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Original Paper

Long Non-Coding MALAT1 Functions as a Competing Endogenous RNA to Regulate Vimentin Expression by Sponging miR-30a-5p in Hepatocellular Carcinoma

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Key Words
Hepatocellular carcinoma • MALAT1 • miR-30a-5p • Vimentin

Abstract
Background/Aims: Hepatocellular carcinoma (HCC) has a high morbidity as well as mortality and is believed to be one of the most prevalent cancers worldwide. The long non-coding RNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is involved in numerous cancers, including HCC. This study aimed to explore the role of MALAT1 in HCC progression. ***Methods:*** The expression levels of MALAT1 and Vimentin in HCC tissues and relative pair-matched adjacent normal liver tissues were analyzed by RT-PCR, and immunohistochemistry. Using bioinformatics analysis and dual-luciferase assay, we examined the correlation between MALAT1 and miR-30a-5p. Dual-luciferase assay and western blotting suggested that Vimentin was a target of miR-30a-5p. A wound healing assay and transwell assays were employed to determine the effect of MALAT1 and miR-30a-5p on cell migration and invasion in HCC. ***Results:*** Our data demonstrated that the levels of MALAT1 and Vimentin were upregulated in HCC tissues and that miR-30a-5p was a direct target of MALAT1. Silenced MALAT1 and overexpressed miR-30a-5p each inhibited cell migration and invasion. Additionally, dual-luciferase assay and western blotting demonstrated that MALAT1 could competitively sponge miR-30a-5p and thereby regulate Vimentin. ***Conclusion:*** Our data suggest that MALAT1 acts as an oncogenic lncRNA that promotes HCC migration and invasion. Therefore, the MALAT1-miR-30a-5p-Vimentin axis is a potential therapeutic target and molecular biomarker in HCC.

Y. Pan and S. Tong contributed equally to this work.

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Introduction

Hepatocellular carcinoma (HCC) is the one of the most common types of primary liver cancer in world with increasing morbidity and mortality are increasing [1]. Hepatitis B virus (HBV), hepatitis C virus (HCV) infection, alcoholic liver disease, and nonalcoholic steatohepatitis (NASH) are well-established risk factors for HCC [2, 3]. Although the survival of HCC patients has improved, the relative 5-year survival rate of liver cancer remains low, frequency of recurrence and metastasis remains high [4]. Therefore, it is important to explore the molecular mechanisms critical to the pathogenesis of HCC. This study provides data that may benefit HCC therapeutics development and improve survival. Recent studies have demonstrated that MALAT1 is involved in HCC progression [5].

Long non-coding RNAs (lncRNAs) have a length greater than 200 nucleotides [6, 7], and many studies have shown that these molecules play a pivotal role in many diseases, including cancer [8]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a highly conserved and widely expressed lncRNA that is over 8 kb in length [9], and regulates proliferation as well as metastasis in many cancers [10, 11]. Recently, some studies have reported a new regulatory model in which lncRNA can act as a competing endogenous RNA (ceRNA) and crosstalk with mRNAs by competitively binding their common miRNAs [12]. LncRNA works as "sponge" in miRNA sequestration, leaving mRNA intact, thus modulating the expression at the post-transcriptional level [13]. To date, MALAT1 has been demonstrated to be a ceRNA that modulates some miRNAs, for instance, miR-195 [13], miR-363-3p [14], and miR-183 [15] in cancer.

In this study, the expression of MALAT1 was found to be upregulated in HCC tissues, and knockdown of MALAT1 inhibited cell migration and invasion. Moreover, luciferase reporter assays demonstrated that MALAT1 acts as a ceRNA sponge for miR-30-5p. Furthermore, miR-30a-5p was able to regulate its downstream target, Vimentin, a compound implicated in HCC migration and invasion. Our study suggests that MALAT1 regulates HCC migration and invasion, and may provide a potential target for HCC therapy.

Materials and Methods

Patients and samples

Human HCC tumor tissue cDNA arrays (cDNA-HLivH60PG01) and human HCC tumor tissue arrays (HLivH030PG03) were purchased from Shanghai Outdo Biotech (Shanghai, China). The cDNA arrays included thirty HCC tissues and relative pair-matched adjacent normal liver tissues, and HCC tumor tissue arrays including thirty tissues and relative pair-matched adjacent normal liver tissues.

Cell culture

Human liver cancer cell lines HepG2, Huh7 and HEK-293T cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The colon cancer cell lines HCT116 wild-type and Dicer-deficit (HCT116-/-) were kindly donated by Professor B. Vogelstein of Johns Hopkins University. HCT116 and HCT116-/- were maintained in McCoy's 5A medium and the other cell lines were grown in RPMI-1640 medium. All culture media contained 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific) and 100 U/ml penicillin/streptomycin (Gibco, Thermo Fisher Scientific), and were incubated at 37°C with 5% CO_2 95% air.

Immunohistochemistry

Whole tissue samples were paraffin-embedded and cut into 4-μm thick tissue sections, bried at 65°C for 12 h, and then cooled at 4°C. Sections were dewaxed, washed with PBS, and repaired in sodium citrate (pH = 6.0). Subsequently, sections were incubated in 0.3% hydrogen peroxide at room temperature for 15 min, then blocked in 10% fetal calf serum for 0.5 h and incubated with primary antibody diluted 1:100 at 4°C overnight. The section was subsequently incubated with secondary antibody at 37°C for 30 min

and developed using DAB. After washing three times with PBS, the section was stained using hematoxylin for 3 min and sealed for imaging. The stained slides were viewed and photographed using an OLYMPUS BX60 microscope (Olympus Corp., Tokyo, Japan), with all images acquired with using the same conditions. The final score represents the average of the percentage of stained area [scored as 0 (without staining), 1 (<25%), 2 (25-50%), 3 (50-75%) and 4 (>75%)].

