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LINC00205 promotes proliferation, migration and invasion of HCC cells by targeting miR-122-5p

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ABSTRACT

Long non-coding RNAs (lncRNAs) have been identified as crucial regulators in the tumorigenesis and progression of hepatocellular carcinoma (HCC). Recently, long intergenic non-protein coding RNA 205 (LINC00205) has been identified as a prognostic biomarker in HCC. However, the biological role of LINC0205 and its potential molecular mechanism are poorly investigated. Here, we found that the expression of LINC00205 was dramatically up-regulated in HCC tissues compared to adjacent nontumor tissues. Furthermore, the level of LINC00205 in both Hep3B and Huh7 cells was prominently higher than that in normal hepatic cell line LO2. Notably, the high expression of LINC00205 was strongly correlated with tumor size ≥5 cm, venous infiltration and advanced tumor stages. Functionally, LINC00205 knockdown obviously repressed the proliferation, migration and invasion of Hep3B and Huh7 cells *in vitro*. An inverse correlation between LINC00205 and miR-122-5p was detected in HCC tissues. Interestingly, LINC00205 knockdown increased the level of miR-122-5p in both Hep3B and Huh7 cells. Mechanistically, luciferase reporter assay demonstrated LINC00205 acted as a competing endogenous RNA (ceRNA) by directly interacting with miR-122-5p. More importantly, miR-122-5p overexpression significantly restrained the proliferation, migration and invasion of HCC cells. Collectively, our study provides solid evidence to support the oncogenic role of LINC00205 in HCC, which may be benefit for the improvement of HCC therapy.

1. Introduction

Hepatocellular carcinoma (HCC), the most common primary liver cancer, accounts for the 3rd commonest cancer-related deaths worldwide [1]. In recent decades, the therapeutic strategies have been improved [2]. However, the prognosis of HCC is still unsatisfied. Thus, it is imperative to investigate the molecular mechanism underlying HCC progression and develop therapeutic targets.

Long non-coding RNAs (lncRNAs) are identified as a group of transcripts over 200 nucleotides with poor protein coding potential and exert impressive roles in physiological and pathological processes including cancer [3,4]. The aberrant expression of lncRNAs has been widely detected in human cancer and modulate the expression of target genes involved in carcinogenesis and tumor metastasis [5,6]. Notably, accumulating studies have demonstrated the diagnostic and prognostic value of lncRNAs in HCC and deregulation of lncRNAs contributes to hepatocarcinogenesis and tumor progression [7–10]. For instance, up-regulation of lnc-PCDH9-13:1 expression is detected in tumor tissues, plasma and saliva of patients with HCC and salivary lnc-PCDH9-13:1 is recognized as a candidate biomarker for early HCC [7]. LncRNA MCM3AP antisense RNA 1 (MCM3AP-AS1) functions as an oncogene which contributes to HCC cell proliferation, cell cycle progression and apoptosis resistance by modulating miR-194-5p/forkhead box A1 (FOXA1) axis [8]. LncRNA cancer susceptibility candidate 2 (CASC2) plays a tumor suppressive role in HCC progression via sponging miR-367 to promote F-box and WD repeat domain containing 7 (FBXW7) expression [9]. Moreover, lncRNA down syndrome critical region 8 (DSCR8) functions as a competing endogenous RNA (ceRNA) by

Abbreviations: HCC, hepatocellular carcinoma; LncRNAs, long non-coding RNAs; MCM3AP-AS1, MCM3AP antisense RNA 1; FOXA1, forkhead box A1; CASC2, cancer susceptibility candidate 2; FBXW7, F-box and WD repeat domain containing 7; DSCR8, down syndrome critical region 8; ceRNA, competing endogenous RNA; FZD7, frizzled-7; LINC00205, long intergenic non-protein coding RNA 205; PDAC, pancreatic ductal adenocarcinoma; TCGA, The Cancer Genome Atlas; TNM, tumor-node-metastasis; MAT2A, methionine adenosyltransferase 2A; PTTG3P, pituitary tumor-transforming 3, pseudogene; CASC9, cancer susceptibility 9; HNRNPL, heterogeneous nuclear ribonucleoprotein L

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directly interacting with miR-485-5p to enhance frizzled-7 (FZD7)-mediated Wnt/β-catenin pathway in HCC [10]. Up to date, long intergenic non-protein coding RNA 205 (LINC00205) is rarely investigated in human cancer. Two studies report that the elevated expression of LINC00205 predicts poor prognosis in HCC and pancreatic ductal adenocarcinoma (PDAC) [11,12]. But, the biological role of LINC00205 and its potential molecular mechanism in HCC progression remain poorly known.

