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Novel long noncoding RNA Linc1749808 promotes hepatocellular carcinoma metastasis by negatively regulating miR-206

Yi LIU[1,#], Yuan GAO[1,#], Si-Yuan WU[1,2], Le MA[1], Chun-Fu ZHU[1], Xu-Dong ZHANG[1,2,*]

[1]*Department of Hepato-Biliary-Pancreatic Surgery, The Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University, Changzhou, Jiangsu, China;* [2]*Clinical College, Nanjing Medical University, Nanjing, Jiangsu, China*

**Correspondence: zxd26244@126.com*
#Contributed equally to this work.

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Notably, a growing number of long noncoding RNAs (lncRNAs) have been recognized to play critical roles in hepatocellular carcinoma (HCC) progression. In this study, we identified a new lncRNA, Linc1749808 (ID: XR_001749808.1) based on microarray data from HCC tissues. Linc1749808 levels in 72 HCC tissues and paracancerous samples were detected by qRT-PCR. The interaction between Linc1749808 and microRNA-206 (miR-206) was assessed by bioinformatic analysis and luciferase assays. Linc1749808 depletion assays, Transwell assays, and miR-206-inhibitor rescue experiments were performed to examine the role of the Linc1749808/miR-206 axis in HCC cells. Our results showed that Linc1749808 was highly expressed in both HCC tissues and cell lines. Linc1749808 expression was significantly correlated with microvascular invasion, metastasis, and prognosis. After the knockdown of Linc1749808, the metastatic potential of 97H and HepG2 cells was attenuated *in vitro* and *in vivo*, but the proliferative capacity did not significantly change. Furthermore, Linc1749808 was found to act as a sponge of miR-206. Inhibition of miR-206 counteracted the effect of Linc1749808 knockdown in 97H cells by regulating YAP1 and epithelial-mesenchymal transition (EMT). In summary, these findings show that Linc1749808 can exacerbate the metastasis of HCC by sponging miR-206.

Key words: hepatocellular carcinoma, long non-coding RNA, microRNA-206, metastasis

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide [1]. Surgical resection is still the main treatment for HCC. However, a considerable number of patients still experience cancer recurrence and metastasis even after radical resection and adjuvant therapy [2]. Metastasis is the most pronounced feature of HCC and is responsible for the high 5-year mortality rate of postoperative HCC (up to 50%) [3]. Therefore, it is important to identify the mechanisms underlying recurrence and metastasis of HCC, as well as precise diagnostic and prognostic biomarkers for early diagnosis and risk assessment.

Long noncoding RNAs (lncRNAs) have been shown to play vital roles in the regulation of the progression of multiple cancers [4] and can act as scaffolds for protein complexes, antagonists for transcriptional regulation and for miRNAs and mRNAs, or guides for recruiting proteins for posttranscriptional regulation [5]. LncRNAs that function as ceRNAs are involved in diverse biological processes in HCC cells, such as tumor cell proliferation, epithelial-mesenchymal transition (EMT), metastasis, and chemoresistance [6, 7]. For instance, the long noncoding RNA H19 can enhance HCC bone metastasis by reducing osteoprotegerin expression via PPP1CA-induced inactivation of the p38-MAPK pathway and the sequestration of miR-200b-3p [8]. The long noncoding RNA ZFPM2-AS1 can regulate miR-139 to promote HCC cell invasion [9]. Recently, the relationship between miR-206 and HCC has been gradually unveiled. Preliminary studies found that miR-206 could not only promote apoptosis by modulating cMET expression [10] but also suppress HCC cell dedifferentiation by targeting EGFR signaling [11]. In addition, other analyses confirmed that lncRNAs can regulate tumor progression by sponging miR-206; for example, the lncRNA MIR4435-2HG functions as an oncogene in colorectal cancer in part by sponging miR-206 to upregulate the YAP1 expression and is likely to be a prognostic biomarker in colorectal cancer [12]. However, the crosstalk between lncRNAs and miR-206 in HCC remains to be clarified.

Here, based on microarray data and in vitro and *in vivo* assays, we revealed a novel lncRNA Linc1749808 (ID: XR_001749808.1, LOC107984584), and investigated its

possible role in the metastasis of HCC. The present research verified that Linc1749808 facilitates EMT and progression in HCC by regulating the miR-206/YAP1 axis, and these results may provide a new direction for the effective prevention and treatment of HCC.

Patients and methods

Clinical ethics and HCC tissues. A total of 72 pairs of fresh human HCC specimens were surgically resected from HCC patients at the Affiliated Changzhou No. 2 People's Hospital of Nanjing Medical University (China) from January 2015 to December 2017. Their clinical data were followed up and recorded. This study was approved by the hospital research ethics committee ([2017]KY013-01). Informed consent was provided from all participants before the surgery. All specimens were diagnosed by an experienced pathologist.

Cell culture. Normal hepatocytes (LO2) and HCC cell lines (HepG2, Hep3B, MHCC97H, SMMC-7221, Huh7) were obtained from the Cell bank of the Chinese Academy of Sciences (Shanghai, China). The normal hepatocyte cell line QSG-7701 was kindly provided by Professor Beicheng Sun (Nanjing University, China). Cells were cultured in the Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, and 100 μg/ml streptomycin in the incubator at 37 °C with 5% CO_2 (Thermo Fisher, USA). Trypsin and ethylenediaminetetraacetic acid were utilized for cell passage.

