Original Article

Suppression of hepatocellular carcinoma progression by long noncoding RNA apolipoprotein C1 pseudogene via the regulation of the microRNA-106b-PTEN axis

Yi Qian[1], Hao Chen[2], Liang Chen[3], Chuang Ge[3], Dongmei Zhu[3], Dinghua Zhou[1]

[1]General Surgery Department, The Sixth Affiliated Hospital of Soochow University, Suzhou, China; [2]Pathology Department, The Second Affiliated Hospital of Nantong University, Nantong, China; [3]General Surgery Department, The Second Affiliated Hospital of Nantong University, Nantong, China

Contributions: (I) Conception and design: D Zhou; (II) Administrative support: D Zhou; (III) Provision of study materials or patients: Y Qian, H Chen; (IV) Collection and assembly of data: Y Qian, L Chen; (V) Data analysis and interpretation: Y Qian, C Ge; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dinghua Zhou, MD. General Surgery Department, The Sixth Affiliated Hospital of Soochow University, Suzhou, China; No. 16 Xinjiekouwai Street, Xicheng District, Beijing 100032, China. Email: dinghua_zhou1234@163.com.

Background: Numerous researches have reported that long noncoding RNAs (lncRNAs) participate in tumor development and progression. LncRNA apolipoprotein C-I pseudogene 1 (*APOC1P1*), a pseudogene located in 19q13.2 between apolipoprotein C-I and apolipoprotein C-IV, is involved in a variety of diseases. However, the role of lncRNA *APOC1P1* in hepatocellular carcinoma (HCC) remains unknown.

Methods: Quantitative polymerase chain reaction (qPCR) was performed to examine the expression of *APOC1P1*, miR-106b, and PTEN (phosphatase and TENsin homolog deleted on chromosome 10) in HCC tissues, adjacent normal tissues, and specific cell lines (LO2, Bel-7407, HCCLM3, MHCC-97H, Hep G2, and Huh-7). Upregulation of *APOC1P1* and downregulation of miR-106b were conducted via application of vector transfection and microRNA (miRNA) inhibitor. Bioinformatics analysis and luciferase reporter assay were used to verify the binding sites of *APOC1P1*, miR-106b, and PTEN. Cell proliferation and invasion were determined with Cell Counting Kit-8 (CCK-8) and Transwell experiments. Subcellular location analysis was used to determine the distribution of *APOC1P1* in cells, and Western blotting was used to detect the expression of PTEN.

Results: It was found that the expressions of *APOC1P1* and PTEN were downregulated, while that of miR-106b was upregulated in HCC tissues and cells. Subcellular location analysis showed that *APOC1P1* was localized in cytoplasm and competitively bound to miR-106b. *APOC1P1* overexpression and miR-106b inhibition suppressed HCC cell proliferation and invasion. qPCR indicated the negative correlation between *APOC1P1* expression and miR-106b expression in HCC tissues and a positive correlation between *APOC1P1* and PTEN.

Conclusions: Our findings suggested that the lncRNA *APOC1P1* inhibits HCC progression by competitively binding to miR-106b, leading to elevated PTEN expression, inhibiting cell proliferation and invasion in HCC cells. These results provide new insights into the diagnosis and therapy of HCC.

Keywords: Hepatocellular carcinoma (HCC); apolipoprotein C-I pseudogene 1 (*APOC1P1*); ceRNA; miR-106b; phosphatase and TENsin homolog deleted on chromosome 10 (PTEN)

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Introduction

Hepatocellular carcinoma (HCC) is the most common type of liver cancer and has a high incidence and mortality worldwide (1). According to the reports, 782,000 cases of HCC were diagnosed and 746,000 HCC-related deaths occurred in 2012, and it is expected that the incidence rate will increase in the future. Among all countries, China has the highest incidence of HCC and accounts for half of the global number of patients with HCC (2). Every year, 300,000 to 400,000 Chinese people die from HCC (3). HCC typically emerges due to cirrhosis induced by viral infection or alcohol consumption. The common treatment methods for HCC, drug administration and surgery, have certain drawbacks (4,5). As curing HCC remains difficult, improving effective treatment for HCC is critical.