In the present study, the correlation between LINC00205 overexpression and HCC progression was disclosed. LINC00205 was identified as a molecular sponge for miR-122-5p to promote the growth of HCC cells. Targeting LINC00205/miR-122-5p axis may be benefit for HCC therapy.

2. Materials and methods

2.1. Patients and tissue samples

A total of 80 pathologically diagnosed HCC tissues and matched tumor-adjacent tissues were obtained the First Affiliated Hospital of Xi'an Jiaotong University. This study was approved by the Ethic Committee of the First Affiliated Hospital of Xi'an Jiaotong University and the informed contents were signed by all enrolled patients. All patients did not receive preoperative therapies. The samples were maintained at -80℃ for later analysis. The clinicopathological features of patients were presented in Table 1.

2.2. Cell culture and transfection

The human normal hepatic cell line LO2, HCC cell lines (Hep3B and Huh7) and human embryonic kidney 293 T (HEK293T) cells were maintained in our laboratory and cultured under standard condition [13,14]. The lentivector-mediated LINC00205 shRNA (shLINC00205) and non-targeting (NT) shRNA were designed and obtained from Geneseed Biotech (Guangzhou, China). Vectors mediated miR-122-5p mimics and corresponding negative control were purchased from GeneCopoeia (Guangzhou, China). The transfection assay was carried out using Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA) following the protocols.

Table 1
Correlation between LINC00205 expression and clinicopathologic characteristics in hepatocellular carcinoma.

Characteristics		n = 80	LINC00205 expression		*P*
			Low (n = 40)	High (n = 40)	
Age (y)	< 50	33	16	17	0.968
	≥50	47	24	23	
Sex	Male	65	31	34	0.390
	Female	15	9	6	
HBV	Absent	26	16	10	0.152
	Present	54	24	30	
Serum AFP level (ng/mL)	< 20	28	17	11	0.160
	≥20	52	23	29	
Tumor size (cm)	< 5	25	17	8	0.030*
	≥5	55	23	32	
No. of tumor nodules	1	66	35	31	0.239
	≥2	14	5	9	
Cirrhosis	Absent	37	22	15	0.116
	Present	43	18	25	
Venous infiltration	Absent	48	29	19	0.022*
	Present	32	11	21	
Edmondson-Steiner grading	I + II	54	30	24	0.152
	III + IV	26	10	16	
TNM tumor stage	I + II	64	36	28	0.025*
	III + IV	16	4	12	

HBV, hepatitis B virus; AFP, alpha-fetoprotein; TNM, tumor-node-metastasis.
* Statistically significant.

2.3. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from tissues and cell lines using RNeasy Mini Kit (Qiagen, Valencia, CA, USA), and was reversely transcribed to cDNA using a TIANScript RT Kit (Tiangen biotech, Beijing, China). The qRT-PCR was carried out using The ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with a QuantiTect SYBR Green PCR Kit (Qiagen). qPCR primers for LINC00205, GAPDH, miR-122-5p and U6 were designed and obtained from GeneCopoeia Inc. The levels of LINC00205 and miR-122-5p were normalized to GAPDH and U6, respectively.

2.4. Cell proliferation detection

Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Dojindo, Japan) assay was used to evaluate the cell viability of HCCs after transfection following the manufacturer's instructions. The results were determined by measuring the light absorbance at 450 nm on a microplate reader (TECAN, Salzburg, Austria). For colony formation assay, 500 HCC cells were added into 6-well plates and then cultured for two weeks. The colonies were fixed with 4% paraformaldehyde for 20 min, stained with 0.1% (w/v) crystal violet for 30 min, and were quantified with the Image J software.

2.5. Transwell assay

HCC cells were added into the upper chambers of Matrigel-uncoated (cell migration) or -coated Transwells (BD Biosciences, San Jose, CA, USA). Serum-free medium was added to the upper chambers, and medium with 10% FBS was added into the lower chambers. The plated cells were allowed to migrate for 24 h, and at 24 h after incubation, the migrated or invaded cells (on the bottom of the filters) were fixed using 4% paraformaldehyde and stained with 0.5% crystal violet for 30 min. The number of migrated or invaded HCCs were counted under a light microscope by randomly selecting five fields.