Real time-polymerase chain reaction (RT-PCR). Total RNAs extracted from tissues and cells were prepared by TRIzol reagent according to the manufacturer's instructions (Invitrogen, USA). Extracted RNA was utilized for reverse transcription to obtain the complementary deoxyribose nucleic acids (cDNAs) (Takara, Japan), followed with RT-qPCR analysis through SYBR Premix Ex Taq™ kit (Takara). The $2^{-\Delta\Delta Ct}$ method was applied to evaluate the expression of a target gene. U6 and β-actin were the normalized internal references. The sequences of primers used in this study are listed in Table 1. The microRNA measure kit for miR-206 was purchased from GenePharma (Shanghai, China) and the level of miR-206 was tested according to the manufacturer's instructions. Each of the samples was parallel performed in triplicate.

Table 1. Primers for quantitative RT- PCR.

Gene name	All Patients	Sequence (5'---3')
Linc1749808	Forward Primer	GCACCTAGAGATACTGAGACCTC
	Reverse Primer	AATCTTGGACACAGGAATACAGT
miR-206	Forward Primer	GCCCGCTGGAATGTAAGGAAGT
	Reverse Primer	CCAGTGCAGGGTCCGAGGT
β-actin	Forward Primer	GTCTTCCCCTCCATCGTG
	Reverse Primer	AGGGTGAGGATGCCTCTCTT
U6	Forward Primer	CTC GCTTCGGCAGCACA
	Reverse Primer	AACGCTTCACGAATT TGCGT

Western blot analysis. Total cells protein was extracted with RIPA lysis buffer (Beyotime, China) and tested the protein concentration (Beyotime, China). Then equal protein samples were separated by SDS-PAGE and transferred to PVDF membranes (Millipore, USA). After the blocking, different antibody diluents were used for incubation: β-actin (20536-1-AP, 1:2000), N-cadherin (#13116, 1:1000), and E-cadherin (#3195, 1:1000) Cell Signaling Technology, USA. The immunocomplexes were visualized using electrochemiluminescence reagent (Millipore, USA) according to the manufacturer's protocol.

Transfection. Short hairpin RNAs (Sh-Linc1749808) were purchased from Corues Biotechnology (Nanjing, China) (shRNA 1: GATCCGCTGGCGGAGACAGAGTAATTTCA AGAGAATTACTCTGTCTCCGCCAGCTTTTT). The shRNA sequences and the negative control shRNAs were cloned into lentivirus vector GV248 labeled with luciferase (Corues Biotechnology). miR-206 inhibitor or mimics were obtained from GenePharma Biotechnology (Shanghai, China). HCC cells were seeded in 6-well plates and cultured to 50% cell density per pore. A mixture of 1 μg non-specific control (NC), plasmids, 185 μl DMEM, and 5 μl Lipofectamine 3000 reagent was used to transfect these cells. After 48 h, the cells were collected for the following experiments.

Cell counting kit-8 (CCK-8) assay. Stable cells were seeded into 96-well plates with 2×10^3 cells each well. After incubation for different times (0, 24, 48, or 72 h), the cells were treated with CCK-8 (Beyotime, China) reagent for 2 h at 37 °C. The absorbance at a wavelength of 450 nm/well was measured by a microplate reader.

Transwell migration and invasion assay. 24-well Transwell chambers (3422, Corning, USA) were precoated with Matrigel (BD Biosciences, USA) (Matrigel-free ones were used for the migration assay). HCC cells were resuspended in a serum-free medium and seeded into the upper chamber (5×10^4 for cell migration, 1×10^5 for cell invasion). Then 600 μl medium supplemented with 10% FBS was applied to the lower chamber. After incubated at 37 °C for 24 h, cells that did not migrate or invade stayed in the top chamber and were wiped with cotton swabs. Then, the cells on the lower surface were fixed with 4% methanol for 15 min and dyed in 0.5% crystal violet for 20 min. The random fields were photographed under the microscope, and the migrated or invaded cells in fields were counted.

Dual-luciferase reporter assay. Wild-type and mutant Linc1749808 reporter vector (WT-Linc1749808, Mut-Linc1749808) containing miR-206 mimics or NC binding site mimics, were synthesized by RiboBio Biotechnology. The synthesized reporter Linc1749808-plasmids were co-transfected with miR-206 mimics or NC by Lipofectamine 3000™ into 293T. Both Firefly and Renilla luciferase activities were determined with the Dual-Glo Luciferase Assay system (Promega, USA) and fluorometer. Firefly luciferase signals were normalized with Renilla luciferase signal values.

Fluorescence in situ hybridization (FISH). RNA FISH was performed using the fluorescent in situ hybridization kit to clear the cellular localization of Linc1749808, according to the manufacturer's protocol (RiboBio Biotechnology, Guangzhou, China). The nucleus was counterstained with DAPI. Cy3-labeled Linc1749808 probes and positive control ones were incubated and then observed with the confocal microscope.

Nude mouse model of transplanted HCC. BALB/c nude mice (aged 4–5 weeks) were purchased from the Model Animal Research Center of Yangzhou University and fed in specific pathogen-free laboratory animal isolation cages. Animal testing was approved by the Ethics Committee of Nanjing Medical University. The nude mice were randomly divided into the sh-NC group and the sh-Linc1749808 group (n=6). 97H cells (1×10^6 cells/50 μl) were injected into the tail vein of each mouse under anesthesia to generate the metastasis model. The vital signs of the mice were observed regularly after injection. Five weeks later, the mice were imaged with the IVIS Lumina II system (Caliper Life Science, USA), and the tumors that developed in the lungs were dissected and embedded in paraffin. The sections were stained with H&E to assess the number of metastatic nodules.

