Neoplasma 2022; 69(1): 136–144

doi:10.4149/neo_2021_210815N1158

LINC00536 promotes hepatocellular carcinoma progression via the miR-203b-5p/VEGFA axis

Li-Gang WANG[1,#], Hai-Yang SONG[1,#], Kun-Peng ZHANG[2,#], Li-Ming LIU[3], Yu-Tian JIANG[1], Sheng LIU[1,*]

[1]Department of Interventional Therapy, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China; [2]Department of Catheterization Room, The Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China; [3]Department of Radiology, Branch Court of the Affiliated Yantai Yuhuangding Hospital of Qingdao University, Yantai, Shandong, China

**Correspondence: wligang1983@163.com*
[#]Contributed equally to this work.

Received August 15, 2021 / Accepted November 8, 2021

LncRNAs exert comprehensive effects in regulating the initiation and deterioration of hepatocellular carcinoma (HCC). However, the specific expression profiles and functional mechanisms of LINC00536 in HCC need to be disclosed. The study is intended to clarify the leverage of LINC00536 in HCC and investigate the potential mechanisms for the regulatory role of LINC00536 in the progression of HCC. In our study, LINC00536 was overexpressed in tumor samples of HCC patients and was related to poor prognosis. LINC00536 knockdown impaired cell viability, proliferation, migration, and invasion. LINC00536 can directly bind with miR-203b-5p, trimming the miR-203b-5p expression levels. VEGFA designates as a target of miR-203b-5p. Rescue research indicated that the miR-203b-5p inhibition or VEGFA overexpression could reverse the impaired cell phenotypes induced by LINC00536 knockdown. The in vivo experiments upheld the LINC00536/miR-203b-5p/VEGFA axis in HCC. Conclusively, LINC00536 could promote HCC deterioration via tuning the miR-203b-5p/VEGFA axis. This research may provide theoretical evidence for LINC00536 to get a gratifying therapeutic target for HCC.

Key words: hepatocellular carcinoma, LINC00536, miR-203b-5p, VEGFA

As one of the most prevalent malignancies, hepatocellular carcinoma (HCC) exhibits a high frequency of metastasis and mortality worldwide [1]. Most patients are ineligible for curative resection due to inadequate methodologies for early diagnosis, making the 5-year survival rate <25% [2]. Although apparent advances have been made in novel therapies for HCC, the prognosis of this malignant disease remains unsatisfactory [3].

Long non-coding RNAs (lncRNAs) are defined as transcripts with a length >220 nucleotides, with foremost properties in regulating the transformation of various malignancies and play critical roles in tumor progression, including prostate cancer, colorectal cancer, lung cancer, and gastric cancer [4–8]. For example, MIR22HG is a critical inducer of the Wnt/β-catenin signaling pathway, and that its targeting may represent a novel therapeutic strategy in glioblastoma patients [9]. Inhibition of lncRNA H19 expression might serve as a novel molecular therapeutic target for aortic aneurysm disease [10]. Lately, increasing evidence has unveiled that lncRNAs are dysregulated in HCC and exert comprehensive effects in the development and progression of HCC via diverse mechanisms. For example, lncRNA MCM3AP-AS1 can facilitate HCC progression via regulating the miR-194-5p/FOXA1 signaling pathway [11]. LncRNA TP53TG1 impaired cancer proliferation and metastasis by targeting the PRDX4/β-catenin pathway in HCC [12]. Recently, a newly identified lncRNA LINC00536 was considered to be involved in deteriorating breast cancer, ovarian cancer, and bladder cancer [13–15]. These studies suggest that LINC00536 exhibits a potential role as a relative gene and a promising therapeutic target in cancer. However, the specific expression profiles and functional mechanisms of LINC00536 in HCC are required to be explored.

Accumulating evidence suggests that vascular endothelial growth factor (VEGF) exerts oncogenic effects in various malignancies via modulating diverse signal pathways [16]. VEGFA is an isoform of VEGF that employs regulatory effects on receptors including VEGFR1 and VEGFR2 [16]. Previous reports have demonstrated that VEGFA can facilitate cancer cell growth and metastasis via governing cell

proliferation, epithelial mesenchyme transition, cell cycle, and cell apoptosis in HCC [17–20]. Several clinical trials have evaluated the effect of VEGFA inhibition in HCC and shown encouraging results [21, 22]. But the upstream regulatory role of LINC00536 on VEGFA remains largely unknown.

Here, we uncovered the alteration of LINC00536 expression and further disclosed the functional regulatory mechanisms of LINC00536 in HCC. We defined and validated the association between LINC00536 and its target miR-203b-5p. We also found that LINC00536 could regulate HCC progression via the miR-203b-5p/VEGFA axis.

Patients and methods

Ethical statement and patients. The study was conducted under the approval of the Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University, which had carefully reviewed the study design. The HCC tissue samples and corresponding normal controls were obtained from HCC patients (n=58) during surgery at The Affiliated Yantai Yuhuangding Hospital of Qingdao University. The normal control tissues were 5 cm away from tumor tissues. Among the patients, 32 of them were males and 26 of them were females. The mean age of these patients was 68.7 years. Informed consent forms were supplied by the involved participants.

Cell culture. HCC cells, including SK-Hep-1, Hep3B, Huh7, and MHCC97L and immortalized liver cells (THLE-21) were bought from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultivated under standard protocols using Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific. USA) in a 37 °C, 5% CO_2 humidified atmosphere.

Cell transfection of short hairpin RNA (shRNA) and miRNA inhibitors. All the transfection assays were performed with Lipofectamine 3000 (L3000008, Thermo Fisher Scientific) following the manufacturer's instructions. In brief, SK-Hep-1 and Hep3B cells were firstly seeded into the 6-well plates. After reaching 60% confluence, we transfected the cells with short hairpin RNA (shRNA) targeting LINC00536 (sh-LINC00536#1 and sh-LINC00536#2), and inhibitor (miR-203b-5p and miR-203b-5p inhibitor) accordingly. We got corresponding si-NC and NC inhibitor as the negative controls. All the oligonucleotides were purchased from RiboBio (Guangzhou, China) that also designed the oligonucleotides.