Plasmid construction

The fragment of MALAT1 containing the predicted miR-30a-5p binding site and the 3'UTR of Vimentin was amplified from human cell genomic DNA and was then cloned into a psi-CHECK-2 vector (Promega, Madison, WI, USA) using the XhoI and NotI sites. Mutated plasmids were generated using the QuickChange® Site-Directed Mutagenesis kit (Stratagene, Agilent Technologies, Wilmington, DE, USA). In order to detect the function of Vimentin, the full-length of Vimentin was constructed into pcDNA 3.1 plasmid (Invitrogen, Carlsbad, CA, USA). All constructs were constructed using the In-Fusion® Clone Kit (Clontech).

2.5. RNA extraction and real-time PCR

For real-time PCR analyses, total RNA was extracted from cells using Trizol® reagent (Ambion®, Life-Technologies). Clearance of DNA contamination in RNA samples and cDNA synthesis was performed using the PrimeScript® RT reagent Kit with gDNA Eraser according to the manufacturer's instructions (TaKaRa Bio). Real-time PCR was subsequently performed using the ABI-7500 System employing SYBR® Select Master Mix (Applied Biosystems®, Life-Technologies). Primer sequences were:

MALAT1: F, 5-AAAGCAAGGTCTCCCCACAAG-3

MALAT1: R,5-GGTCTGTCTAGATCAAAAGGCA-3

Vimentin: F,5-AACGTGCGAGTGTCTAACGG-3

Vimentin: R,5-CCCTCTAGGGGTTTGTGATTCT-3

GAPDH: F: 5-GAGTCAACGGATTTGGTCGT-3

GAPDH R: 5-GACAAGCTTCCCGTTCTCAG-3

The specific RT-PCR primers for miR-30a-5p and U6 were purchased from Ribo (RiboBio Co., Ltd., Guangzhou, China).

Western Blot Analysis

Whole-cell lysate preparation and western blot analysis were performed as previously described [16]. Briefly, whole cells were lysed using RIPA lysis buffer with 1% protease inhibitor. The protein concentration of the extracts was quantified using bicinchoninic acid reagent (BCA) (Thermo Fisher Scientific), and protein samples were separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked in 5% non-fat milk for 2 h, then incubated with primary antibody against Vimentin (Cell Signaling Technology, USA) overnight at 4 °C. Then the membrane was incubated with horseradish peroxidase-conjugated secondary antibodies (Thermo Fisher, Life-Technologies, Carlsbad, CA, USA) at room temperature for 2 h. The protein bands were detected using enhanced chemiluminescence (ECL) reagent (Thermo Scientific, USA), and identified and quantified using Image Lab Software (Version 2.0; Bio-Rad Laboratories, Berkeley, CA, USA).

Immunofluorescence assay

HCC cells were fixed with 4% paraformaldehyde for 10 min, and then permeabilized in 0.1% Triton X-100 for 10 min. The cells were washed and incubated with 10% goat serum in PBS for 2 h at room temperature, and then incubation with antibodies against Vimentin (5741, Cell Signaling Technology, Danvers, MA, USA) at a dilution of 1:100 at 4°C overnight. Following washing, cells were incubated with FITC-conjugated secondary antibody (A0562, Beyotime, Nantong, China) for 2 h at room temperature. The cells were then washed and incubated with PI 10 min. Cells were observed and images were captured by fluorescence microscopy (Olympus IX71, Japan).

Luciferase reporter assay

Luciferase reporter assays were performed as previously described [17]. HEK293T cells were seeded into 6-well plates and co-transfected with the appropriate plasmids and miR-30a-5p mimics. Twenty-four hours after transfection, cells were harvested and firefly as well as *Renilla* luciferase activities were

measured using the Luciferase Reporter Gene assay kit from Beyotime Institute of Biotechnology (Nantong, China). *Renilla* luciferase was used as an internal control and the RLU (relative light units) ratio of firefly luciferase relative to *Renilla* luciferase was calculated.

Wound healing migration assay

HCC cells were seeded into 6-well plates and grown to 90% confluence. Twenty-four hours after transfection, a vertical wound was created using a 200-μl pipette tip. Then the cells were washed with PBS three times and medium without serum was added into the wells. After 24 h incubation, the wound was observed and random fields in each well were selected for imaging. The images were analyzed by ImageJ and the distance of wound closure was used to estimate the migration ability.

Transwell invasion assay

Cell invasive ability was assessed using 24-well Transwell plates (Corning). For the invasion assay, before cell seeding, the Matrigel was diluted in serum-free medium to a concentration of 1 mg/ml. Diluted Matrigel (100 μl) per well was added into the top chamber and incubated for 4 h at 37 ˚C. Then 24 h after transfection, 200, 000 cells per well were seeded into the top chamber and maintained in serum-free medium. Medium (600 μl) containing 10% FBS was added into the bottom chamber. After incubation for 6 h at 37 °C, cells that migrated through the pore polycarbonate membrane were fixed with methanol and stained with 0.05% crystal violet. Then, images of the stained cells were captured using microscopy (Olympus IX71, Japan).