Statistical analysis. All numerical results are expressed as the mean ± standard deviation (SD). Student's t-tests were applied to calculate statistical significance between two groups with normal data distribution. A p-value <0.05 was considered statistically significant. Some figures were prepared with GraphPad Prism 6.

Results

High expression of Linc1749808 in HCC. To investigate the expression profile of lncRNAs in HCC, microarray analysis was performed on three pairs of liver cancer tissues and their adjacent non-tumor samples (Figures 1A, 1B). From the lncRNAs with the most significant fold changes (fold >2.5, p<0.05), we selected the top 3 differentially upregulated lncRNAs and detected their expression in 72 liver cancer tissues. We further selected Linc1749808 for in-depth analysis because of its differential value and novelty (Figure 1C).

Clinical information was collected to verify whether the aberrant expression of Linc1749808 was associated with the clinicopathological characteristics of HCC. According to the median expression value of Linc1749808 in 72 liver cancer tissues, the cases were divided into a high expression (n=36) and a low expression (n=36) group. The differences in clinical characteristics were determined and are presented in Table 2. Linc1749808 expression was significantly correlated with tumor size (p=0.012), microvascular invasion (p=0.016), and metastasis (p=0.029). Furthermore, the level of Linc1749808 was negatively associated with HCC-specific survival according to Kaplan-Meier analysis (p=0.01, Figure 1D).

As Linc1749808 has not been previously reported in human disease, we conducted an experiment to confirm that Linc1749808 does not encode protein and its expression in liver cell lines. On the basis of its gene sequence, we used the RNA online prediction website (Coding Potential Calculator) to confirm that Linc1749808 cannot be translated into protein (Figure 1E). Linc1749808 was upregulated in most HCC cell lines (97H, HepG2, and Huh7) in comparison with normal liver cell lines (LO2 and QSG-7701) (Figure 2A). FISH-based detection of subcellular localization indicated that the Linc1749808 transcript is mainly located in the cytoplasm of 97H cells (Figure 2B).

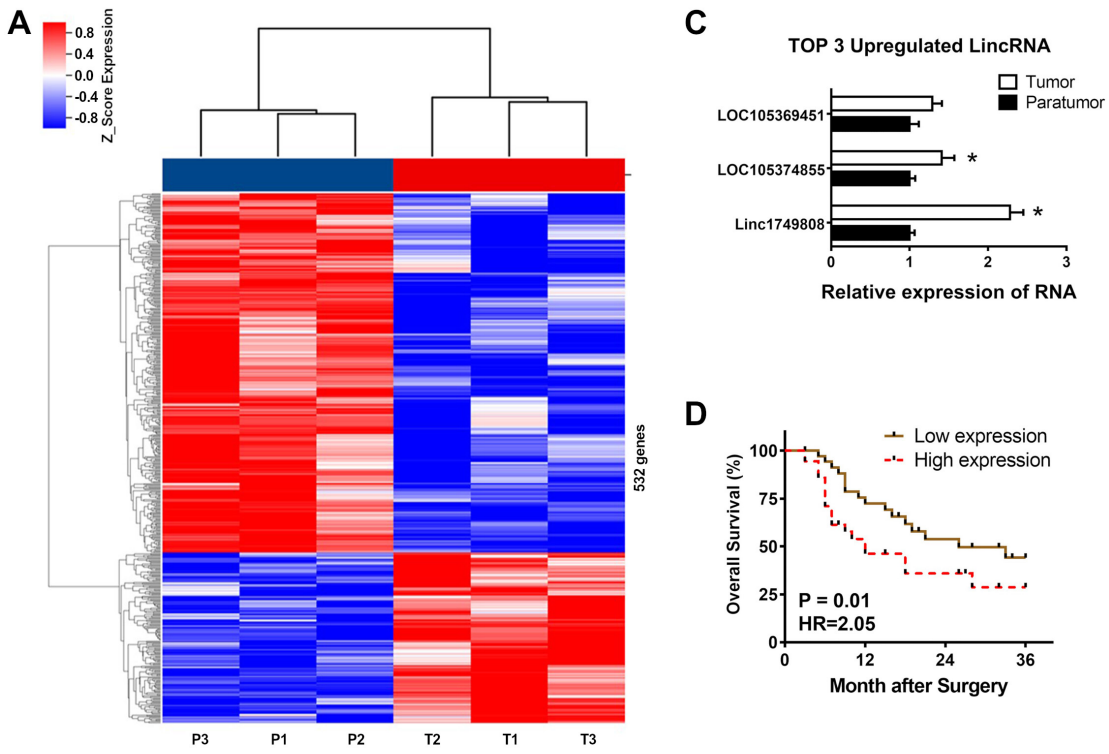
Linc1749808 promotes cell proliferation and invasion *in vitro* and *in vivo*. To detect whether Linc1749808 can regulate the physiological function of HCC, we selected the high Linc1749808-expressing cell lines (97H and HepG2) for the following experiments. As displayed in Figures 2C and 2D, the knockdown efficiency of sh-Linc1749808 was detected by RT-qPCR, and shRNA1 was employed for subsequent studies of the Linc1749808 effects on HCC growth. Although the clinical data showed that Linc1749808 was related to tumor size, there was no significant change in cell proliferation after interference with Linc1749808 expression (as assessed by CCK-8 assay, Figures 2E, 2F).