Cell Counting Kit-8 (CCK-8) assay. Briefly, transfected HCC cells (SK-Hep-1 and Hep3B) were plated in a 96-well plate at a density of 2×10^3 cells/well. After culturing for 24 h, 48 h, and 72 h, 10 μl/well of the CCK-8 (Dojindo Molecular Technologies, Japan) solutions were added to the plates. After incubation for 90 min, the 450 nm absorbance was detected using a spectrophotometer (Thermo Fisher Scientific).

Transwell assay. Invasion Chambers (Corning, Inc.) were used to perform Transwell assay. For migration, transfected cells in 0.2 ml serum-free DMEM were added into the upper chamber of a Transwell plate with a cell density of 2×10^5, along with DMEM containing 20% FBS in the lower part. Twenty-four hours later, the cells that migrated into the lower chamber were fixed with 4% PFA, followed by the staining with 0.1% crystal violet. The fixation and staining assays were performed at room temperature. For invasion, the same procedures were employed except that the Transwell plates were coated with Matrigel (Corning, Inc.). After staining, cells were observed under an IX71 inverted optical microscope (Olympus Corporation, ×20 magnification).

RNA extraction and the qRT-PCR. The total RNA from the involved cells was extracted with TRIzol® Reagent (Thermo Fisher Scientific) and then reverse-transcribed into cDNA with PrimeScript RT master mix (RR036A; Takara Bio). Subsequently, the real-time quantitative PCR was performed with the SYBR Premix Ex Taq II kit (DRR081A; Takara Bio). GAPDH and U6 were regarded as internal references accordingly. The RNA relative levels were established with $2^{-\Delta\Delta Ct}$ methods.

Western blot. Total proteins of the cells were extracted following standard protocols. Then, proteins were loaded onto the 10% SDS gel, followed by separating with electrophoresis and transferring to PVDF membranes. Next, corresponding primary antibodies VEGFA (Cat. ab52917, Abcam, USA), GAPDH (Cat. ab9485, Abcam, USA, dilution ratio: 1:1000) were used to incubate the membranes, followed by the secondary HRP rabbit IgG antibodies (Cat. ab6721, Abcam, USA, 1:5000) treating the membranes. Finally, the protein bands were analyzed with enhanced chemiluminescence (407207, Millipore).

Dual-luciferase reporter assay. LINC00536-WT vector or LINC00536-mutant (MUT) vector was constructed using pGL-3 control vector (Promega Corporation, Madison, USA) with the existence or missing a binding site for miR-203b-5p in the 3'-untranslated region (3'-UTR). The VEGFA-WT vector or VEGFA-MUT vector was constructed in the same way. The designed vectors were used to transfect the cells as described above and then luciferase activity was determined.

RNA pull-down. Cells were transfected with biotin-coupled LINC00536 or lnc-NC (Biotin-LINC00536, Biotin-lnc-NC) for 48 h. Then, cell lysates were collected after centrifuging and then incubated with magnetic beads (Thermo Fisher Scientific, Inc.). The miR-203b-5p enrichment was determined.

Tumor formation experiment *in vivo*. BALB/C nude mice were purchased from the Cancer Institute of the Chinese Academy of Medical Science (Beijing, China). The stably transfected cells were injected subcutaneously into each mouse. The behavior and food intake of the mice were monitored every day to maintain their health. Tumor volume was computed every week. After inoculation for 35 days, in strict accordance with the principles of animal welfare, mice were euthanized by cervical dislocation. Each tumor weight was isolated and measured. The research protocol was

approved by the Medical Ethics Committee of The Affiliated Yantai Yuhuangding Hospital of Qingdao University.

Statistical analyses. Data are expressed as mean ± standard deviation (SD). Student's t-test was used to uncover the difference between the two groups. The difference among the three groups was assessed using ANOVA and the subsequent Tukey's postdoc test. All the analyses were performed using GraphPad Prism 8.0 (GraphPad, La Jolla, CA, USA). A two-sided $p<0.05$ indicated a statistically significant difference.

Results

LINC00536 was upregulated in HCC tissues and cell lines, and high LINC00536 predicted poor survival of HCC patients. Firstly, we used GSE138178 microarrays to identify differential lncRNA expression profiles in cancerous and para-cancerous tissues (fold change ≥2.0 or ≤0.5, $p<0.05$). In these tissues, top 100 differential lncRNA expressions were disclosed, as is shown in the heatmap (Figure 1A) and the volcano plot (Figure 1B). Among them, one of the potential candidates, LINC00536, was identified (Figure 1C). To determine LINC00536 levels *in vitro*, we detected LINC00536 expression levels in HCC cell lines, including SK-Hep-1, Hep3B, Huh7, and MHCC97L cells, and normal liver cells (THLE-21). We found LINC00536 levels were markedly upregulated in HCC cells compared with normal cells (Figure 1D). We further examined clinical outcomes of differential LINC00536 expression in HCC patients. We found that high LINC00536 expression was related to poor survival in HCC (Figure 1E). These results indicated LINC00536 was upregulated in HCC tissues and cell lines and high LINC00536 predicted poor survival of HCC patients.

LINC00536 played an oncogenic role to enhance cell malignant phenotypes in HCC cells. To uncover the biomedical function of LINC00536, we constructed stable LINC00536 knockdown Hep3B and SK-Hep1 cell lines with sh-LINC00536#1 and sh-LINC00536#2. As we can see here, LINC00536 was markedly downregulated in Hep3B and SK-Hep1 cell lines with LINC00536 knockdown (Figure 2A). We then explored cell viability, migration, and invasion with LINC00536 knockdown in HCC cells. LINC00536 knockdown abated cell growth significantly in Hep3B and SK-Hep1 cells (Figure 2B). Colony formation assays noted that LINC00536 knockdown markedly decreased colony formation of HCC cells (Figure 2C). Transwell assays exhibited that the metastasis ability of HCC cells was substantially inhibited with LINC00536 knockdown (Figures 2D, 2E). These findings suggested that LINC00536 played an oncogenic role to enhance cell malignant phenotypes in HCC cells.