Xenograft mouse model

All animal experiments were approved by the Animal Care and Use Committee of Harbin Medical University. Approximately 1×10^7 HepG2 cells stably expressing control shRNA or shRNA-MALAT1 were subcutaneously injected in male BALB/c nude mice (4 – 6 weeks old). Tumor volume and size were measured every 7 days, and tumor volume was calculated using the formula: $V = (\text{length} \times \text{width}^2)/2$. After 28 days the mice were sacrificed, and their tumors were excised and fixed in 4% paraformaldehyde for 24 h. These were then analyzed by H&E staining and immunohistochemical staining.

Statistical analysis

All results are expressed as mean ± standard deviation (S.D.). Student's t-test was used for comparisons between two groups. Significance of correlations was determined using the Pearson's test. Differences were considered statistically significant if $P < 0.05$. All statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Each experiment was repeated at least three times.

Results

MALAT1 and Vimentin are overexpressed in HCC tissues and cells

To investigate the roles of MALAT1 and Vimentin in hepatocellular carcinoma, we detected the expression levels in 30 pairs of HCC tissues and relative pair-matched adjacent normal tissues by RT-PCR analysis. As shown in Fig. 1A, B, MALAT1 and Vimentin were significantly increased in HCC tissues compared to adjacent normal tissues. Our results demonstrated MALAT1 expression is positively correlated with Vimentin levels (Fig. 1C). Additionally, IHC results showed that Vimentin expression was significantly increased in HCC tissues as compared to those in adjacent normal tissues (Fig. 1D). A panel of immortal human liver cell line and HCC cell lines were selected to examine the MALAT1 and Vimentin expression levels. The levels of MALAT1 and Vimentin were significantly increased in HCC cell lines (Fig. 1E, F). Subsequently, we knocked down the expression of MALAT1 in Huh7 cells using three different siRNAs against MALAT1. The effect of siRNA-MALAT1-1 was found to be most significant (Fig. 1G), so siRNA-MALAT1-1 was used in the following experiments.