The clinical data also indicated that Linc1749808 might participate in HCC metastasis. Thus, we next explored the

Table 2. Expression levels of Linc1749808 and summary of the clinicopathological characteristics of liver cancer patients.

Variable	Linc1749808 expression		
	High	Low	p-value
Total	36	36	
Age (years)			0.628
≥60	23	21	
<60	13	15	
Gender			0.599
Male	27	25	
Female	9	11	
AFP (μg/l)			0.434
≤400	10	13	
>400	26	23	
Tumor size (cm)			**0.012***
≤5	11	21	
>5	25	15	
Microvascular Invasion			**0.016***
Yes	19	9	
No	17	27	
Metastasis			**0.029***
Yes	13	5	
No	23	31	
Edmondson Stage			0.276
I–II	7	11	
III–IV	29	25	

Notes: statistical analysis was performed using the χ^2-test, p<0.05 in bold was considered significant. Abbreviations: AFP-alpha-fetoprotein



E

Result for species name : hg19 with job ID :1588740215

Data ID	Sequence Name	RNA Size	ORF Size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
0	XR_001749808.1	740	165	0.8125	-0.377457208202	0.0018380602822752	no

Figure 1. Linc1749808 is significantly upregulated in HCC. A) Clustering analysis of the microarray results. LncRNA chip results from 3 human liver cancer and adjacent tissues samples were analyzed. B) A quantile algorithm was used for standardization, and differential genes were analyzed according to the magnitude of the difference (greater than 2) and the corresponding p-values ($p<0.05$). A total of 171 lncRNAs were upregulated in the LncRNA chip results, and Linc1749808 was identified from these lncRNAs. C) RT-PCR verification of the top 3 lincRNAs with the highest expression in 72 liver cancer specimens was performed (the expression in paracancerous cells were used as the standard value), and the expression of Linc1749808 in liver cancer was significantly higher than that in adjacent tissues ($p<0.05$). D) Kaplan-Meier analysis (36 months) was performed to analyze whether the expression of Linc1749808 was related to the survival duration of HCC patients after surgery. E) An online database was used to confirm that Linc1749808 is a non-coding RNA.