Long noncoding RNA (lncRNA) is a transcript including more than 200 nucleotides that does not code for any protein (6). Regardless of whether lncRNAs are in the nucleus or the cytoplasm, they form regulatory networks with other RNAs, such as microRNAs (miRNAs), or proteins to affect the downstream molecules and play key roles in many of the biological processes of diseases (7,8). An abundance of research has shown that lncRNAs participate in some biological processes of HCC, including proliferation, invasion, ferroptosis and metastasis, etc. (9,10). For instance, one study shows that HCC ferroptosis associative lncRNA (HEPFAL) is shown to mediate the ubiquitination of SLC7A11 to promote ferroptosis in HCC (11). Stabilization of β-catenin mRNA is promoted by lncRNA UFC1 via binding with the mRNA stabilizing protein HuR, a protein reported for mRNA stabilization, thus activating Wnt signaling and consequent HCC progression (12). While miRNA sponge lncRNA XIST acts as a tumor suppressor by interaction with miR-92b leading to repression of HCC proliferation and metastasis (13). Although these studies are illuminating, much more dysregulated lncRNAs require further investigation for providing new insights into HCC pathogenesis and novel tools for the early diagnosis and treatment of HCC.

The lncRNA apolipoprotein C-I pseudogene 1 (*APOC1P1*) is located in 19q13.2 between apolipoprotein C-I and apolipoprotein C-IV. Han *et al.* (14) identified that lncRNA *APOC1P1* can inhibit the inflammatory response in malignant bile duct cell carcinoma. In another study, *lncRNA APOC1P1* was found to be overexpressed in breast cancer (15) and was confirmed to regulate the pathogenesis of cholangiocarcinoma (16). Considering the relationship between cholangiocarcinoma and HCC, we hypothesized whether *lncRNA APOC1P1* plays certain mechanism in HCC development and progression. However, the role of *lncRNA APOC1P1* in HCC remains underexamined.

In this study, we aimed to determine whether *lncRNA APOC1P1* participates in the progression of HCC and to clarify any related mechanisms. Our findings may provide novel biomarkers and therapeutic targets for HCC. We present this article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2189/rc).

Highlight box

Key findings

- *lncRNA APOC1P1* inhibits hepatocellular carcinoma (HCC) progression by competitively binding to miR-106b, leading to elevated phosphatase and TENsin homolog deleted on chromosome 10 (PTEN) expression, inhibiting cell proliferation and invasion in HCC cells.

What is known and what is new?

- *APOC1P1* was reported to regulate the pathogenesis of cholangiocarcinoma and breast cancer.
- Upregulated *APOC1P1* inhibited HCC progression. *APOC1P1* was shown to regulate PTEN expression by targeting miR-106b to suppress HCC progression.

What is the implication, and what should change now?

- These results provide new insights into the diagnosis and therapy of HCC.

Methods

Clinical samples and cell lines

Matched pairs of cancerous and normal tissue from 50 patients with HCC were obtained from the Second Affiliated Hospital of Nantong University. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by Ethics Committee of the Second Affiliated Hospital of Nantong University (No. 2020KT014). The study mainly uses biological specimens obtained in previous clinical diagnosis and treatment, and is a secondary use of biological specimens, which is approved by the ethics committee without informed consent. The clinical characteristics of the HCC patients are summarized in *Table 1*.

The human HCC cell lines (Bel-7404, HCCLM3, MHCC-97H, Hep G2, and Huh-7) and normal human

Table 1 Correlation between APOC1P1 expression and clinicopathologic features of patients with statistical analyses were carried out using Pearson χ^2 test

Characteristic	Total	APOC1P1 (n)		χ^2 value	P value
		Low (n=32)	High (n=18)		
Gender				0.006	0.921
Male	38	27	11		
Female	12	5	7		
Age (years)				0.014	0.781
≤60	21	17	4		
>60	29	15	14		
Size (cm)				0.023	0.716
<5	31	26	5		
≥5	19	6	13		
Lymphatic metastasis (yes/no)				4.126	0.035*
Yes	16	9	7		
No	34	23	11		
TNM stage				3.823	0.048*
I and II	29	20	9		
III and IV	21	12	9		

* P<0.05 was considered significant. APOC1P1, apolipoprotein C-I pseudogene 1; TNM, tumor, node, metastasis.

hepatocytes LO2 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium (product code: 11960044; Corning, Corning, NY, USA) supplemented with 10% fetal bovine serum (FBS) (product code: 10099141C; Corning, Corning, NY, USA) and 1% penicillin and streptomycin (product code: 15140122; Gibco, Thermo Fisher Scientific, Grand Island, NY, USA) in a humidified incubator at 37 ℃ with a 5% CO_2 atmosphere.