LINC00536 knockdown upregulated miR-203b-5p expression while suppressing VEGFA expression levels in the HCC cells. LncRNAs can exert their regulatory effects via multiple mechanisms including modulating transcriptional factors, splicing factors, and histone expression [23].

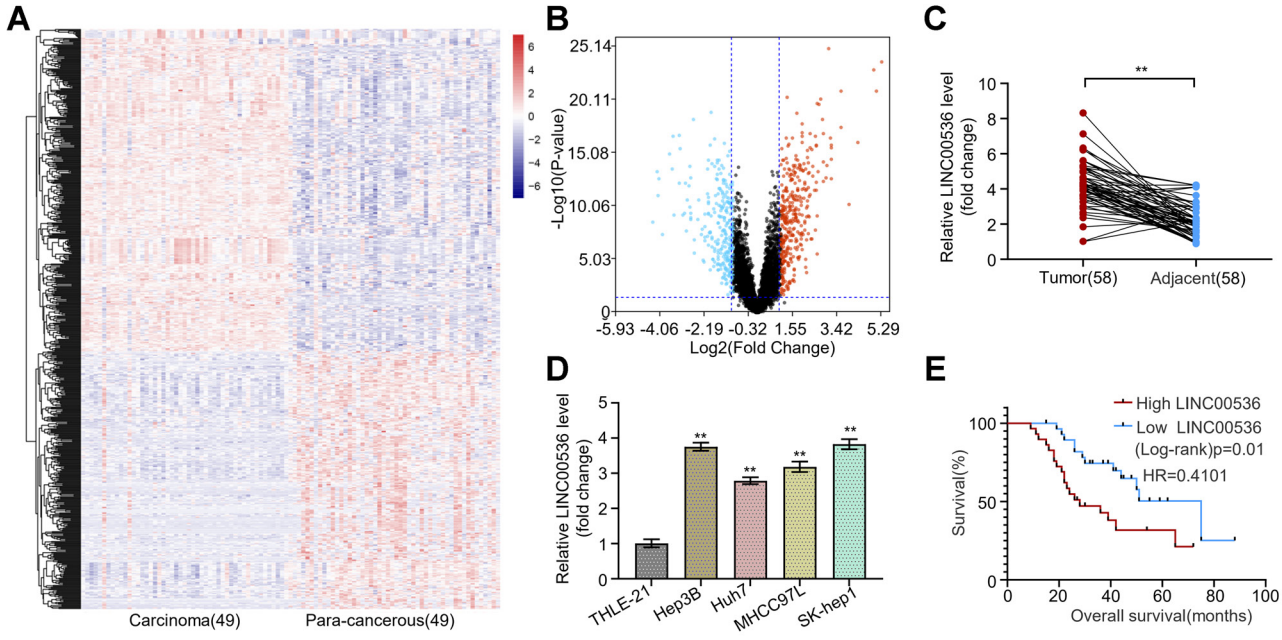

Figure 1. LINC00536 expression was upregulated in HCC tissue and cell lines. A) Differential expression of lncRNAs in cancerous and para-cancerous tissues was analyzed with a heatmap in GSE138178. B) Differential expression of lncRNAs in cancerous and para-cancerous tissues was analyzed with a volcano plot in GSE138178. C) Differential expression of LINC00536 was measured in tumor and adjacent normal tissues in 58 HCC patients. D) Differential expression of LINC00536 was determined in HCC cells and normal liver cells. E) Kaplan-Meier plot was used to analyze the survival rate of patients with different expression of LINC00536. All the experiments were conducted three repeated times. **p<0.01

To unveil the regulatory mechanism of LINC00536 in HCC, we investigated the locational distribution and identified the potential downstream targets. Cytoplasmic and nuclear RNA fractions were separated from the involved cells. We performed qRT-PCR to illuminate the locational distribution of LINC00536 in the HCC cells. LINC00536 was localized mainly in the cytoplasm in HCC cells (Figure 3A). Numerous studies have indicated that lncRNAs can regulate the downstream signaling pathways by acting as a miRNA sponge to tune miRNA expression [24, 25]. We used starBase V3.0 to calculate the downstream potential miRNAs of LINC00536. We identified that miR-203b-5p was a putative downstream miRNA that contained binding sites of LINC00536 (Figure 3B). We further upheld the direct binding of LINC00536 and miR-203b-5p. The luciferase assay disclosed that miR-203b-5p overexpression decreased the activity of LINC00536-WT vectors, but marginally changed the activity of LINC00536-Mut vectors in HEK-293T (Figure 3C). RNA pull-down assays also demonstrated that biotin-labeled LINC00536 captured more miR-203b-5p compared with biotin-labeled NC in the involved cells (Figure 3D). We then predicted the downstream targets of miR-203b-5p with miRanda bioinformatic analysis and found VEGFA was a potential target of miR-203b-5p (Figure 3E). The luciferase report validated the direct association of miR-203b-5p and VEGFA, showing that miR-203b-5p overexpression descended the activity of VEGFA-WT vectors, but it did not influence the luciferase activity of VEGFA-Mut vectors in HEK-293T (Figure 3F). Subsequently, miR-203b-5p and VEGFA levels were studied with LINC00536 knockdown to validate the regulatory axis of LINC00536/miR-203b-5p/VEGFA. The results showed that LINC00536 knockdown significantly upregulated miR-203b-5p expression levels (Figure 3G), but suppressed VEGFA expression levels in the HCC cells (Figure 3G). Together, these results stated that LINC00536 regulated the miR-203b-5p/VEGFA signal axis through the miRNA sponge.