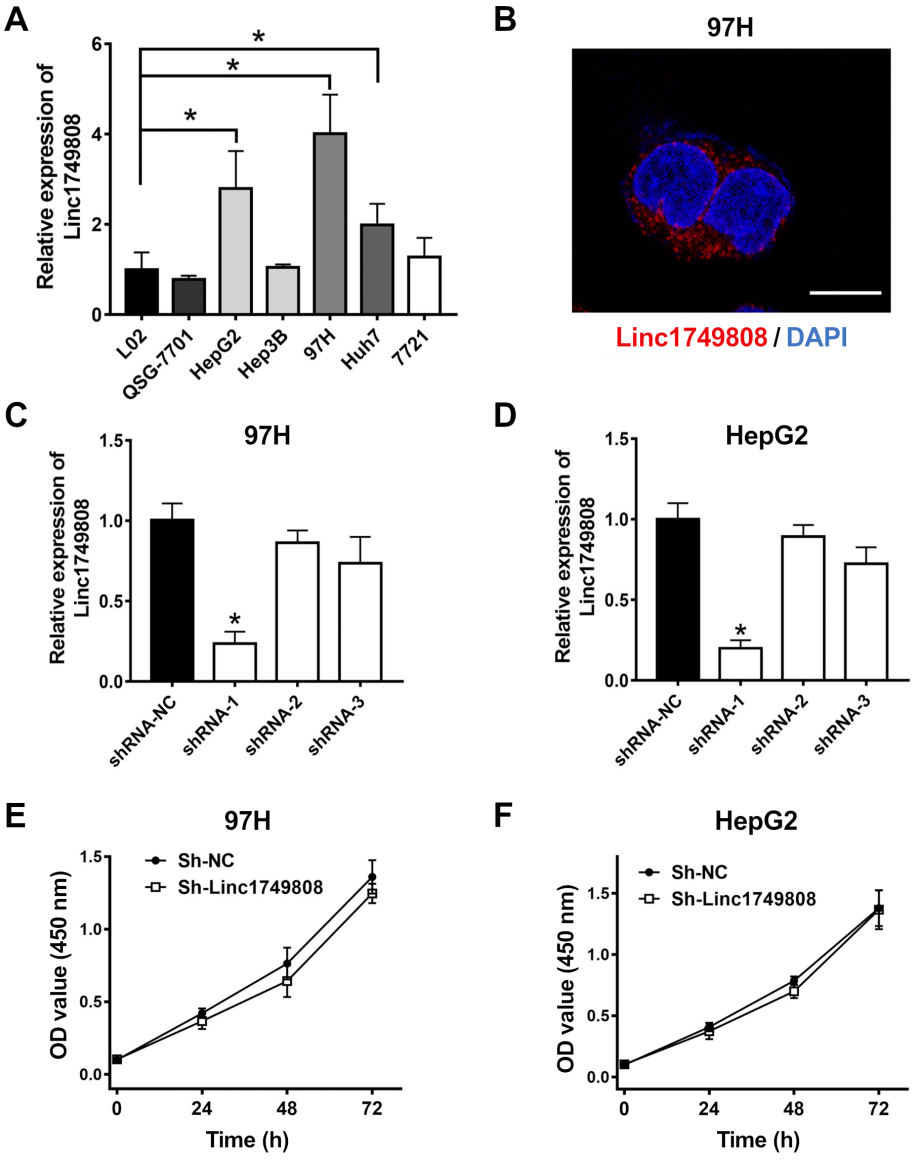


Figure 2. The expression of Linc1749808 in HCC cells. A) The expression of Linc1749808 in liver cancer cell lines (Huh7, Hep3B, HepG2, 7721, and MHCC97H), compared with normal liver cell lines (LO2 and QSG-7701). B) Linc1749808 was primarily located in the cytoplasm of 97H cells (as assessed with fluorescent in situ hybridization (FISH)), magnification ×400, scale bar: 20 μm. C, D) Three shRNA-transfected 97H and HepG2 cell lines were used to verify that shRNA-1 was the most efficient for Linc1749808 interference ($p<0.05$). E, F) CCK-8 analysis between the control group and shRNA interference group. Cell proliferation was similar after Linc1749808 interference in 97H and HepG2 cells.

functional role of Linc1749808 in cell metastasis with a Transwell assay. Compared with the negative controls, 97H and HepG2 cells with stable knockdown of Linc1749808 had decreased migration and invasion (Figure 3A). There is a close relationship between tumor metastasis and EMT. Subsequently, the results of qRT-PCR and western blot analysis revealed that the level of the epithelial marker E-cadherin was upregulated in Linc1749808 knockdown HCC cells, while the expression of the mesenchymal marker N-cadherin was significantly repressed (Figures 2B, 2C). Thus, we speculate that Linc1749808 might be involved in the process of EMT.

To extend our investigations *in vivo*, we generated a xenograft model via tail vein injection to investigate the role of Linc1749808 in the lung metastasis of HCC cells. The mice were divided into a Linc1749808 knockdown group and a control group (n=6). Via fluorescence intensity and HE staining analyses, the volume and number of lung-metastatic nodules were found to be significantly decreased in the Linc1749808 knockdown group (Figure 2D). All

these *in vitro* and *in vivo* experimental results suggest that Linc1749808 promotes the metastasis of HCC cells.

Linc1749808 acts as a competing endogenous RNA (ceRNA) interacting with miR-206. Understanding the function of Linc1749808 in HCC metastasis will help to delineate the underlying mechanism of the interaction between lncRNAs, EMT, and HCC. As reported, lncRNAs are able to modulate the expression of non-protein-coding transcripts such as microRNAs [13].

With the help of a microRNA target scanning algorithm [14] and the RNA22-online database [15], the 6 highest-scoring potential miRNAs (miR-206, miR-3132, miR-3916, miR-342-5p, miR-583, and miR-31-5p) were identified and validated by RT-PCR. Subsequently, among the candidate miRNAs, miR-206 was selected for further testing because it showed high expression in 97H cells following the downregulation of Linc1749808 (Figure 4A). In addition, the RT-PCR results of HCC and paratumor tissues showed that the expression of miR-206 was lower in tumor tissues than in paratumor tissues and had a negative correlation with the level of Linc1749808 (Figures 4B, 4C). Thus, we hypothesized that miR-206 was very likely to be sponged by Linc1749808.

We used a microRNA target-scanning algorithm to predict miRNA targets and found a putative binding site of Linc1749808 in miR-206 (Figure 4D). Then, we constructed luciferase reporters containing the wild-type 3'UTR sequence of Linc1749808 and Mut-Linc1749808. The results showed that co-transfection of miR-206 mimics and WT-Linc1749808-3'UTR reduced the luciferase activity by nearly 70% (Figure 4E). Conversely, the co-transfection of miR-206 and Mut-Linc1749808 did not change the luciferase activity. As such, miR-206 was sponged and directly downregulated by Linc1749808 in liver cancer cells.

Linc1749808 regulates EMT by serving as a sponge for miR-206. Since previous studies showed that miR-206 could regulate tumor progression in different cancers [16], we investigated whether Linc1749808 could promote the development of HCC via miR-206. The PCR results showed