2.6. Luciferase reporter assays

The fragment of LINC00205 was amplified by PCR using human genomic DNA and the corresponding mutated fragment (mt LINC00205) was generated using a QuickChange II Site-Mutagenesis kit (Agilent, San Jose, CA, USA). Then these fragments were sub-cloned into pGL3 reporter vector (Promega, Madison, USA). For the luciferase reporter assays, HEK293 T cells were seeded in a 6-well plate and co-transfected with pGL3 reporter vector containing wt or mt LINC00205 and miR-122-5p mimics or negative control. Cells were collected at 48 h after co-transfection and analyzed for luciferase activity using a Dual-luciferase reporter kit (Promega) [15].

2.7. Statistical analysis

All the data analysis was performed using the GraphPad Prism Software (Version 8.0, GraphPad Software, La Jolla, CA, USA). All the experimental data were shown as mean ± standard deviation (SD). The statistical significance between different treatment groups were determined by Student's *t*-test or ANOVA. Chi-square test was used to reveal the clinical significance of LINC00205. Pearson correlation test was carried out to confirm the correlation between LINC00205 and miR-122-5p expression. The level of statistical significance was set at $P < 0.05$.

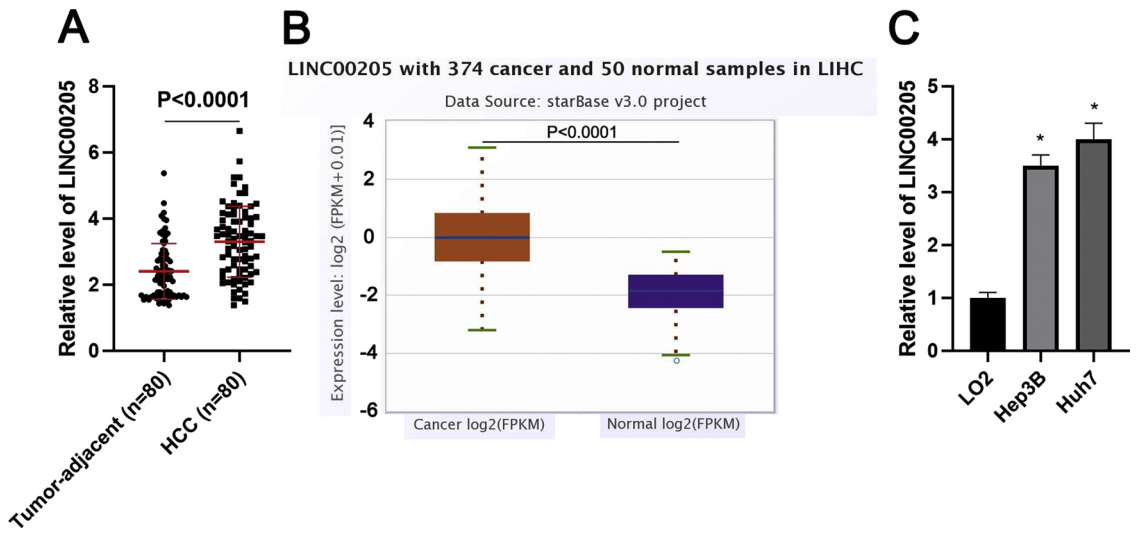

Fig. 1. The expression of LINC00205 in HCC. (A) The expression of LINC00205 in HCC tissues (n = 80) and matched noncancerous tissues (n = 80) was detected by qRT-PCR. (B) TCGA data from starBase V3.0 online platform indicated that the expression of LINC00205 in HCC tissues (n = 374) was significantly higher than that in normal liver tissues (n = 50). (C) The levels of LINC00205 between HCC cell lines (Hep3B and Huh7) and LO2 cells were analyzed by qRT-PCR. n = three independent repeats. *P < 0.05.