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

Total RNA was extracted from tissues and cell lines using TRIzol Reagent (product code: 15596026; Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) according to the manufacturer's instructions. The primer sequences of these genes in this study are summarized in *Table 2*. The complement DNA (cDNA) was synthesized from RNA with the Revert Aid First-Strand cDNA Synthesis Kit (K1621; Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was then performed on a LightCycler 480 using SYBR Green Master Mix (product code: 4913914001; Roche Diagnostics, Basel, Switzerland). The relative gene expression level was determined using the $2^{-\Delta\Delta Ct}$ approach with 18S ribosomal RNA and U6 as the normalization references.

Table 2 The primer sequences used in qRT-PCR

Gene	Primer sequence
lncRNA APOC1P1	F GGTCCTGGTGGTGGTTCTGTC
	R CTCCTTCACTTTCCGAAATGTCTC
miRNA-106b	F TTTTCGCCCTTAGCGTGAAGA
	R GAGGCAGTCGAAGCTCTCG
PTEN	F GTTTACCGGCATCAAAT
	R CCCCCACTTTAGTGCAGT
18S	F GTAACCCGTTGAACCCCATT
	R CCATCCAATCGGTAGTAGCG
U6	F TCCCTTCGGGGACATCCG
	R AATTTTGGACCATTTCTCGATTTGT

qRT-PCR, quantitative real-time polymerase chain reaction.

Plasmid construction and cell transfection

PCR products of *lincRNA APOC1P1* were cloned into pcDNA3.1 vector. Small interfering RNA (siRNA), short hairpin RNA (shRNA), and miRNA mimics and inhibitors were purchased from RiboBio (RiboBio, Guangzhou, China). All of the transfection experiments were conducted with Lipofectamine 3000 (product code: L3000008; Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA). qRT-PCR was used to measure the transfection efficiency.

Cell Counting Kit 8 assay and cell invasion assay

Cell Counting Kit-8 (CCK-8) (product code: CA1210; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) assay was used to measure cell viability. Cells (1×10^3) were planted into the 96-well plant and cultured at 37 ℃ in a humidified incubator. After incubation for 4 h, 10 μL of CCK-8 was added into the each well. The absorbance was measured at 450 nm with a microplate reader.

Cell invasive ability was measured with a Transwell assay. Cells (5×10^4) were cultured in serum-free DMEM and seeded

into the upper chambers (product code: CLS3450; Corning, Tewksbury, MA, USA). In the bottom chamber, medium with 10% FBS and 90% DMEM was added. Cells were left invade for 24 h at 37 ℃. The upper cavity was wiped with a cotton swab from the culture dish for nonmigrated cells. The cells were then fixed with 4% paraformaldehyde for 10 min at room temperature and stained with 0.1% crystal violet for 20 min at room temperature. Counts of invaded cells were used to estimate the cell invasion ability.

APOC1P1 subcellular location analysis

Cells were fixed in 4% paraformaldehyde for 30 min and then washed with phosphate-buffered saline (PBS). Triton X-100 (product code: 9002-93-1, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was used to treat the fixed cells. The cells were incubated with a fluorescent in situ hybridization (FISH) kit (RiboBio, Guangzhou, China). The PARIS Kit (Invitroge, Thermo Fisher Scientific, Vilnius, Lithuania) was used to separate cytoplasmic and nuclear RNA.

Bioinformatics analysis

The differential expression of *APOC1P1* in HCC tissues was analyzed via The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/). Kaplan-Meier curve analysis of TCGA and Gene Expression Omnibus (GEO) was used to define the association of *APOC1P1* with time to progression. Differentially expressed messenger RNA (mRNA) datasets were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Luciferase reporter assay

Luciferase reporter assay was carried out to examine the interactions between *APOC1P1* and miR-106b. pSI-Check2 vector (Riobio, Guangzhou, China) was participated in the cloning of full length *APOC1P1* as *pSI-Check2-APOC1P1* wild vector or *pSI-Check2-APOC1P1* mutant vector was constructed. Mutant or wild type for *APOC1P1* was contransfected with miR-106b mimics or control. Meanwhile, mutant or wild type for phosphatase and TENsin homolog deleted on chromosome 10 (PTEN) was also constructed with pSI-Check2. Both pSI-Check2-PTEN wild vector or pSI-Check2-PTEN mutant vector was contransfected with miR-106b mimics. A Dual Luciferase Assay kit (Promega Corp., Fitchburg, WI, USA) was used to detect the Firefly and Renilla luciferase activities 2 days post-transfection following the manufacturer's instructions.