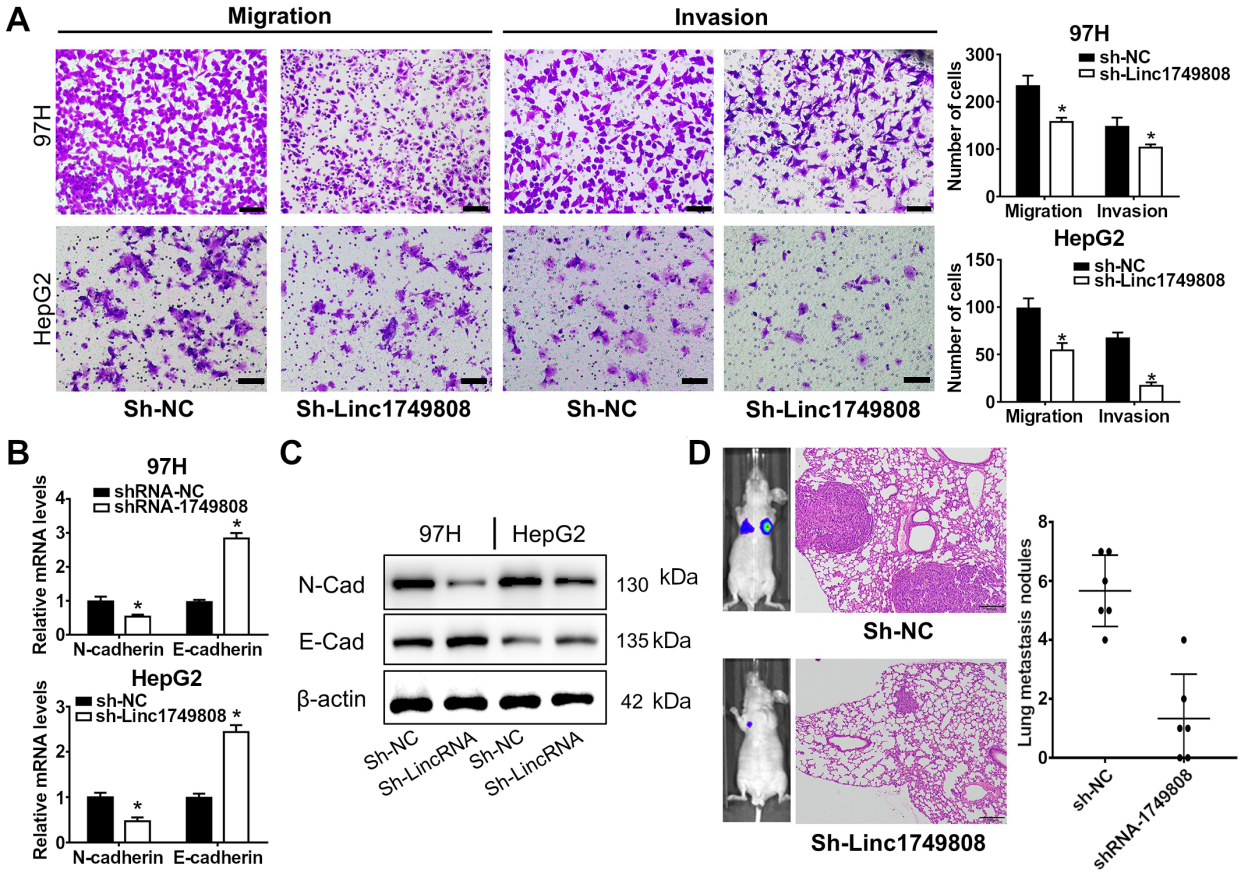


Figure 3. Linc1749808 promotes HCC cell migration and invasion *in vitro* and *in vivo*. A) Left panels: Inhibition of cell migration and invasion after Linc1749808 interference in 97H and HepG2 cells and after stable transfection of the negative control (NC). Scale bar: 100 μm. Right panels: The number of cells that passed through the compartment was counted in 3 random visual fields. All data are shown as the mean ± SD (*p<0.05). All experiments were repeated at least three times; B, C) EMT-related signaling markers (E-cadherin and N-cadherin) were modulated by Linc1749808 interference (*p<0.05). D) Representative figures (right) and the calculated number (left) of mice with pulmonary metastasis in tail vein xenograft model mice injected with 97H cells.