LINC00536 promoted HCC proliferation and metastasis via the miR-203b-5p/VEGFA axis. To further validate the role of LINC00536 in HCC malignant pheno-

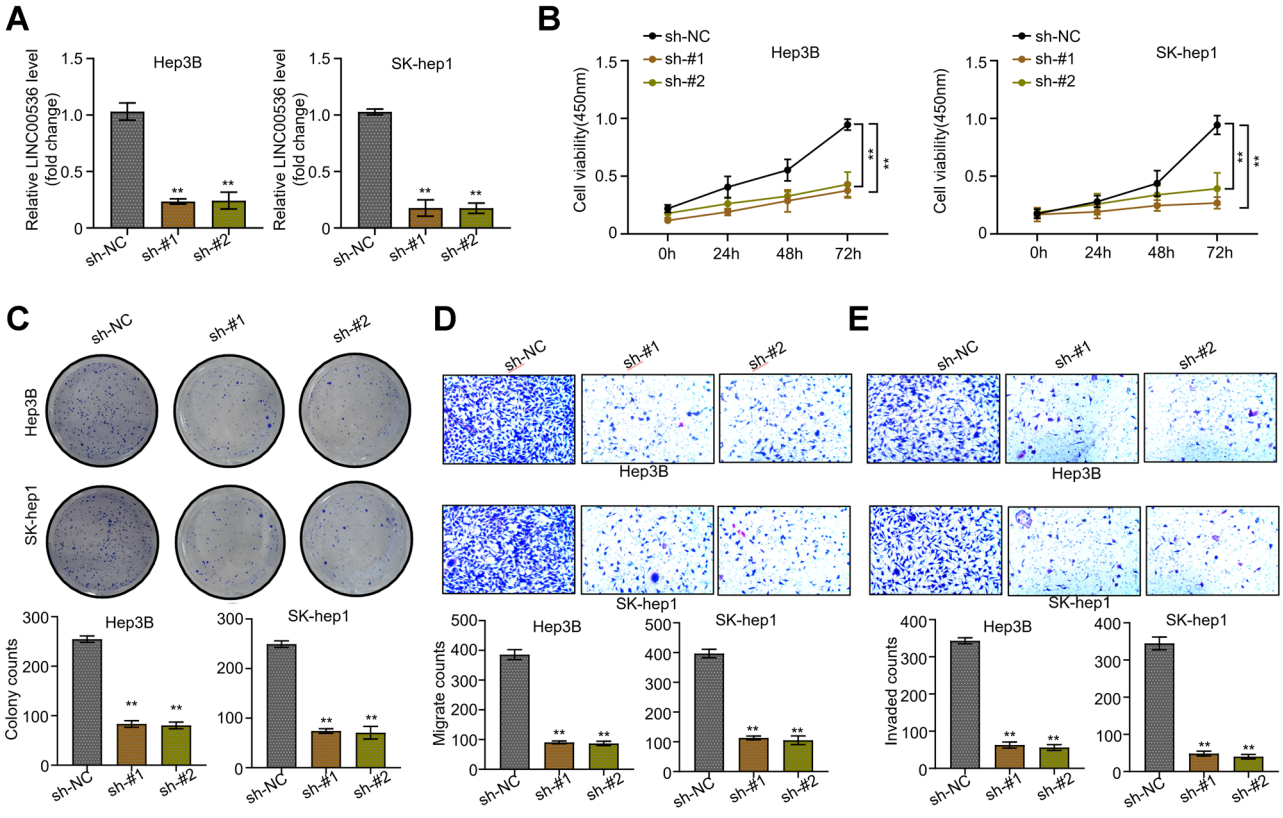

Figure 2. Knockdown of LINC00536 inhibited HCC cell proliferation and metastasis *in vitro*. A) LINC00536 levels were measured by qRT-PCR in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#2. B) Cell viability was detected using the CCK-8 assay in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#2. C) Colony formation was measured (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#2. D) Cell migration was determined (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#2 through Transwell assays. E) Cell invasion was determined (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#2 through Transwell assays. All the experiments were conducted three repeated times. **p<0.01