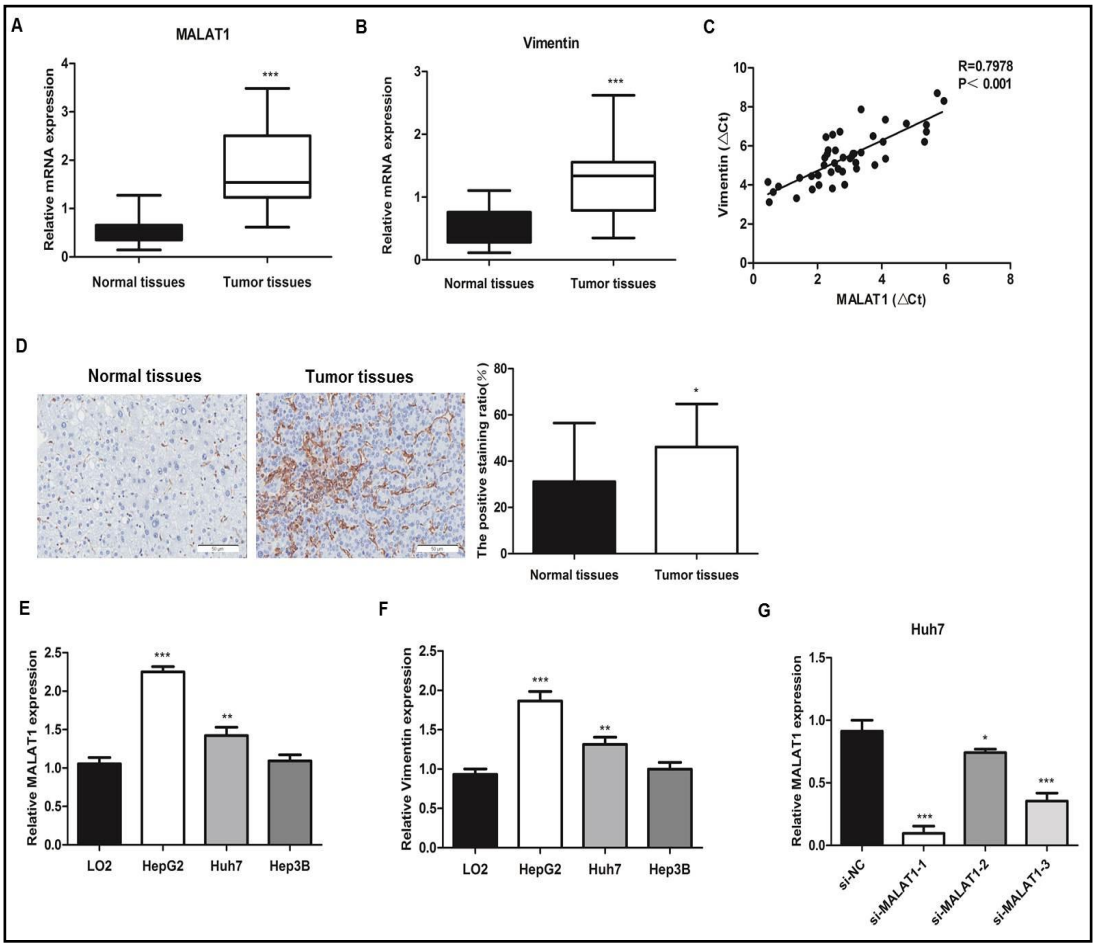

Fig. 1. MALAT1 and Vimentin are overexpressed in HCC tissues and cells. (A) Relative expression of MALAT1 and (B) Vimentin in HCC tissues and paired adjacent tissues (n = 30) were detected by qRT-PCR. Relative levels were normalized to those of GAPDH. (C) Pearson's correlation between MALAT1 and Vimentin. (D) The positive staining ratio of of Vimentin in HCC tissues and paired adjacent tissues (n = 15) (Immunohistochemistry, 20×), quantification on the right. *p<0.05, ***p<0.001 vs Normal tissues. (E) Relative expression of MALAT1 and (F) Vimentin in HCC cell lines were detected by qRT-PCR and compared with those in the normal liver cell line LO2. Relative levels were normalized to those of GAPDH. (n = 3), **p<0.01, ***p<0.001vs LO2. (G) Interference efficiency of si-MALAT1. Data are presented as means ± SD of three independent experiments. (n = 3), *p<0.05, ***p<0.001vs si-NC.

MALAT1 negatively regulates miR-30a-5p in HCC cells

As described in the introduction, an increasing amount of studies have reported that lncRNAs can act as a ceRNAs or molecular sponges regulating miRNAs. Computational analysis using LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr/index.php?r =lncbasev2) predicted that miR-30a-5p binding sites in MALAT1. The potential binding site shown in Fig. 2A, wild type and mutated binding sites were cloned into luciferase reporter plasmids, generating the plasmids MALAT1-WT and MALAT1-MUT, respectively. Co-transfection of plasmids and miR-30a-5p mimics in 293T cells, detected that the over-expression of miR-30a-5p significantly decreased the MALAT1-WT than MALAT1-MUT. miR-30a-5p expression was significantly downregulated in HCC tissues compared with that in adjacent normal tissues (Fig. 2B). Interestingly, MALAT1 levels correlated negatively with miR-30a-5p levels (Fig. 2C). Next, the expression of miR-30a-5p was explored in a panel of immortal human liver cell line and HCC cell lines. The levels of miR-30a-5p was significantly decreased in HCC cell lines (Fig. 1D). Furthermore, the expression of miR-30a-5p was up-