3. Results

3.1. LINC00205 is highly expressed in HCC

The expression of LINC00205 in 80 pairs of HCC and matched nontumor tissues was detected by qRT-PCR. The results demonstrated that the expression of LINC00205 in HCC tissues was markedly higher than that in corresponding nontumor tissues (P < 0.0001, Fig. 1A). Moreover, The Cancer Genome Atlas (TCGA) data from starBase V3.0 online platform [16,17] also indicated that LINC00205 expression in HCC tissues was 4.73-fold higher than that in normal liver tissues (P < 0.0001, Fig. 1B). Additionally, the upregulation of LINC00205 expression was confirmed in both Hep3B and Huh7 cells compared to normal hepatic cell line LO2 (P < 0.05, Fig. 1C). Next, HCC specimens were grouped into low and high LINC00205 groups according to the median level of LINC00205. As shown in Table 1, high level of LINC00205 was closely correlated with tumor size ≥5 cm (P = 0.030), venous infiltration (P = 0.022) and advanced tumor-node-metastasis (TNM) stages (P = 0.025). Thus, our results suggested that LINC00205 was overexpressed in HCC and correlated with poor prognostic features.

3.2. LINC00205 knockdown inhibits HCC cell proliferation, migration and invasion

To investigate the biological function of LINC00205 in HCC cells, LINC00205 expression was dramatically knocked down by a specific shRNA in Hep3B and Huh7 cells (P < 0.05, Fig. 2A). CCK-8 assay revealed that LINC00205 knockdown prominently repressed the viability of HCC cells (P < 0.05, Fig. 2B). Furthermore, the colonies formed by HCC cells were markedly reduced by knockdown of LINC00205 (P < 0.05, Fig. 2C). Additionally, LINC00205 silencing obviously suppressed the migration and invasion of HCC cells (P < 0.05, Fig. 2D). These data suggested that LINC00205 exerted a tumor-promoting role in HCC.

3.3. LINC00205 inversely modulates miR-122-5p expression in HCC cells

To explore the molecular mechanism underlying the oncogenic role of LINC00205 in HCC cells, the candidate targets of LINC00205 were predicted using starBase V3.0 online platform [16,17]. Among these predicted miRNAs, only miR-122-5p expression was significantly downregulated (P < 0.0001, Supplementary Fig. 1) and inversely correlated LINC00205 level in HCC tissues from TCGA database (r = −0.364, P < 0.0001, Fig. 3A). Moreover, an inverse correlation between miR-122-5p and LINC00205 expression was further confirmed in our HCC tissues (r = −0.384, P = 0.0004, Fig. 3B). Next, we found that knockdown of LINC00205 significantly reduced the level of miR-122-5p in both Hep3B and Huh7 cells (P < 0.05, Fig. 3C). More importantly, miR-122-5p overexpression dramatically reduced the fluorescence intensity of vector containing wt LINC00205 (P < 0.05, Fig. 3D). However, ectopic expression of miR-122-5p had no impact on the luciferase activity of vector carrying mt LINC00205 (Fig. 3D). Thus, these evidences supported that LINC00205 functioned as a molecular sponge for miR-122-5p.

3.4. MiR-122-5p suppressed the proliferation, migration and invasion of HCC cells

To further study whether miR-122-5p mediates the biological role of LINC00205 in HCC cells, miR-122-5p expression was overexpressed by transfecting its mimics in Hep3B and Huh7 cells (P < 0.05, Fig. 4A). CCK-8 assay revealed that miR-122-5p overexpression prominently repressed the viability of HCC cells (P < 0.05, Fig. 4B). Furthermore, the colonies formed by HCC cells were markedly reduced by overexpression of miR-122-5p (P < 0.05, Fig. 4C). Additionally, restoration of miR-122-5p expression obviously suppressed the migration and invasion of HCC cells (P < 0.05, Fig. 4D and Supplementary Fig. 2). Collectively, our results indicated that miR-122-5p played a tumor suppressive role in HCC, which was consistent with LINC00205 knockdown.

4. Discussion

The aberrant expression of lncRNAs are implicated in the diagnosis and prognosis of HCC [18,19]. For instance, lncRNAs ENSG00000258332.1 and LINC00635 are enriched in serum exosomes from HCC patients and function as diagnostic and prognostic biomarker [20]. Moreover, studies suggest that lncRNA MCM3AP-AS1 is strongly correlated with poor clinical outcomes of HCC [8,21]. Currently, we