Western blotting

Total proteins were collected with cell lysis buffer (product code: P70100; NCM Biotech, Newport, RI, USA). Proteins were separated with sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (product code: IPFL00010; MilliporeSigma, Burlington, MA, USA). Membranes were sealed with 5% nonfat milk and then treated with primary antibodies at 4 ℃ overnight. After a wash with PBS, secondary antibody (product code: SA00001-2; Proteintech, Rosemont, IL, USA) was incubated with membranes for 1 h in the dark. The antibodies of PTEN (product code: ab170941; Abcam, Cambridge, UK) and GAPDH (product code: ab181602; Abcam, Cambridge, UK) were purchased from Abcam. The antibodies were diluted by buffer. The dilution radio of primary antibodies was 1:1,000, while that of the secondary antibodies was 1:10,000. GAPDH served as the internal control. The data were detected using an enhanced chemiluminescence (ECL) detection system.

Statistical analysis

All data are presented as the mean and standard deviation (SD) and were analyzed with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA). The analysis of the relative expression of HCC tissues and multiple groups was completed with the independent-samples *t*-test and one-way analysis of variance (ANOVA), respectively. A P value <0.05 was considered to indicate a statistically significant difference.

Results

APOC1P1 was downregulated in HCC and was associated with overall survival

The first results showed that *APOC1P1* was downregulated in 50 HCC tissues in comparison with the adjacent tissues (P=0.002; *Figure 1A*). To determine the molecular role of *APOC1P1* in HCC, we used qRT-PCR to determine the expression of *APOC1P1* in 6 different cell lines including 1 normal cell line (LO2) and 5 HCC cell lines (Bel-7404, HCCLM3, MHCC-97H, Hep G2, and Huh-7). We found that the expression of *APOC1P1* was considerably decreased