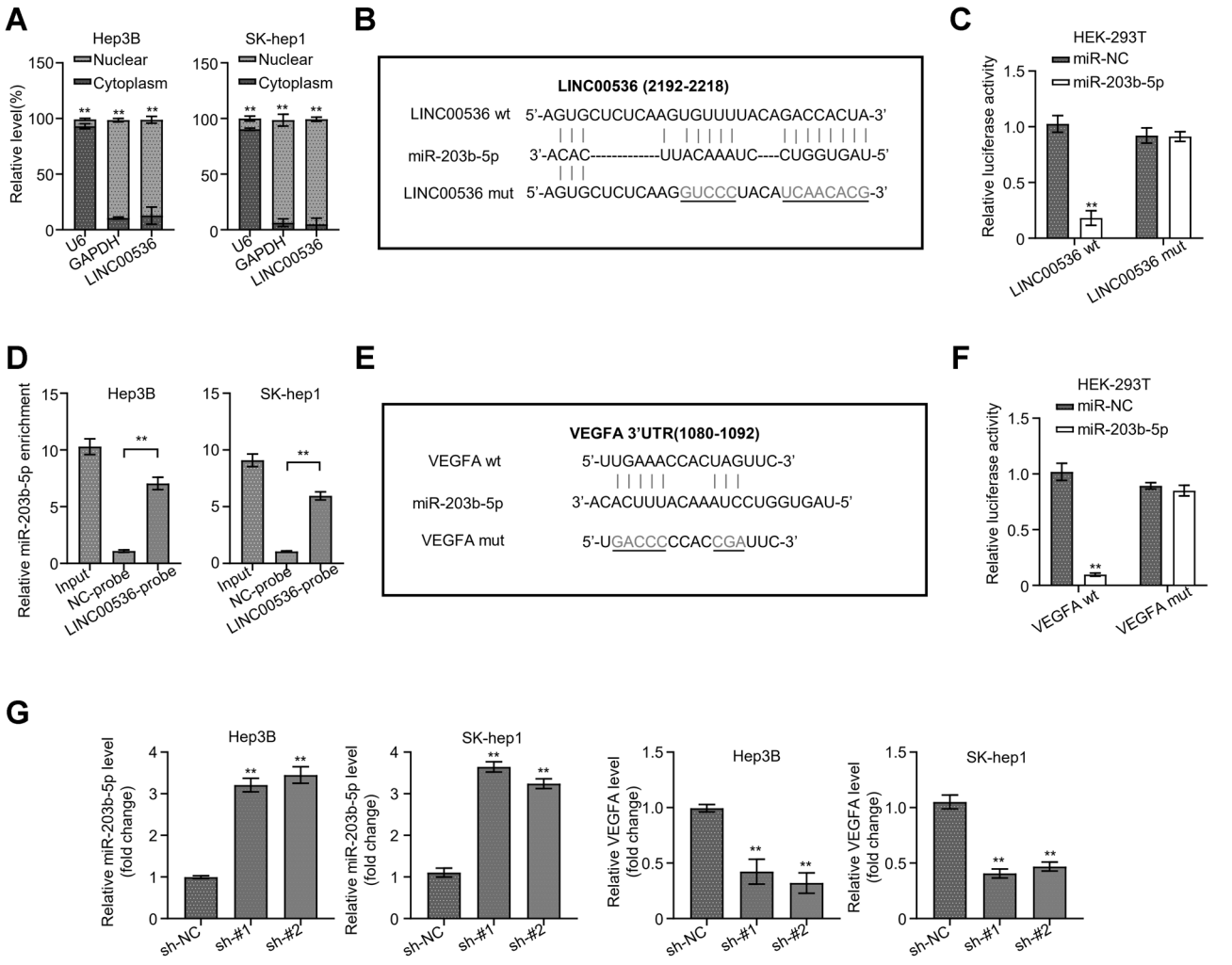

Figure 3. LINC00536 regulated the miR-203b-5p/VEGFA signal axis by miRNA sponge. A) Cytoplasmic and nuclear RNA fractions were isolated from SK-Hep-1 and Hep3B cells. LINC00536 was located mainly in the cytoplasm. B) The binding sites between LINC00536 and miR-203b-5p were predicted by lncBase. C) The binding between LINC00536 and miR-203b-5p was validated using the dual-luciferase reporter assay. D) The binding between LINC00536 and miR-203b-5p was validated using an RNA pull-down assay. E) The binding sites between miR-203b-5p and VEGFA were predicted by miRanda. F) The binding between miR-203b-5p and VEGFA was validated using the dual-luciferase reporter assay. G) The expression of miR-203b-5p and VEGFA was measured by qRT-PCR in SK-Hep-1 and Hep3B cells with sh-NC, sh-LINC00536#1, sh-LINC00536#2. All the experiments were conducted three repeated times. **p<0.01

types via the miR-203b-5p/VEGFA axis, the rescue experiments were executed. We found that LINC00536 knockdown significantly decreased VEGFA expression levels in SK-Hep-1 and Hep3B cells, while the suppressive effect was evidently reversed after the cells were co-transfected with sh-LINC00536#1 and miR-203b-5p inhibitor, or sh-LINC00536#1 and VEGFA overexpression plasmid (Figures 4A, 4B). We further verified the cell proliferation and migration with rescue experiments. The CCK-8 assay showed that cell viability was inhibited with LINC00536 knockdown, but the effect was recovered from co-transfecting with sh-LINC00536#1 and miR-203b-5p inhibitor, or sh-LINC00536#1 and VEGFA overexpression plasmid (Figure 4C). The colony formation assay also revealed that cell colony formation was impaired with LINC00536 knockdown but was rescued with co-transfecting with sh-LINC00536#1 and miR-203b-5p inhibitor, or sh-LINC00536#1 and VEGFA overexpression plasmid (Figure 4D). Consistently, the metastatic ability of SK-Hep-1 and Hep3B cells was markedly destructed by LINC00536 knockdown, but the influence was significantly recovered after the cells were co-transfected with sh-LINC00536#1 and miR-203b-5p inhibitor, or sh-LINC00536#1 and VEGFA overexpression plasmid (Figures 4E, 4F). Collectively, LINC00536 hastened HCC proliferation and metastasis via the miR-203b-5p/VEGFA axis.