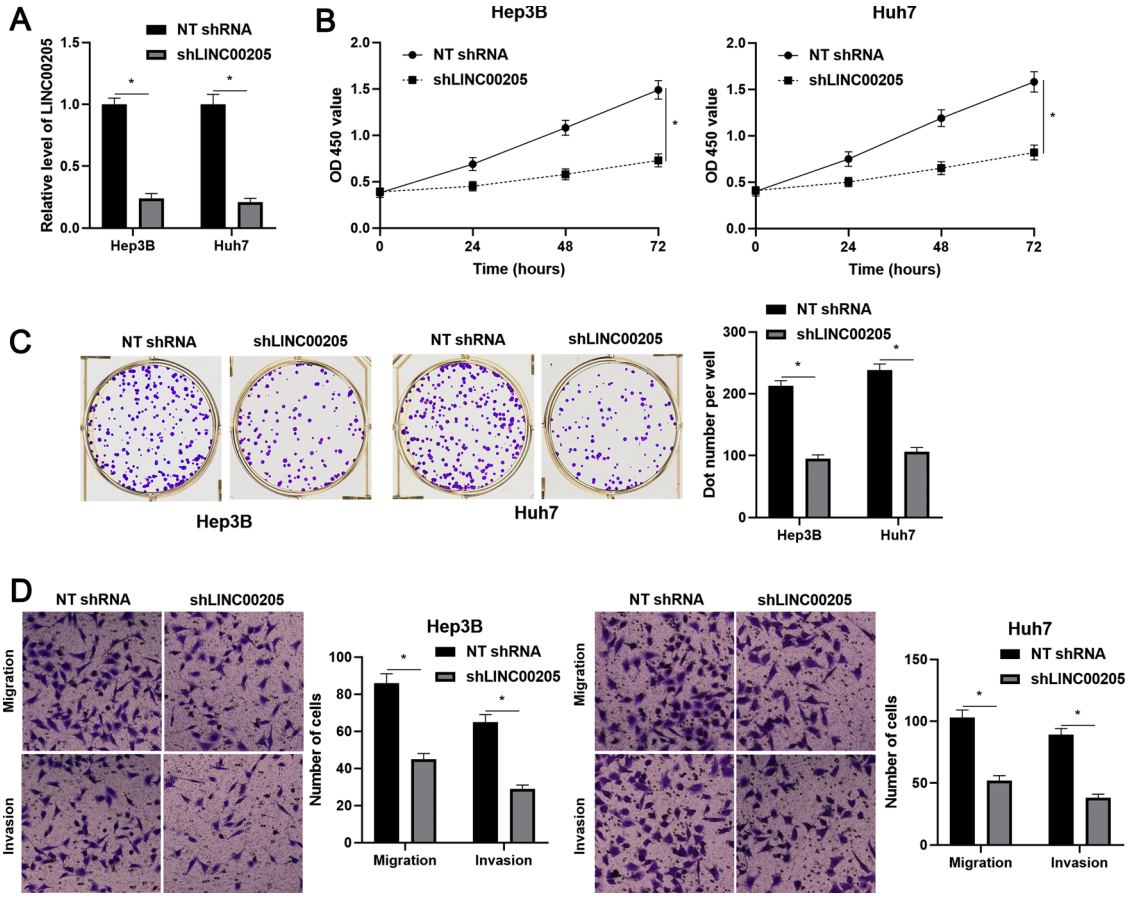

Fig. 2. LINC00205 knockdown suppresses the proliferation, migration and invasion of HCC cells. (A) Hep3B and Huh7 cells that were transfected with lentivector-mediated LINC00205 shRNA (shLINC00205) or non-targeting (NT) shRNA were detected by qRT-PCR for LINC00205 expression. (B) CCK-8 assay revealed that LINC002056 knockdown inhibited the viability of HCC cells. (C) The colonies formed by HCC cells were decreased by LINC00205 knockdown. (D) LINC00205 knockdown repressed the migration and invasion of HCC cells as suggested by Transwell assays. n = three independent repeats. *P $<$ 0.05.

firstly found the upregulation of LINC00205 expression in HCC. The high level of LINC00205 was closely associated with larger tumor size, more venous infiltration and advanced tumor stages. A recent study has reported that LINC00205 overexpression predicts poor prognosis of HCC base on the analysis of TCGA data [11]. Additionally, another study also identifies LINC00205 as a prognostic biomarker for PDAC [12]. Thus, these results indicated a promising prognostic value for predicting poor survival of HCC patients.