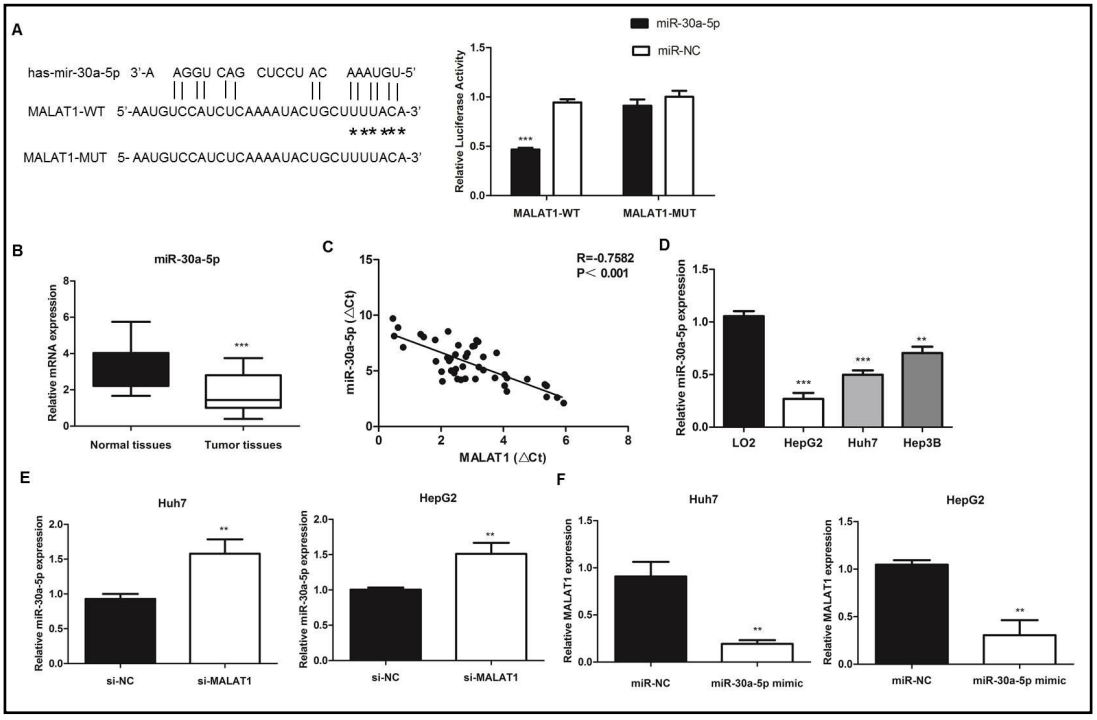

Fig. 2. MALAT1 negatively regulates miR-30a-5p in HCC cells. (A) Complementarities of sequences between miR-30a-5p and MALAT1. Luciferase assay showed miR-30a-5p could target MALAT1. Luminescence (a.u.) was normalized based on Renilla luciferase activity, from the psi-CHECK-2 plasmid. (n = 3), ***p<0.001 vs psi-CHECK-2-MALAT1-WT. (B) Relative expression of miR-30a-5p in HCC tissues and paired adjacent tissues (n = 30) were detected by qRT-PCR. Relative levels were normalized to those of GAPDH. (C) Pearson's correlation between MALAT1 and miR-30a-5p. ***p<0.001 vs Normal tissues. (D) Relative expression of miR-30a-5p in HCC cell lines were detected by qRT-PCR and compared with those in the normal liver cell line LO2. Relative levels were normalized to those of GAPDH. (n = 3), **p<0.01, ***p<0.001 vs LO2. (E) The expression of miR-30a-5p was up-regulated after silencing MALAT1 in Huh7 and HepG2 cells. (F) The expression of MALAT1 was decreased after transfecting the miR-30a-5p mimic in Huh7 and HepG2 cells. Data are presented as means ± SD of three independent experiments. (n = 3), *p<0.05, **p<0.01 vs si-NC and miR-NC.

regulated in si-MALAT1-transfected HCC cells (Fig. 2E), while MALAT1 was down-regulated in miR-30a-5p mimics-transfected HCC cells (Fig. 2F). These results demonstrated that MALAT1 correlated negatively with miR-30a-5p.

miR-30a-5p effects HCC cell invasion and migration via targeting of Vimentin

Bioinformatic analysis TargetScan (www.targetscan.org) predicted that Vimentin might be a target of miR-30a-5p. Therefore, we used dual luciferase assay to show that overexpression of miR-30a-5p significantly decrease Vimentin-WT more than Vimentin-MUT (Fig. 3A). Western blot analysis indicated that Vimentin expression levels were decreased in miR-30a-5p mimics-transfected HCC cells (Fig. 3B). The same results were detected by immunofluorescence assay (Fig. 3C). As Vimentin is a mesenchymal marker in EMT, and EMT is a process involving in cancer cells invasion and migration, we used wound healing and invasion assays to assess the invasive and migratory abilities of HCC cells. Results from these assays demonstrated that miR-30a-5p inhibits the invasive and migratory abilities of HCC cells (Fig. 3D, E). However, overexpression of Vimentin could abolished this effect (Fig. 3F, G). Collectively, these data manifested that miR-30a-5p inhibits HCC cells invasion and migration by regulating Vimentin.