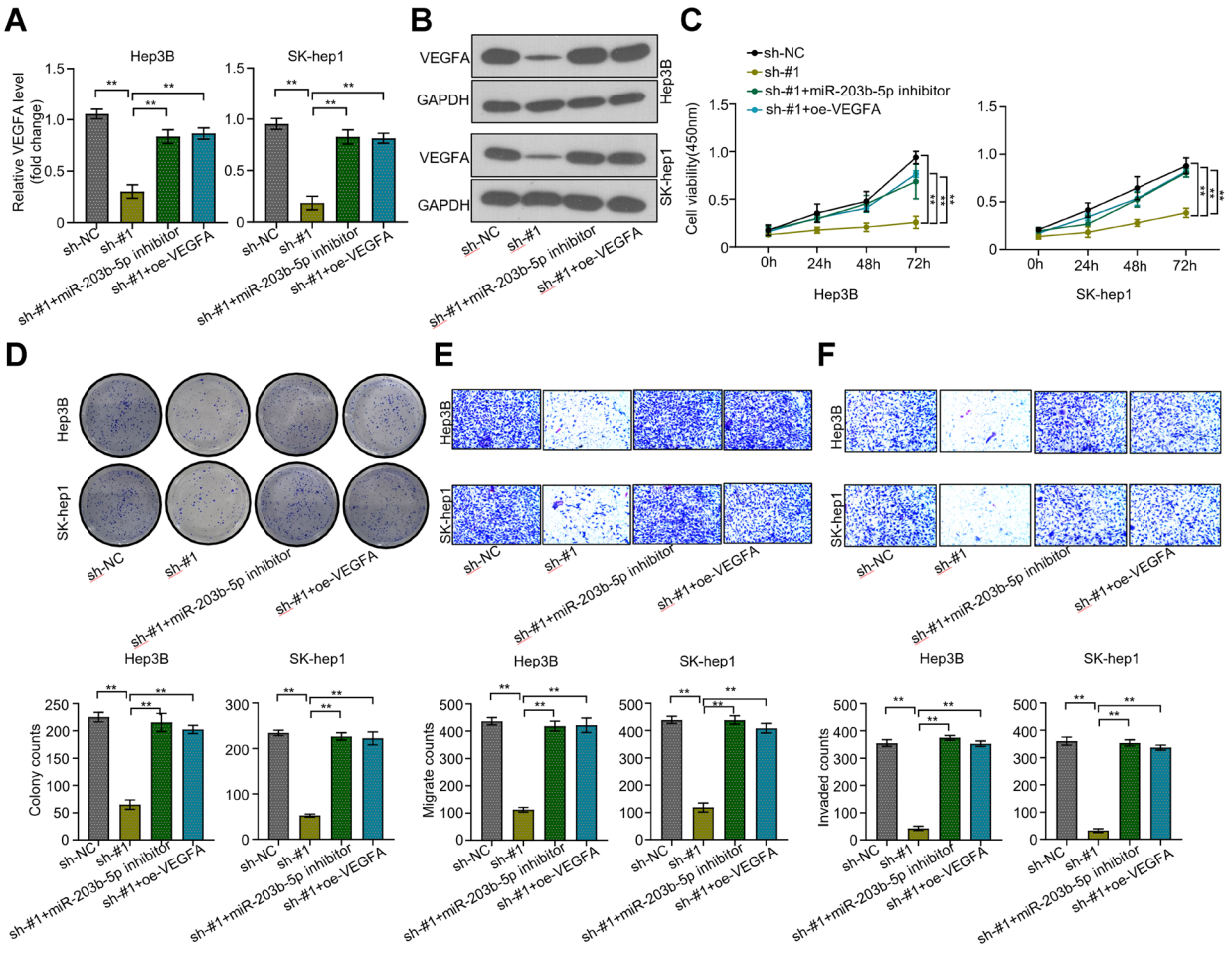

Figure 4. LINC00536 promoted HCC proliferation and metastasis by regulation of the miR-203b-5p/VEGFA axis. A) The expression of VEGFA was determined by qRT-PCR in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. B) The expression of VEGFA was determined by western blotting in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. C) Cell viability was detected using CCK-8 assay in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. D) Colony formation was measured (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. E) Cell migration was determined (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. F) Cell invasion was determined (upper) and quantified (lower) in SK-Hep-1 and Hep3B cells transfected with sh-NC, sh-LINC00536#1, sh-LINC00536#1+miR-203b-5p inhibitor, and sh-LINC00536#1+VEGFA overexpression plasmid. All the experiments were conducted three repeated times. **p<0.01

LINC00536 knockdown inhibited HCC cell proliferation via downregulating VEGFA *in vivo*. To further prove the functional role of LINC00536 *in vivo*, we established xenografts using nude mice. The decreased tumor volume and weight were found in nude mice with LINC00536 knockdown (Figures 5A–5C). We also determined the VEGFA levels in tumor tissues. We revealed VEGFA was markedly downregulated in nude mice with LINC00536 knockdown (Figure 5D). These results indicated that LINC00536 knockdown inhibited HCC cell proliferation via downregulating VEGFA *in vivo*.

Discussion

Emerging evidence has shown that numerous dysregulated lncRNAs are identified in HCC tumor tissues and many of them exert a fundamental effect in the initiation, development, and deterioration of the malignancy [4–7]. However, the biological function and mechanisms of potential critical lncRNAs have to be elucidated. In this study, we identified a group of lncRNAs with differential expression levels in GSE138178 microarrays. Notably, we found LINC00536 showed a higher expression level in HCC tissues and cell

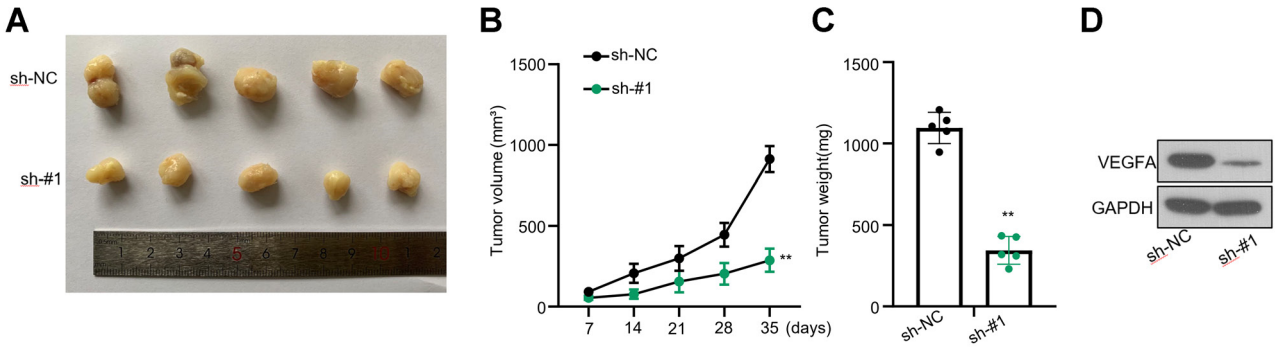

Figure 5. Knockdown of LINC00536 inhibits HCC cell proliferation *in vivo*. A) Effects of LINC00536 knockdown on tumor growth *in vivo*. Images of tumor formation in nude mice (n = 10) injected subcutaneously with SK-Hep-1 cells with LINC00536 knockdown or vector. B) Tumor volume was detected in mice injected subcutaneously with SK-Hep-1 cells with LINC00536 knockdown or vector. C) Tumor weight was detected in mice injected subcutaneously with SK-Hep-1 cells with LINC00536 knockdown or vector. D) The expression of VEGFA was determined by western blotting in mice injected subcutaneously with SK-Hep-1 cells with LINC00536 knockdown or vector. All the experiments were conducted three repeated times. **p<0.01

lines. Interestingly, high LINC00536 expression indicated a poor prognosis for HCC patients. These results indicated a fundamentally oncogenic role of LINC00536 in HCC.