More and more studies provide evidences to support the crucial roles of lncRNAs in the carcinogenesis and metastasis of HCC. Fox example, lncRNA SNHG6 exerts a tumor promoting role in hepatocarcinogenesis by acting as a molecular sponge for miR-1297 to promote methionine adenosyltransferase 2A (MAT2A) [22]. LncRNA pituitary tumor-transforming 3, pseudogene (PTTG3P) contributes to tumor growth and metastasis of HCC via enhancing PTTG1 expression and activating PI3K/AKT pathway [23]. Additionally, lncRNA cancer susceptibility 9 (CASC9) regulates the proliferation and apoptosis of HCC cells by interacting with heterogeneous nuclear ribonucleoprotein L (HNRNPL) and subsequently modulating AKT pathway [24]. Currently, the biological function of LINC00205 in human cancer is still unclear. Here, we found that LINC00205 knockdown impaired HCC cell proliferation, migration and invasion through functional experiments, which provided strong evidence to support an oncogenic role of LINC00205 in HCC. Increasing studies demonstrate that lncRNAs act as ceRNAs to regulate miRNAs and their downstream targets [25,26].

LncRNA-MUF directly interacts with miR-34a to promote Snail expression in HCC [25]. And lncRNA Linc00176 functions as a ceRNA by sponging miR-8 and miR-185 to enhance the proliferation and survival of HCC cells [26]. Our study firstly recognized LINC00205 as a molecular sponge for miR-122-5p in HCC. LINC00205 directly interacting with miR-122-5p and inversely regulated its abundance in HCC cells. Recently, LncRNAs ANRIL and LINC01296 are also identified as molecular sponge for miR-122-5p in HCC [27,28]. Previous studies have revealed that the tumor suppressive role of miR-122-5p in the proliferation, migration and invasion of HCC cells [27–29]. And the current study further demonstrated that miR-122-5p inhibited HCC cell proliferation, migration and invasion, suggesting that miR-122-5p possibly mediated the biological role of LINC00205 in HCC.

To conclude, we identified a novel oncogenic lncRNA LINC00205 and provide evidence to support the interaction between LINC00205 and miR-122-5p in HCC. LINC00205/miR-122-5p axis might be potential therapeutic targets for HCC.

5. Conclusions

Taken together, our results in this study indicate that LINC00205 expression is upregulated in HCC tissues and cells compared to that in normal controls. The upregulation of LINC00205 is correlated with poor prognostic features of HCC patients. Knockdown of LINC00205 results in reduced abilities of cell proliferation, migration and invasion by targeting miR-122-5p. LINC00205/miR-122-5p axis may be a novel target for HCC targeted therapy.