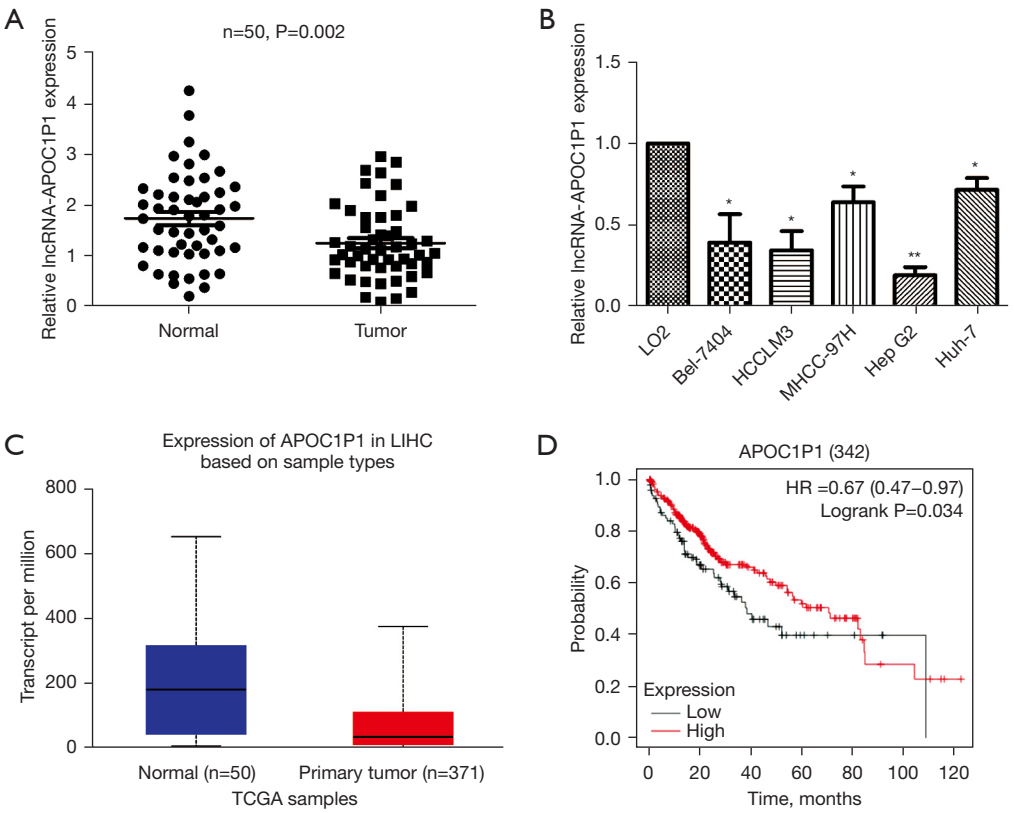

Figure 1 APOC1P1 was significantly down regulated in HCC tissues and cell lines. (A) Relative expression of APOC1P1in HCC tissues and adjacent normal tissues. (B) Relative expression of APOC1P1 in HCC cell lines and normal liver cells. (C) Expression of APOC1P1 in TCGA samples. (D) Kaplan-Meier curve which downloaded from TCGA database revealed the relationship between APOC1P1 expression and overall survival. *, P<0.05; **, P<0.01. APOC1P1, apolipoprotein C-I pseudogene 1; LIHC, liver hepatocellular; TCGA, The Cancer Genome Atlas; HR, hazard ratio; HCC, hepatocellular carcinoma.

in HCC cell lines compared with normal cells (P<0.05; *Figure 1B*). Significantly, *APOC1P1* expression in HCCLM3 cells and Hep G2 cells were lower than that in other cell lines. HCCLM3 cells and Hep G2 cells were used in the subsequent experiments. Our results were confirmed by the TCGA database (*Figure 1C*). In addition, the Kaplan-Meier curve revealed the relationship between *APOC1P1* expression and overall survival (*Figure 1D*). The above results indicated that *APOC1P1* was downregulated in HCC and associated with good prognosis.

APOC1P1 overexpression inhibited the tumor cell proliferation and invasion

To investigate the biological role of *APOC1P1* in HCC cells, we conducted *APOC1P1* overexpression in HCCLM3 cells and Hep G2 cells through shRNA transfection. The qRT-PCR results indicated that the *APOC1P1* expression of *APOC1P1* was markedly upregulated in HCCLM3 cells and Hep G2 cells which were transfected by plasmid compared with control cells (P<0.05; *Figure 2A*). CCK-8 assay showed that *APOC1P1* overexpression markedly reduced cellular viability of HCCLM3 cells and Hep G2 cells compared to control cells (P<0.05; *Figure 2B*). Moreover, we found that *APOC1P1* overexpression also affected cell invasion. The results of Transwell invasion assay confirmed that upregulated *APOC1P1* obviously decreased invasion ability (*Figure 2C*). These findings indicated that *APOC1P1* inhibited the progression of HCC cells.