Several previous studies also expressed that LINC00536 was aberrantly overexpressed in breast cancer, ovarian cancer, and bladder cancer. According to previous reports, LINC00536 could be used as a prognostic biomarker and showed satisfactory diagnostic performance in predicting patients with poor prognosis in breast and ovarian cancer [13, 14]. In bladder cancer, Li et al. found that LINC00536 was aberrantly overexpressed in bladder cancer and promoted malignant phenotypes through regulating the Wnt3a/β-Catenin signaling pathway [15]. Here, we also validated an oncogenic role of LINC00536 in HCC cells and we found that LINC00536 knockdown markedly suppressed malignant phenotypes. This stated that LINC00536 might also be a potential diagnostic, therapeutic, and prognostic biomarker in HCC.

The underlying mechanisms of lncRNAs in disease initiation, development, and progression include modulating transcriptional factors, splicing factors, and histone expression [23, 26–28]. For instance, MIR31HG in senescence depending on its localization and can as a potential therapeutic target in the treatment of senescence-related pathology [29]. The function of lncRNAs serving as a miRNA sponge and then regulating miRNA expression has gained wide attention. In this study, we also found that LINC00536 could exert the tumor promotive effect via acting as a miRNA sponge by directly binding to miR-203b-5p. Notably, several previous reports also suggested a tumor depressor of miR-203b-5p in several cancers including HCC [30–33]. Yet, potential mechanisms of miR-203b-5p acting as a tumor suppressor remain unclear. Through bioinformatic analysis and luciferase assays, we identified and validated a downstream target of miR-203b-5p, VEGFA in HCC cells. Studies have shown that VEGFA can accelerate cancer cell growth, migration, and invasion through regulating cell proliferation, epithelial mesenchyme transition, cell cycle, and cell apoptosis in HCC. But upstream regulatory networks of VEGFA need to be elucidated. Through rescue assays, we confirmed LINC00536 could modulate VEGFA via sponging miR-203b-5p. We also validated the biological function of the LINC00536/miR-203b-5p/VEGFA axis *in vivo*, which further consolidated the critical role of the LINC00536/miR-203b-5p/VEGFA signaling pathway in HCC.

In conclusion, a novel lncRNA LINC00536 was shown to be overexpressed in HCC, correlating with shorter survival. LINC00536 knockdown suppressed cancerous behaviors of HCC cells. In biological mechanism, we revealed that LINC00536 played a role of an oncogene by exerting a role of a miR-203b-5p sponge, thus in turn, promoting the downstream VEGFA expression. Our findings reported that LINC00536 might be a promising target for the diagnosis and therapeutics of HCC.

Acknowledgments: This project was supported by the project The expression and clinical significance of miRNA-203 in liver cancer before and after TACE treatment (No. 2021YD003).

References

[1] SUNG H, FERLAY J, SIEGEL RL, LAVERSANNE M, SOERJOMATARAM I et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021; 71: 209–249. https://doi.org/10.3322/caac.21660

[2] BRUIX J, DA FONSECA LG, REIG M. Insights into the success and failure of systemic therapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2019; 16: 617–630. https://doi.org/10.1038/s41575-019-0179-x

[3] FORNER A, REIG M, BRUIX J. Hepatocellular carcinoma. Lancet 2018; 391: 1301–1314. https://doi.org/10.1016/S0140-6736(18)30010-2

[4] KHANDELWAL A, BACOLLA A, VASQUEZ KM, JAIN A. Long non-coding RNA: A new paradigm for lung cancer. Mol Carcinog 2015; 54: 1235–1251. https://doi.org/10.1002/mc.22362

[5] DONG Y, LI X, LIN Z, ZOU W, LIU Y et al. HOXC-AS1-MYC regulatory loop contributes to the growth and metastasis in gastric cancer. J Exp Clin Cancer Res 2019; 38: 502. https://doi.org/10.1186/s13046-019-1482-7

[6] ZHANG Z, FU C, XU Q, WEI X. Long non-coding RNA CASC7 inhibits the proliferation and migration of colon cancer cells via inhibiting microRNA-21. Biomed Pharmacother 2017; 95: 1644–1653. https://doi.org/10.1016/j.biopha.2017.09.052

[7] LI JB, LIU F, ZHANG BP, BAI WK, CHENG W et al. LncRNA625 modulates prostate cancer cells proliferation and apoptosis through regulating the Wnt/beta-catenin pathway by targeting miR-432. Eur Rev Med Pharmacol Sci 2017; 21: 2586–2595.

[8] WANG M, MAO C, OUYANG L, LIU Y, LAI W et al. Long noncoding RNA LINC00336 inhibits ferroptosis in lung cancer by functioning as a competing endogenous RNA. Cell Death Differ 2019; 26: 2329–2343. https://doi.org/10.1038/s41418-019-0304-y

[9] HAN M, WANG S, FRITAH S, WANG X, ZHOU W et al. Interfering with long non-coding RNA MIR22HG processing inhibits glioblastoma progression through suppression of Wnt/beta-catenin signalling. Brain 2020; 143: 512–530. https://doi.org/10.1093/brain/awz406

[10] LI DY, BUSCH A, JIN H, CHERNOGUBOVA E, PELISEK J et al. H19 Induces Abdominal Aortic Aneurysm Development and Progression. Circulation 2018; 138: 1551–1568. https://doi.org/10.1161/CIRCULATIONAHA.117.032184

[11] WANG Y, YANG L, CHEN T, LIU X, GUO Y et al. A novel lncRNA MCM3AP-AS1 promotes the growth of hepatocellular carcinoma by targeting miR-194-5p/FOXA1 axis. Mol Cancer 2019; 18: 28. https://doi.org/10.1186/s12943-019-0957-7

[12] CHEN B, LAN J, XIAO Y, LIU P, GUO D et al. Long noncoding RNA TP53TG1 suppresses the growth and metastasis of hepatocellular carcinoma by regulating the PRDX4/β-catenin pathway. Cancer Lett 2021; 513: 75–89. https://doi.org/10.1016/j.canlet.2021.04.022

[13] ZHANG Y, YE Q, HE J, CHEN P, WAN J et al. Recurrence-Associated Multi-RNA Signature to Predict Disease-Free Survival for Ovarian Cancer Patients. Biomed Res Int 2020; 2020: 1618527. https://doi.org/10.1155/2020/1618527