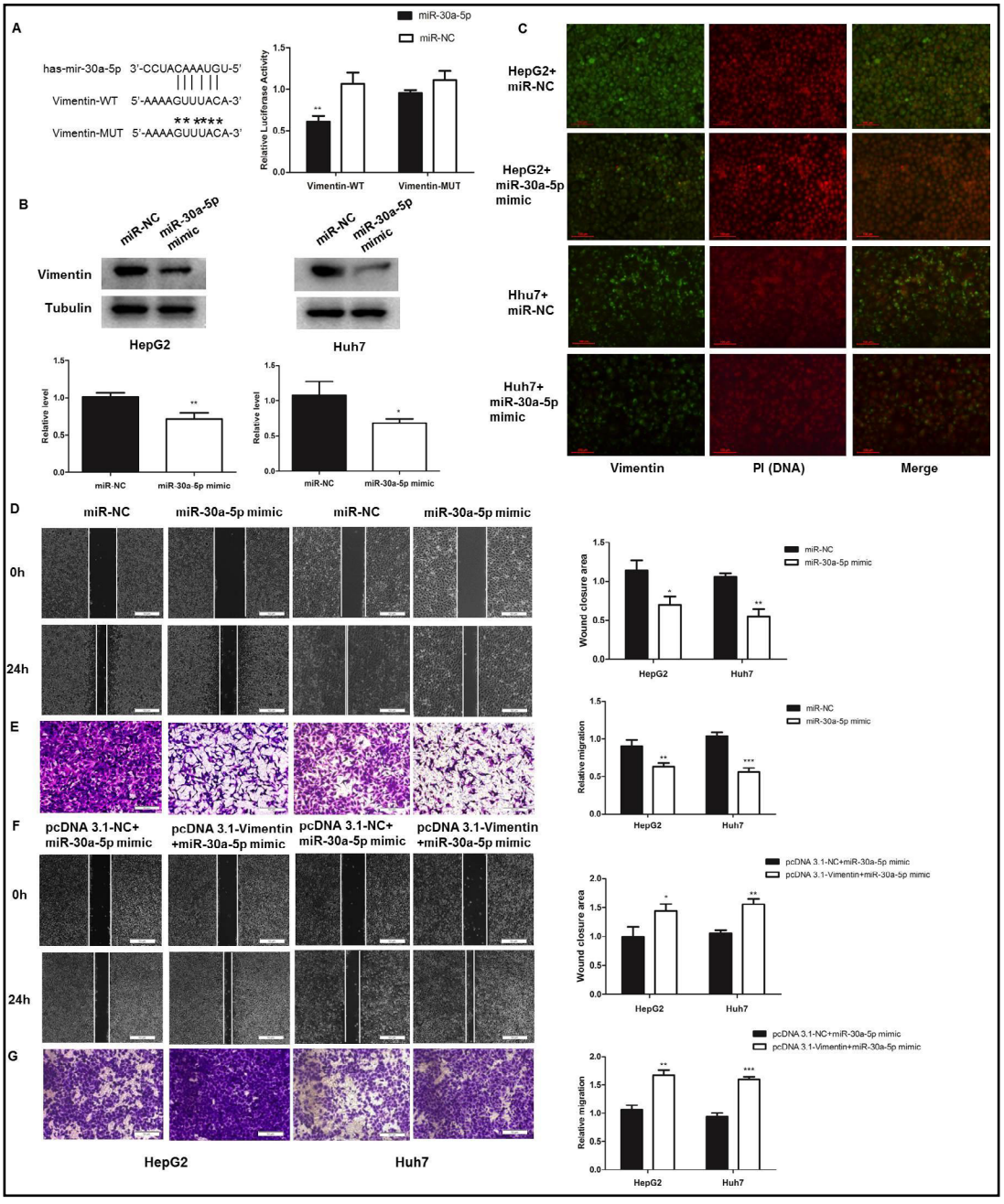

Fig. 3. miR-30a-5p effects HCC cell invasion and migration via targeting Vimentin. (A) Complementarities of sequences between miR-30a-5p and Vimentin. Luciferase assay showed miR-30a-5p could target Vimentin. Luminescence (a.u.) was normalized based on Renilla luciferase activity, from the psi-CHECK-2 plasmid. (n = 3), **p<0.01 vs psi-CHECK-2- Vimentin-WT. (B) The protein levels of Vimentin in Huh7 and HepG2 cells transfected with miR-NC and miR-30a-5p mimics. (C) Representative images of HCC cells stained with antibody to Vimentin (green). Red indicates PI staining (10×). (D and E) Wound healing migration assay and transwell invasion assay were evaluated in HCC cells transfected with miR-NC and miR-30a-5p mimic (20×), quantification on the right. (n = 3), *p<0.05, **p<0.01, ***p<0.001 vs miR-NC. (F and G) Wound healing migration assay and transwell invasion assay were evaluated in HCC cells transfected with pcDNA 3.1-NC + miR-30a-5p mimic and pcDNA 3.1-Vimentin + miR-30a-5p mimic (20×), quantification on the right. Data are presented as means ± SD of three independent experiments. (n = 3), *p<0.05, **p<0.01, ***p<0.001 vs pcDNA 3.1-NC + miR-30a-5p mimic.