APOC1P1 regulated PTEN by targeting miRNA-106b as a competing endogenous RNA

We conducted subcellular location analysis to characterize

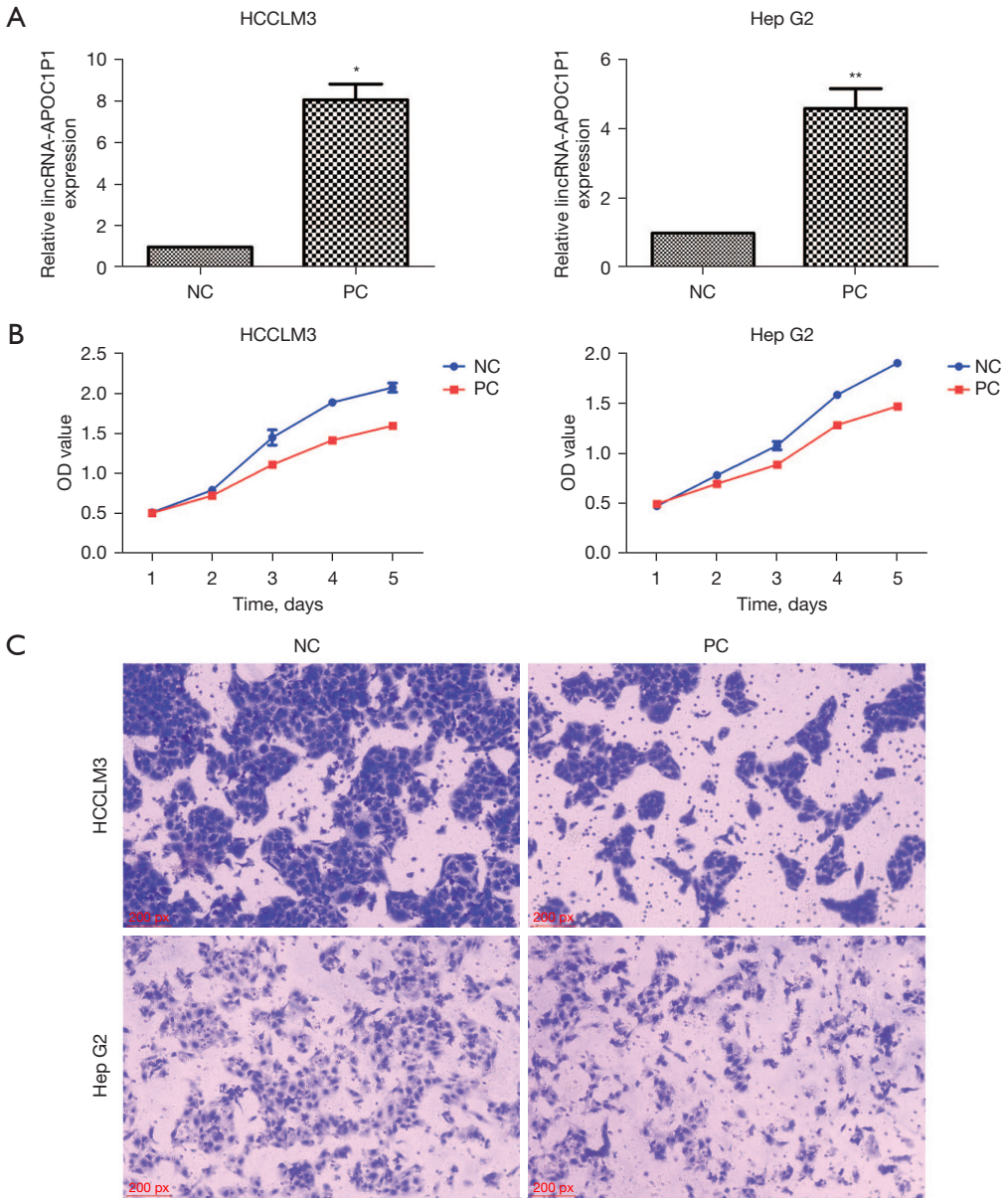

Figure 2 APOC1P1 overexpression suppressed the cell proliferation and invasion of HCC cells. (A) Relative expression of APOC1P1in HCCLM3 cells and Hep G2 cells transfected with pc-APOC1P1. (B) CCK-8 assay revealed the proliferation of HCCLM3 cells and Hep G2 cells transfected with pc-APOC1P1. (C) Transwell assay was applied to measure the effect of upregulated APOC1P1 expression on the invasion of HCCLM3 cells and Hep G2 cells (crystal violet staining). *, P<0.05; **, P<0.01. APOC1P1, apolipoprotein C-I pseudogene 1; NC, negative control; PC, positive control; OD, optical density; CCK-8, Cell Counting Kit-8.

the distribution of *APOC1P1* in cells. FISH and subcellular fractionation analysis results showed that *APOC1P1* was abundant in the cytoplasm (*Figure 3A,3B*). We then used two GEO datasets, GSE108724 and GSE10694, with 2687 mRNAs and 6 miRNAs being aberrantly expressed in GSE108724 and 69 miRNAs being differentially expressed in GSE10694 (*Figure 4A,4B*). A volcano plot revealed the upregulated and downregulated miRNAs of GSE108724 and GSE10694 (*Figure 4C,4D*). We identified three upregulated miRNAs (miR-106b, miR-222 and miR-221) by identifying the intersection of miRNAs of the two datasets. PCR results showed that miR-106b expression was significantly lower