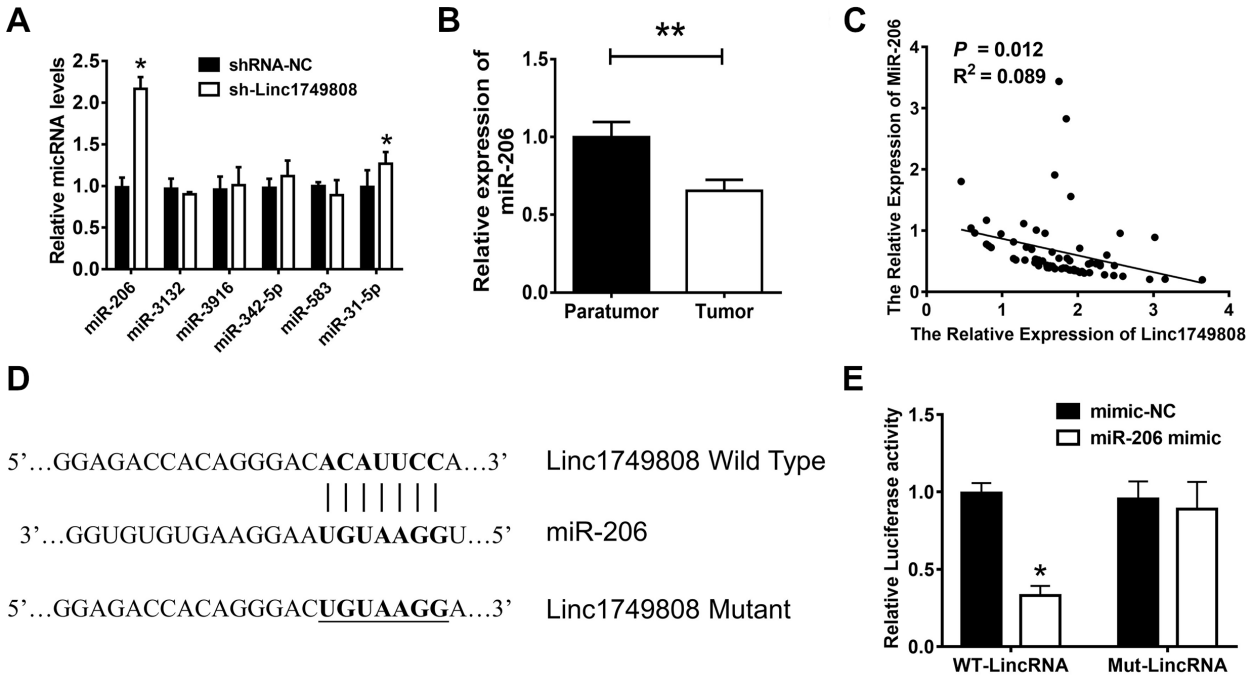


Figure 4. Linc1749808 functions as a sponge for miR-206. A) miRNAs (miR-206, miR-3132, miR-3916, miR-342-5p, miR-583, and miR-31-5p) expression was detected after shRNA-Linc1749808 transfection of 97H cells. B) Relative miR-206 expression in clinical samples was detected by RT-PCR and normalized to U6 expression. C) Pearson's correlation analysis was used to show the relationship between miR-206 and Linc1749808 expression in HCC. D) Bioinformatics analysis was used to identify a potential binding site between miR-206 and Linc1749808, and a plasmid carrying a mutated construct was generated, as presented. E) Luciferase reporter assays were used to validate the Renilla luciferase activity of the WT and Mut-Linc1749808 constructs in cells co-transfected with miR-206 mimics compared with NC mimic (*p<0.05).

that the upregulation of miR-206 induced by sh-Linc1749808 transfection could be reversed by a miR-206 inhibitor, but this pattern was not seen with administration of inhibitor-NC (Figure 5A). Through Transwell assays (Figure 5B), we found that the sh-Linc1749808-mediated suppression of migration and invasion was blocked by adding the miR-206 inhibitor to 97H cells. Preliminary studies have shown that miR-206 is involved in the development of liver cancer and it effectively inhibits the proliferation and metastasis of liver cancer through various downstream pathways, such as the YAP1, CDK14, PTP1B, and MAP3K1 pathways [12, 17–19]. We assessed the downstream pathway factors related to miR-206 after interfering with Linc1749808 through RT-PCR experiments. The results displayed that only YAP1 and MAP3K1 showed decreased expression with the downregulation of Linc1749808 and increased expression with the inhibition of miR-206 (Figure 5C). The same trend was also found in the levels of proteins related to EMT (E-cadherin and N-cadherin, Figure 5D).

Discussion

Globally, cancer mortality remains a huge public health challenge. Although advances have been made in understanding the genomic changes in many cancers, the molecular mechanisms underlying the development of HCC are poorly understood [20].

Abnormally expressed lncRNAs participate in the development, invasion, and metastasis of HCC through different pathways and different mechanisms [21, 22]. For example, Linc-KILH potentiates EMT through the KRT19 phosphorylation/Notch1 pathway to promote liver cancer metastasis [23]. LncRNAs are also key factors in the chemoresistance of HCC. Depletion of LINC01234 might efficiently decrease the resistance of HCC cells to cisplatin via the microRNA-31-5p/MAGEA3 axis [24]. LINC01352 can restrain HBV-related HCC by targeting miR-135b/APC [25]. Moreover, lncRNAs are also used in tumor screening and as biomarkers for determining prognosis [3]; for example, HOTTIP/HOXA13 is used as an indicator of prognosis in liver cancer [26]. These findings guided and support the significance and value of our follow-up research.

In the current study, we identified Linc1749808 through lncRNA microarray screening of liver cancer tissues. We first demonstrated that Linc1749808 was significantly upregulated in HCC tissues. In the analysis of clinical characteristics, Linc1749808 was closely related to tumor size, microvascular invasion, metastasis, and survival rate. Furthermore, Linc1749808 was located in the cytoplasm and highly expressed in most liver cancer cell lines. In CCK-8, Transwell