[14] LI H, GAO C, LIU L, ZHUANG J, YANG J et al. 7-lncRNA Assessment Model for Monitoring and Prognosis of Breast Cancer Patients: Based on Cox Regression and Co-expression Analysis. Front Oncol 2019; 9: 1348. https://doi.org/10.3389/fonc.2019.01348

[15] LI R, ZHANG L, QIN Z, WEI Y, DENG Z et al. High LINC00536 expression promotes tumor progression and poor prognosis in bladder cancer. Exp Cell Res 2019; 378: 32–40. https://doi.org/10.1016/j.yexcr.2019.03.009

[16] KASEB AO, HANBALI A, COTANT M, HASSAN MM, WOLLNER I et al. Vascular endothelial growth factor in the management of hepatocellular carcinoma: a review of literature. Cancer 2009; 115: 4895–4906. https://doi.org/10.1002/cncr.24537

[17] SUZUKI K, HAYASHI N, MIYAMOTO Y, YAMAMOTO M, OHKAWA K et al. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res 1996; 56: 3004–3009.

[18] YI ZY, FENG LJ, XIANG Z, YAO H. Vascular endothelial growth factor receptor-1 activation mediates epithelial to mesenchymal transition in hepatocellular carcinoma cells. J Invest Surg 2011; 24: 67–76. https://doi.org/10.3109/08941939.2010.542272

[19] HUYNH H, FARGNOLI J, AYERS M, SOO KC, KOONG HN et al. Brivanib alaninate, a dual inhibitor of vascular endothelial growth factor receptor and fibroblast growth factor receptor tyrosine kinases, induces growth inhibition in mouse models of human hepatocellular carcinoma. Clin Cancer Res 2008; 14: 6146–6153. https://doi.org/10.1158/1078-0432.CCR-08-0509

[20] YOSHIJI H, KURIYAMA S, HICKLIN DJ, HUBER J, YOSHII J et al. KDR/Flk-1 is a major regulator of vascular endothelial growth factor–induced tumor development and angiogenesis in murine hepatocellular carcinoma cells. Hepatology 1999; 30: 1179–1186. https://doi.org/10.1002/hep.510300509

[21] FOUNTZILAS C, GUPTA M, LEE S, KRISHNAMURTHI S, ESTFAN B et al. A multicentre phase 1b/2 study of tivozanib in patients with advanced inoperable hepatocellular carcinoma. Br J Cancer 2020; 122: 963–970. https://doi.org/10.1038/s41416-020-0737-6

[22] GELLER JI, FOX E, TURPIN BK, GOLDSTEIN SL, LIU X et al. A study of axitinib, a VEGF receptor tyrosine kinase inhibitor, in children and adolescents with recurrent or refractory solid tumors: A Children's Oncology Group phase 1 and pilot consortium trial (ADVL1315). Cancer 2018; 124: 4548–4555. https://doi.org/10.1002/cncr.31725

[23] SCHMITT AM, CHANG HY. Long Noncoding RNAs in Cancer Pathways. Cancer Cell 2016; 29: 452–463. https://doi.org/10.1016/j.ccell.2016.03.010

[24] DENG L, YANG SB, XU FF, ZHANG JH. Long noncoding RNA CCAT1 promotes hepatocellular carcinoma progression by functioning as let-7 sponge. J Exp Clin Cancer Res 2015; 34: 18. https://doi.org/10.1186/s13046-015-0136-7

[25] CAI K, LI T, GUO L, GUO H, ZHU W et al. Long non-coding RNA LINC00467 regulates hepatocellular carcinoma progression by modulating miR-9-5p/PPARA expression. Open Biol 2019; 9: 190074. https://doi.org/10.1098/rsob.190074

[26] MAO C, WANG X, LIU Y, WANG M, YAN B et al. A G3BP1-Interacting lncRNA Promotes Ferroptosis and Apoptosis in Cancer via Nuclear Sequestration of p53. Cancer Res 2018; 78: 3484–3496. https://doi.org/10.1158/0008-5472.CAN-17-3454

[27] ELGUINDY MM, MENDELL JT. NORAD-induced Pumilio phase separation is required for genome stability. Nature 2021; 595: 303–308. https://doi.org/10.1038/s41586-021-03633-w

[28] YANG R, LIU N, CHEN L, JIANG Y, SHI Y et al. GIAT4RA functions as a tumor suppressor in non-small cell lung cancer by counteracting Uchl3-mediated deubiquitination of LSH. Oncogene 2019; 38: 7133–7145. https://doi.org/10.1038/s41388-019-0909-0

[29] MONTES M, LUBAS M, ARENDRUP FS, MENTZ B, ROHATGI N et al. The long non-coding RNA MIR31HG regulates the senescence associated secretory phenotype. Nat Commun 2021; 12: 2459. https://doi.org/10.1038/s41467-021-22746-4

[30] TURASHVILI G, LIGHTBODY ED, TYRYSHKIN K, SENGUPTA SK, ELLIOTT BE et al. Novel prognostic and predictive microRNA targets for triple-negative breast cancer. The FASEB J 2018; fj201800120R. https://doi.org/10.1096/fj.201800120R

[31] WANG B, LI X, ZHAO G, YAN H, DONG P et al. miR-203 inhibits ovarian tumor metastasis by targeting BIRC5 and attenuating the TGFβ pathway. J Exp Clin Cancer Res 2018; 37: 235. https://doi.org/10.1186/s13046-018-0906-0

[32] HUR K, TOIYAMA Y, OKUGAWA Y, IDE S, IMAOKA H et al. Circulating microRNA-203 predicts prognosis and metastasis in human colorectal cancer. Gut 2017; 66: 654–665. https://doi.org/10.1136/gutjnl-2014-308737

[33] ZHANG A, LAKSHMANAN J, MOTAMENI A, HARBRECHT BG. MicroRNA-203 suppresses proliferation in liver cancer associated with PIK3CA, p38 MAPK, c-Jun, and GSK3 signaling. Mol Cell Biochem 2018; 441: 89–98. https://doi.org/10.1007/s11010-017-3176-9