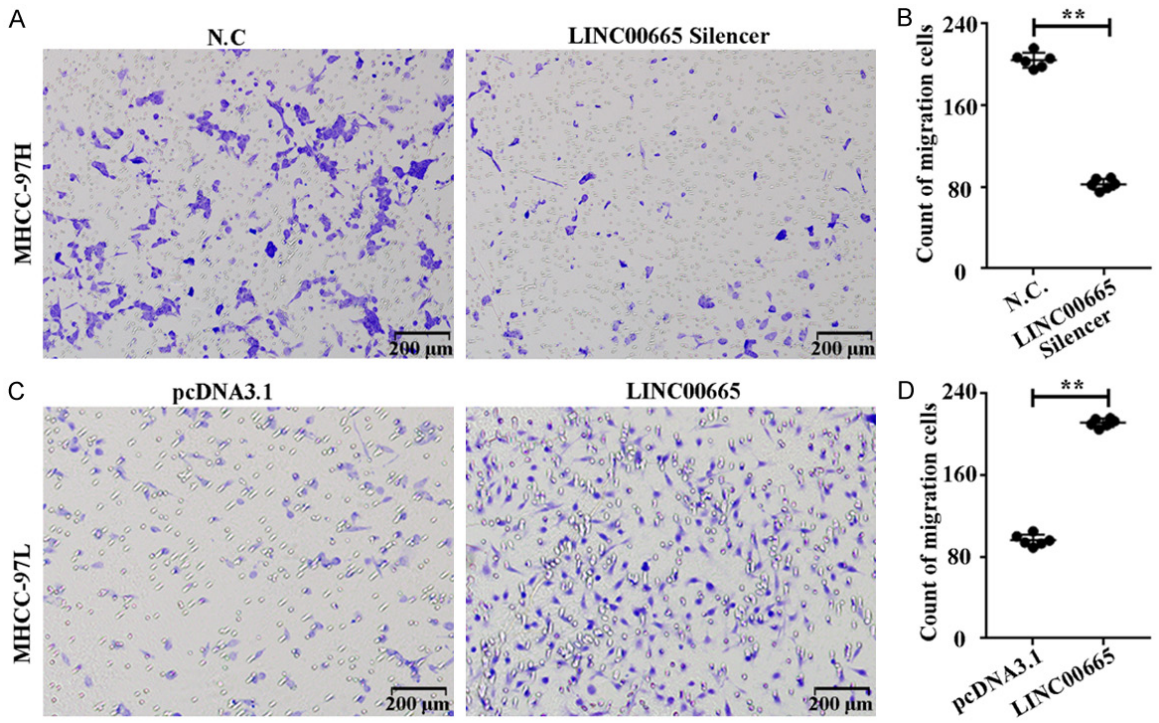


**Figure 5.** LINC00665 enhanced the migration potential of HCC cells. A, B. LINC00665 knockdown significantly decreased the migration ability of MHCC-97H cells. ** means $P < 0.01$ in the t-test. Scale bar = 200 μm. C, D. LINC00665 upregulation significantly enhanced the migration ability of MHCC-97L cells. ** means $P < 0.01$ in the t-test. Scale bar = 200 μm.

nosis [26, 28]. Wen et al. first reported that overexpression of LINC00665 in patients with HCC was significantly associated with sex, tumor grade, stage, and tumor cell type in LIHC sequencing data from TCGA [23]. Ding et al. proved the high expression of LINC00665 in HCC tissues and found that LINC00665 expression was positively correlated with the TNM stage and BCLC stage [21]. By analysis of TCGA LIHC cohort LINC00665 transcriptome data, we found that LINC00665 was significantly upregulated in tumor tissues, and high LINC00665 expression was associated with tumor grade and stage in LIHC patients. Kaplan-Meier survival analysis confirmed that LIHC patients with high LINC00665 expression had a shorter survival time.

Nearly half of new HCC cases occur in China [29]. We analyzed LINC00665 expression in 54 pairs of HCC tissues and matched normal adjacent tissues. We found that LINC00665 was frequently upregulated in HCC tissues, and high LINC00665 expression positively correlated with the AFP level, BCLC stage, and tumor grade of HCC patients. Those patients were of Han ethnicity, originally resided in 15 different provinces around mainland China, and worked or lived in Shenzhen. Our results prove that LINC00665 is a diagnostic and prognostic biomarker for Chinese HCC patients.

In addition, we cloned a full-length 1,371 nt LINC00665 transcript in HCC cells by 5' and 3' rapid amplification of the complementary DNA ends. LINC00665-1,371-nt shared the first two common exons with other isoforms recorded on the UCSC website (https://genome.ucsc.edu/index.html) but varied in the 3' ends. Ding et al. cloned a 1,183-nt LINC00665 transcript [21], fully included in our LINC00665-1371-nt. LINC00665-1,183 overexpression primarily affected the downstream signaling pathways closely related to NF-κB signalling, enhancing HCC cell proliferation potential [21]. LINC00665-1,371-nt overexpression significantly improved the proliferation and cell migration abilities of HCC cells, so this may be a new LINC00665 transcript that functions in HCC progression.

## Conclusion

This study demonstrated that LINC00665 was overexpressed in HCC tissues and cells. High LINC00665 RNA levels were positively correlated with AFP levels, BCLC stage, and tumor grade in Chinese Han nationality HCC patients. LINC00665 overexpression enhanced the proliferation and migration abilities of HCC cells. In contrast, interference with LINC00665 expression reversed this phenomenon. This study indicates that LINC00665 may be a diagnostic and prognostic biomarker in HCC patients.

## Acknowledgements

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## Disclosure of conflict of interest

None.

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