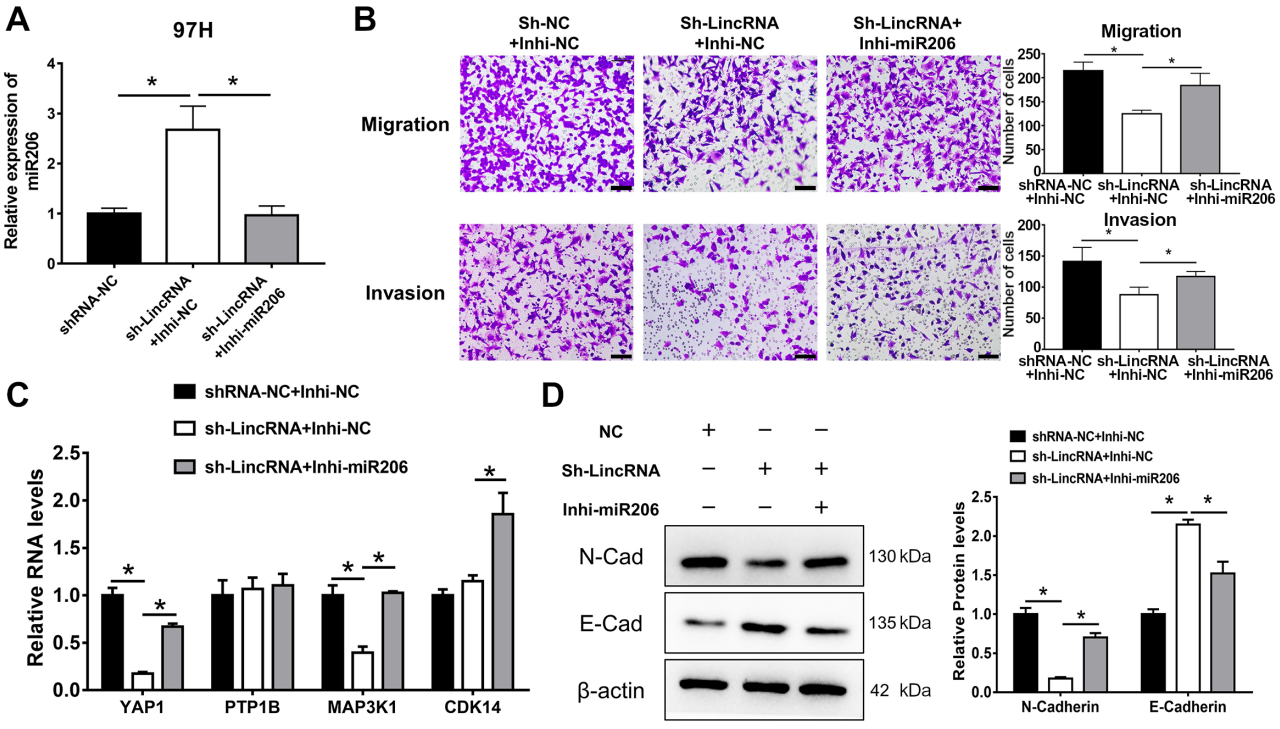


Figure 5. Linc1749808 regulates EMT by serving as a ceRNA for miR-206. A) The miR-206 levels in 97H cells were detected by RT-PCR. Cells were treated with shRNA-NC, shRNA-Linc1749808+inhibitor-NC, or shRNA-Linc1749808+inhibitor-miR-206. B) Cell morphology changes in the cells were detected by Transwell assay. Scale bar: 100 μm. C) Changes in miR-206-related downstream factors (YAP1, PTP1B, MAP3K1, and CDK14) were verified with RT-PCR. D) Western blot analysis of E-cadherin and N-cadherin proteins was performed in the above cell lines, and the levels were normalized to β-actin (right). The IOD was calculated for each band (left). Scale bar: 100 μm. The data are from at least three independent experiments and expressed as the mean ± SD (*p<0.05).

assays, and experiments in animal models, Linc1749808 was found to enhance the migration and invasion of HCC cells. A recent report showed that lncRNAs function as ceRNAs to competitively bind to miRNAs [27]. To illuminate the potential regulatory mechanism through which Linc1749808 affects HCC progression, we performed bioinformatic analysis and predicted that Linc1749808 contains binding sites for miR-206. Then, luciferase reporter assays showed that Linc1749808 could bind to miR-206. Furthermore, the negative correlation between the expression of Linc1749808 and miR-206 in HCC tissues reinforces the above hypothesis. Therefore, we confirmed that Linc1749808 reduces miR-206 expression by acting as a ceRNA. miR-206 was proven to function as a tumor suppressor by targeting different genes in liver cancer [11] and could also be a target of lincRNAs; for example, LINC00707 increases the viability and metastasis of HCC cells by sponging miR-206/CDK14 [19]. In addition, Transwell assays showed that inhibition of miR-206 could reverse the inhibitory effect of sh-Linc1749808 on 97H cell motility. LncRNAs that function as ceRNAs are involved in diverse biological processes in HCC cells, such as tumor cell proliferation, EMT, invasion, metastasis, and chemoresistance [28]. The EMT program is linked to metastasis because it causes the increased motility/invasiveness, related to a mesenchymal cell state, and cell separation from the primary tumor mass can be identified as the first step of the invasion-metastasis cascade [29]. We observed loss of N-cadherin and an increase in E-cadherin expression with downregulation of Linc1749808. However, inhibition of miR-206 caused tumor cells to undergo EMT, which has also been reported in lung cancer [30]. Whether EMT-related genes are directly or indirectly targeted by Linc1749808/miR-206 needs to be further verified experimentally. These results indicate that the Linc1749808/miR-206 axis plays an important role in promoting metastasis in liver cancer. Nevertheless, some of our results were perplexing. On the one hand, the metastasis ability of liver cancer cells was affected by interference with Linc1749808, but there was no obvious change in proliferation. There might be other pathways downstream of Linc1749808 that could block or neutralize the effects of the Linc1749808/miR-206 axis on proliferation. RNA pull-down technology, mass spectrometry, or microarray screening could be used to explore possible binding proteins or synergistic ceRNAs of Linc1749808. In addition, it has been demonstrated that many lncRNAs are promising biomarkers for

cancer diagnosis, prognosis, and treatment. For instance, high expression of lncRNA-ATB was found in the plasma of HCC patients with early recurrence and was proposed as a promising novel biomarker [31]. Linc1749808 was shown to be highly correlated with tumor metastasis and prognosis in this research. Multicenter observational studies on the diagnostic and therapeutic value of Linc1749808 are needed. This research provides preliminary results regarding Linc1749808/miR-206 but has certain limitations. We will perform more in-depth studies in future explorations.

In summary, the present study shows that Linc1749808 acts as a novel oncogene in liver cancer. Furthermore, Linc1749808 acts as a ceRNA to sponge miR-206 and in part regulate HCC metastasis. Therefore, the present findings broaden the current understanding of lncRNA function in HCC and provide useful information for novel biomarkers of HCC prognosis.

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