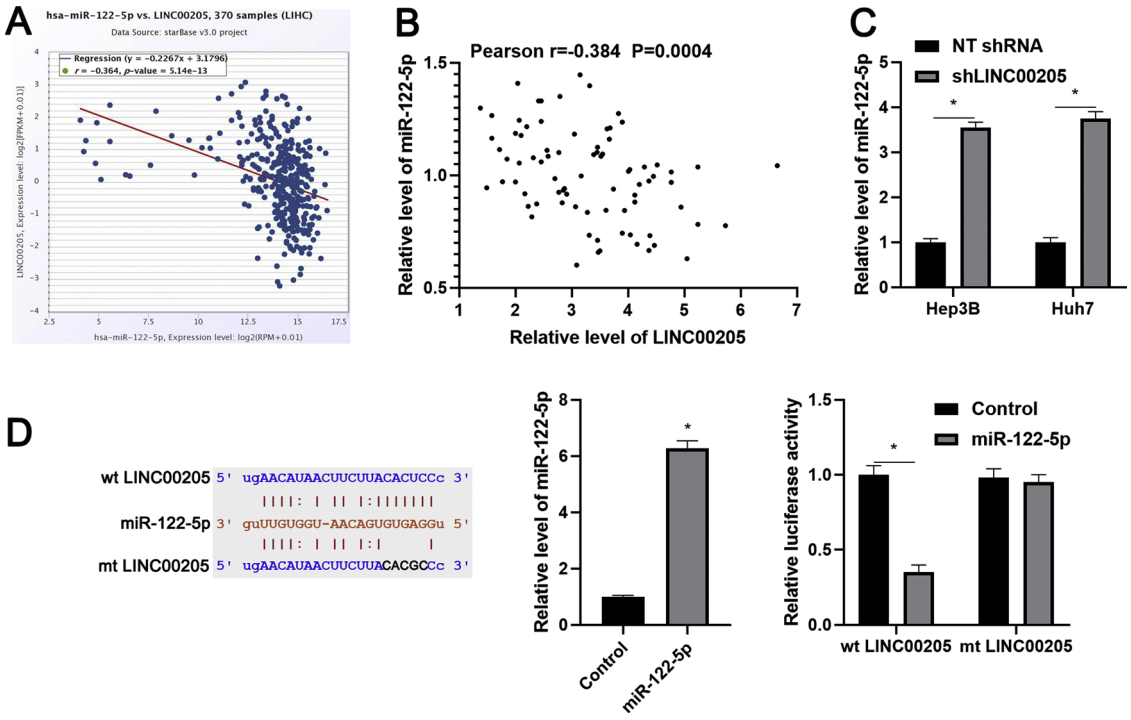

Fig. 3. LINC00205 functions as molecular sponge for miR-122-5p in HCC cells. (A) TCGA data from starBase V3.0 online platform indicated that LINC00205 expression was inversely correlated miR-122-5p level in HCC tissues (n = 370). (B) A negative correlation between LINC00205 and miR-122-5p expression was confirmed in HCC tissues (n = 80) as suggested by Pearson correlation analysis. (C) Hep3B and Huh7 cells that were transfected with lentivector-mediated LINC00205 shRNA (shLINC00205) or non-targeting (NT) shRNA were detected by qRT-PCR for miR-122-5p expression. (D) Co-transfection of miR-122-5p mimics or negative control and wild type (wt) or mutated (mt) LINC00205 vector was performed in HEK293 T cells and the relative intensity was measured. n = three independent repeats. *P < 0.05.

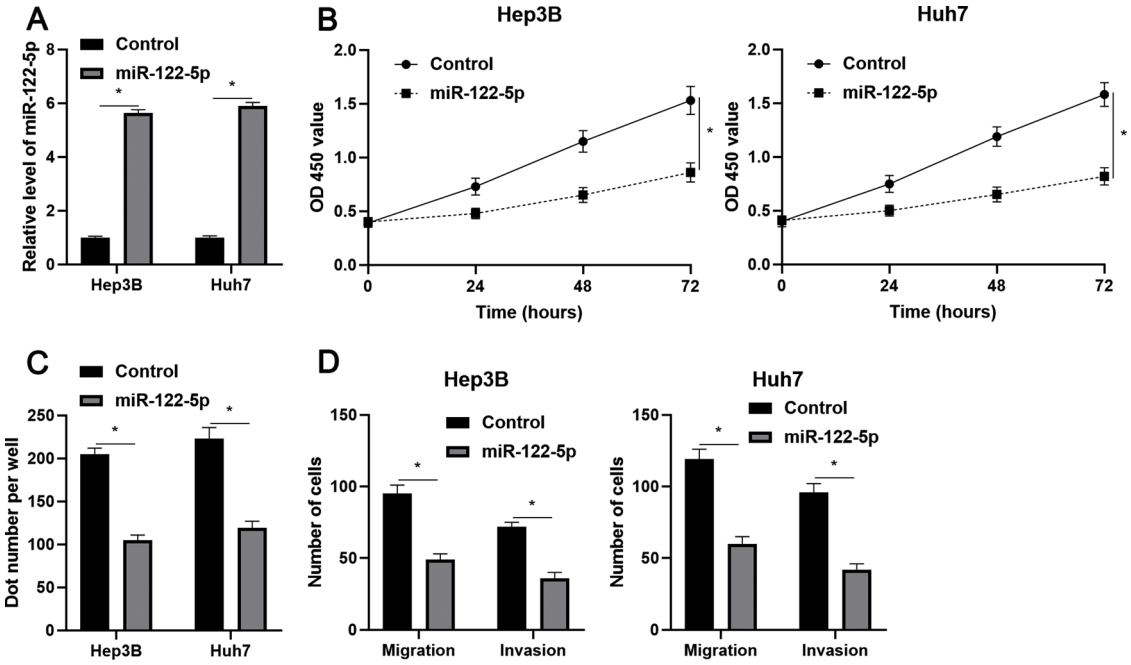

Fig. 4. miR-122-5p overexpression inhibits HCC cell proliferation, migration and invasion. (A) Hep3B and Huh7 cells that were transfected with miR-122-5p mimics or negative control were detected by qRT-PCR for miR-122-5p expression. (B) CCK-8, (C) colony formation and (D) Transwell assays were performed to detect the proliferation, migration and invasion of HCC cells. n = three independent repeats. *P < 0.05.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152515.

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