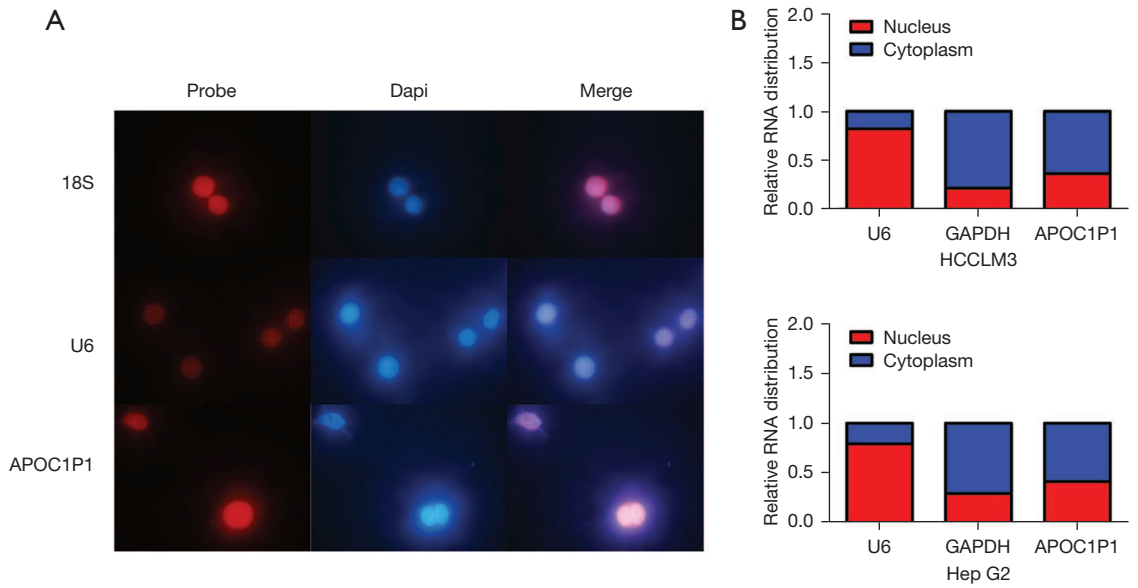

Figure 3 The distribution of APOC1P1 in cells. (A) FISH analysis showed that the APOC1P1 was mainly located in the cytoplasm. (B) PCR was used to detect APOC1P1 subcellular fractionation. APOC1P1, apolipoprotein C-I pseudogene 1; FISH, fluorescent in situ hybridization; PCR, polymerase chain reaction.

than other miRNAs in the control group. Thus, we chose miR-106b as the target gene in subsequent experiments.

To determine the relationship of *APOC1P1* and miR-106b, we detected the expression of miR-106b in cell lines and tumor tissues. The qRT-PCR results indicated that miR-106b was upregulated in HCC cell lines and HCC tissues compared with normal control groups (*Figure 5A,5B*). Luciferase reporter assay confirmed the binding site between *APOC1P1* and miR-106b, suggesting the presence of an lncRNA-miRNA network (*Figure 5C* and Figure S1). In the study by Zhang *et al.* (16), miR-106b had a putative biding site in the 3'-untranslated region (UTR) of PTEN. As expected, our luciferase reporter assay also revealed this relationship between miR-106b and PTEN (*Figure 5D*). Moreover, there was a negative correlation between *APOC1P1* expression and miR-106b expression in HCC tissues (r^2=0.5012; P=0.0062; *Figure 5E*) and a positive correlation between *APOC1P1* and PTEN (r^2=0.5128; P=0.0041; *Figure 5F*). All data suggested that *APOC1P1* regulated PTEN by targeting miRNA-106b as a competing endogenous RNA (ceRNA).

The APOC1P1-miR-106b-PTEN axis modulated HCC cell proliferation and invasion in vitro

PTEN was downregulated in the HCC cell lines, while miR-106b was upregulated in HCC cell lines (*Figure 6A*). miR-inhibitor transfection and *APOC1P1* plasmid construction + miR-inhibitor cotransfection were performed to determine whether miR-106b and PTEN participate in the biological function of *APOC1P1* in HCC (*Figure 6B*). CCK-8 assay indicated that *APOC1P1* overexpression or miR-106b inhibition decreased HCC cell proliferation ability (*Figure 6C*). Moreover, HCC cell proliferation ability was markedly suppressed when *APOC1P1* overexpression and miR-106b inhibition occurred simultaneously. The Western blot results showed that PTEN protein expression of the *pc-APOC1P1* + miR-inhibitor group was significantly higher than that in other groups (*Figure 6D*). Overall, upregulation of *APOC1P1* or downregulation of miR-106b enhanced PTEN expression, which modulated HCC cell proliferation and invasion.

Discussion

Due to its high incidence, frequent recurrence, and poor survival rate, HCC has attracted considerable attention in global research (17). Molecular mechanism studies have been used to identify a vast number of genetic factors that are involved in tumor genesis and development. lncRNA is the most notable of these and has been proven to regulate many diseases. Noticeably, in 2018, lncRNA *APOC1P1*