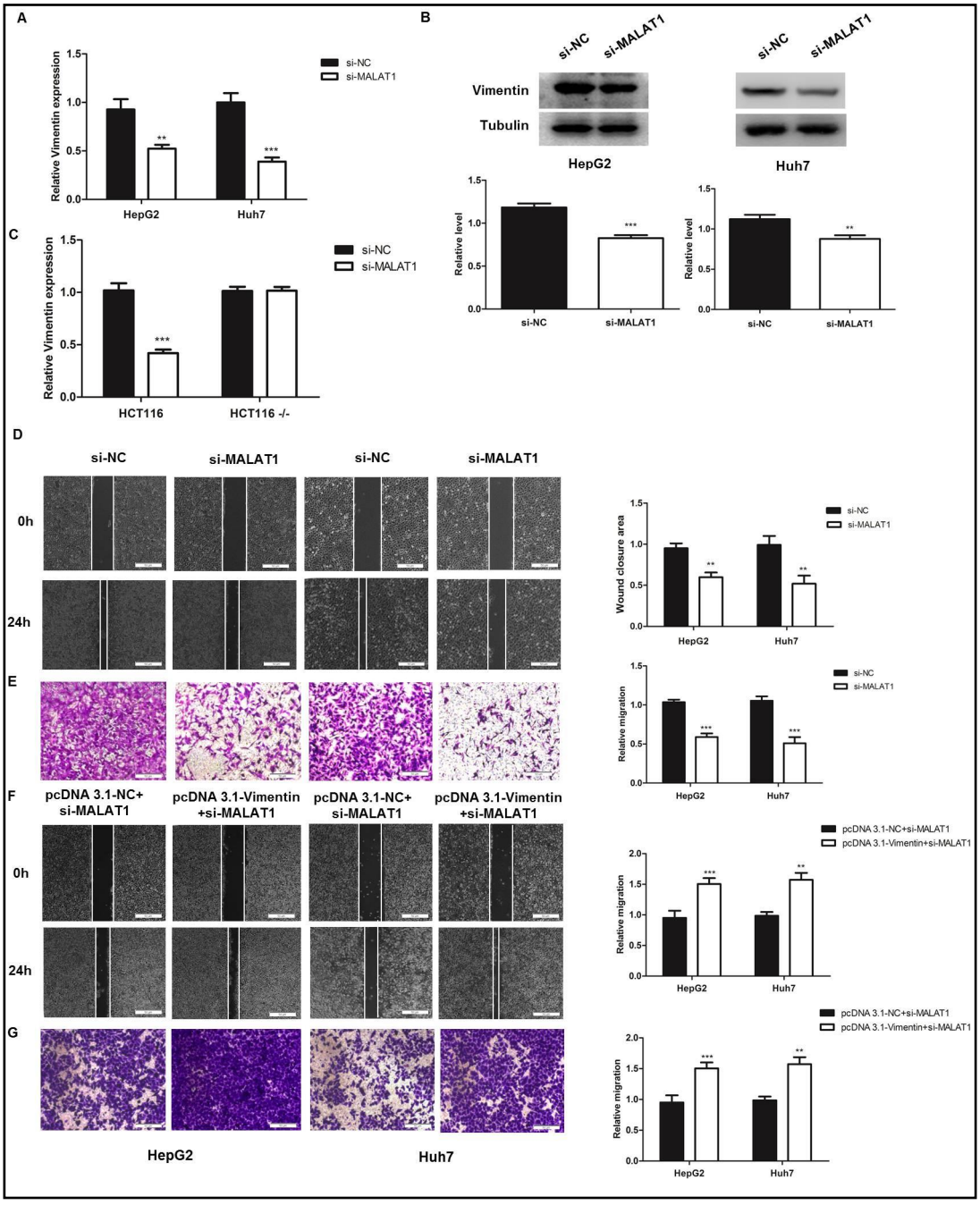

Fig. 4. MALAT1 regulates Vimentin expression and effects HCC cell invasion and migration. (A and B) The mRNA or protein levels of Vimentin in Huh7 and HepG2 cells transfected with si-NC and si-MALAT1. (C) The mRNA levels of Vimentin in HCT116 and HCT116-/- cells transfected with si-NC and si-MALAT1. (D and E) Wound healing migration assay and transwell invasion assay were evaluated in HCC cells transfected with si-NC and si-MALAT1 (20×), quantification on the right. (n = 3), **p<0.01, ***p<0.001 vs si-NC. (F and G) Wound healing migration assay and transwell invasion assay were evaluated in HCC cells transfected with pcDNA 3.1-NC + si-MALAT1 and pcDNA 3.1-Vimentin + si-MALAT1 (20×), quantification on the right. Data are presented as means ± SD of three independent experiments. (n = 3), **p<0.01, ***p<0.001 vs pcDNA 3.1-NC + si-MALAT1.

MALAT1 regulates Vimentin expression and effects HCC cell invasion and migration

It was found that Vimentin is inversely associated with miR-30a-5p, and there is a negative correlation between Vimentin and MALAT1 expression. In order to further explore the regulatory mechanism of MALAT1 on Vimentin expression levels, we measured the RNA and protein levels of Vimentin in si-MALAT1-transfected HCC cells. As demonstrated in Fig. 4A-B, expression of Vimentin was decreased in both mRNA and protein levels in HCC cells transfected with si-MALAT1 (Fig. 4A, B). In HCT116 cells, deficiency of MALAT1 repressed Vimentin expression, but in HCT116-/- cells (HCT116-/- cells do not express the majority of mature miRNAs). The fact that expression of Vimentin was not influenced (Fig. 4C), showed that MALAT1 regulates Vimentin expression via miR-30a-5p. Furthermore, knockdown of MALAT1 inhibits the invasive and migratory abilities of HCC cells (Fig. 4D, E). However, overexpression of Vimentin could abolished this effect (Fig. 4F, G). These results demonstrate that MALAT1 regulates HCC invasion and migration, and up-regulates Vimentin expression by sponging miR-30a-5p.

Inhibition of MALAT1 suppresses HepG2 cells growth in vivo

To investigate the role of MALAT1 in HCC cell growth *in vivo*, HepG2 cells were stably transfected with control sh-NC or sh-MALAT1 were subcutaneously injected in nude mice. Tumors volume and size in sh-NC groups were larger than sh-MALAT1 groups (Fig. 5A, B). Additionally, IHC results revealed that the expression of Vimentin were reduced in sh-MALAT1 groups (Fig. 5C, D). These results indicated that MALAT1 may promotes HCC progression *in vivo*.

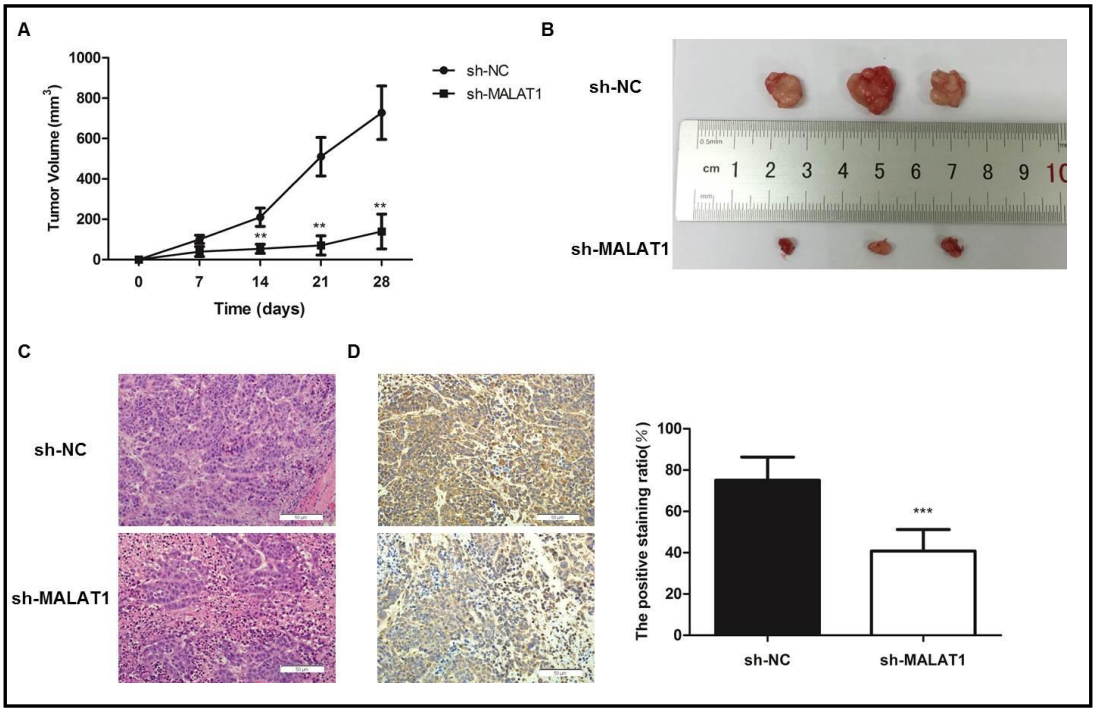

Fig. 5. Inhibition of MALAT1 suppresses HepG2 cells growth in vivo. (A) Tumor volumes were measured every 7 days after injection. (B) Morphological images of the mice with xenograft HepG2 cells transfected with sh-MALAT1 or sh-NC, tumors were removed from nude mice after 28 days injection. (C) H&E showed the features of the tumor sections (20×). (D) Expression of Vimentin of the tumor sections (Immunohistochemistry, 20×), quantification on the right. (n=3), *p<0.05, **p<0.01, ***p<0.001 vs sh-NC.

Discussion

It was demonstrated that MALAT1 was significantly increased in HCC cancer tissues compared to adjacent normal tissues, and inhibition of MALAT1 repressed HCC cell migration and invasion, and decreased the tumor volume *in vivo*. These findings indicate that MALAT1 exerts a carcinogenic effect in HCC cells. Additionally, miR-30a-5p was demonstrated to be a direct target of MALAT1, and miR-30a-5p up-regulation inhibited HCC cell migration and invasion. Moreover, Vimentin was regulated by miR-30a-5p, and Vimentin exhibited a carcinogenic role.

MALAT1 is a crucial lncRNA found in tumor progression [18]. MALAT1 is highly expressed in variety of tumor tissues and cell lines [18]. In this study, we have demonstrated that MALAT1 was upregulated in HCC cancer tissues and cell lines. This finding revealed that MALAT1 might be implicated in the tumorigenesis and development in HCC. Interestingly, the tumor promoter properties of MALAT1 have been found in numerous cancers. MALAT1 regulated cell metastasis but had no impact on proliferation of lung cancer cells [19-22]. Furthermore, MALAT1 was been shown to affects cell viability, proliferation and migration, colony formation, cell cycle distribution, and tumor growth in CaSki cervical cancer cells [23]. MALAT1 has also been reported to be an oncogene in HCC, in addition, MALAT1 was been found play a marker with high sensitivity for HCC at both early and late stages [24], suggesting that it can be used as a potential diagnostic tool for HCC [25]. Moreover, a study reported that inhibition of MALAT1 in HepG2 cells could effectively reduce cell viability, motility, invasiveness, and increase the sensitivity to apoptosis [26]. These findings indicated that MALAT1 may act as an essential role in HCC progression.

To further explore the underlying mechanisms via which MALAT1 suppresses HCC cell activity, we focused on the cross-regulation between miR-30a-5p and MALAT1. An increasing amount of research has revealed that lncRNAs can act as ceRNAs to competitively bind to the miRNAs response elements (MRE) to regulated miRNAs functions [27]. Li *et al*. reported that TUG1 could act as ceRNAs to regulate SIRT1 expression by sponging miR-138-5p in cervical cancer [28]. In the current study we found that MALAT1 is a target of miR-30a-5p by computational analysis, luciferase reporter assays, and RT-PCR. These results suggested that MALAT1 is a ceRNA sponge of miR-30a-5p. Interstingly, our results showed that MALAT1 was a upstream regulator of miR-30a-5p. According to previous studies, lncRNA can regulate miRNA [29, 30], and miRNA can also regulate lncRNA [31, 32]. Therefore, we propose that there could be a mutually inhibitory feedback loop between MALAT1 and miR-30a-5p, which needs further investigation.

miR-30a-5p was reported to be expressed aberrantly and have different roles in many cancers, Wei *et al*. demonstrated that miR-30a-5p was significantly downregulated in colon cancer [33], but it also promoted glioma cell growth invasion by directed binding neural cell adhesion molecule (NCAM), which expression is decreased in glioma cell [34]. These findings indicated that miR-30a-5p plays a dual role in many cancers. Therefore, the biological and molecular mechanisms of miR-30a-5p in HCC need further study. This study used bioinformatics analysis and luciferase reporter assays to show that Vimentin is a target gene of miR-30a-5p. Western blot analyses and immunofluorescence assay showed that overexpression of miR-30a-5p downregulated Vimentin levels. Moreover, overexpression of miR-30a-5p inhibited HCC cell migration and invasion，but overexpression of Vimentin could abolished this effect. Subsequently, RT-PCR and western blot analyses demonstrated that inhibition of MALAT1 repressed Vimentin expression levels in HCC cells. Furthermore, inhibition of MALAT1 downregulated Vimentin expression in HCT116, but had no effect on HCT116-/-. Therefore, the effect of MALAT1 on HCC cells migration and invasion is due, in part, to its act as a molecular sponge of miR-30a-5p that targets Vimentin. Although knockdown of MALAT1 could inhibit HCC cells viability and cell proliferation, overexpression of Vimentin could not abolish this effect.

Conclusion

In summary, our data revealed that miR-30a-5p dampened HCC cells migration and invasion by inhibiting Vimentin, and MALAT1 promoted HCC cell migration and invasion by acting as a ceRNA of miR-30a-5p. These findings suggest that MALAT1 functionality and molecular mechanisms relevant to HCC may provide a potential therapeutic candidate for HCC.

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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