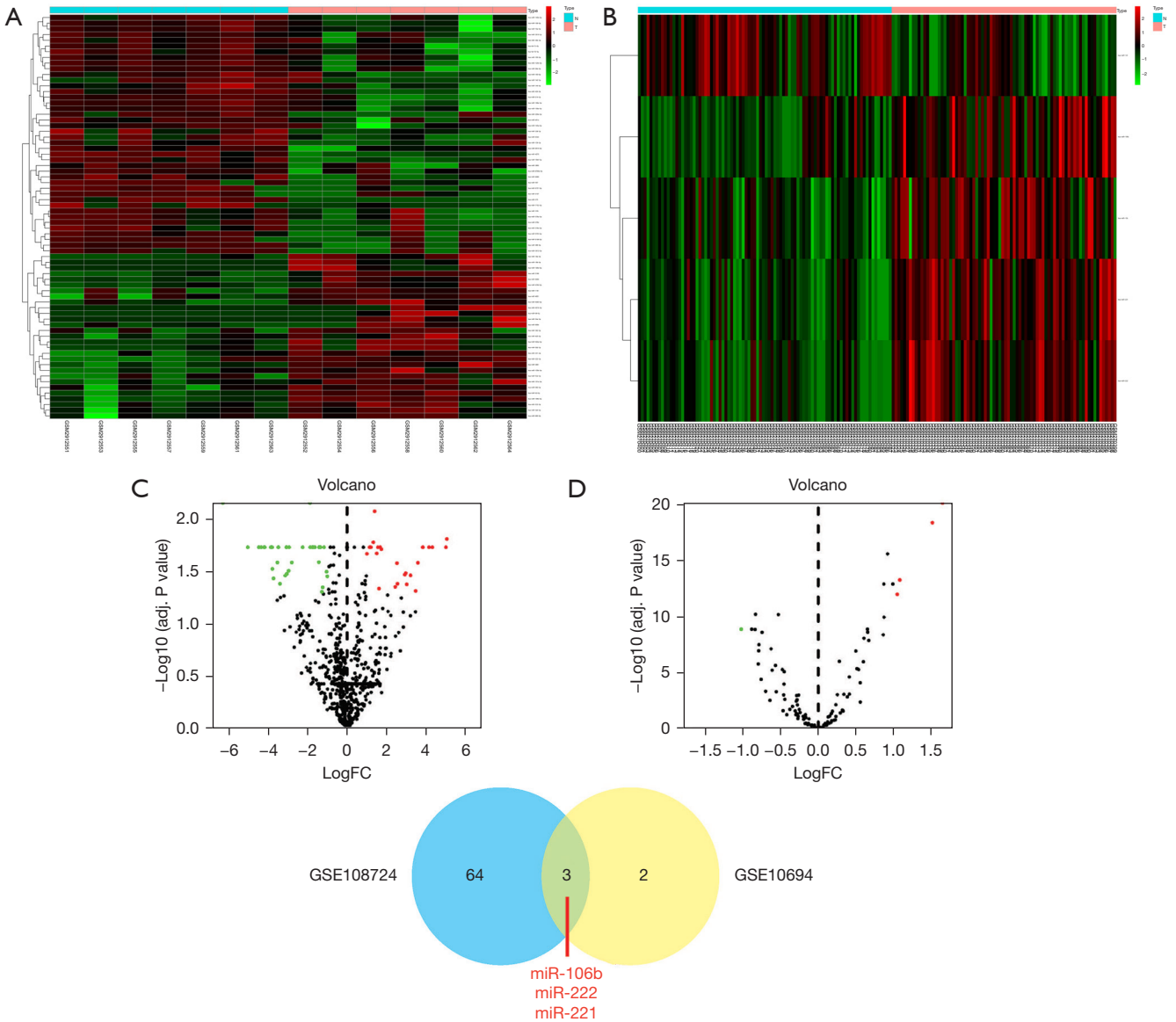

Figure 4 The targets of APOC1P1 in bioinformatics analysis. (A,B) The clustered heatmaps of GSE108724 and GSE10694 showed dysregulated miRNA in HCC tissues. (C,D) Volcano plot showed different miRNAs in TCGA database. Green dots represented downregulated miRNAs while red dots represented upregulated miRNAs. Black dots represented non-differentially expressed miRNAs. Venn diagram of the miRNAs from GSE108724 and GSE10694. FC, fold change; APOC1P1, apolipoprotein C-I pseudogene 1; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

was confirmed to be involved in the inflammation of cholangiocarcinoma (16). Considering the relationship between inflammation and HCC, we hypothesized that *APOC1P1* has a role in HCC.

In our study, we found that *APOC1P1* expression was downregulated in HCC tissues and HCC cell lines. Furthermore, based on TCGA data, it was found that the high expression of *APOC1P1* was associated with good prognosis in patients with HCC. After the overexpression of *APOC1P1* was established, the influence on proliferation and invasion of HCC was observed. The results confirmed that *APOC1P1* was vital in the progression of HCC and may thus serve as a therapeutic target in clinical treatment.

We conducted subsequent experiments to clarify

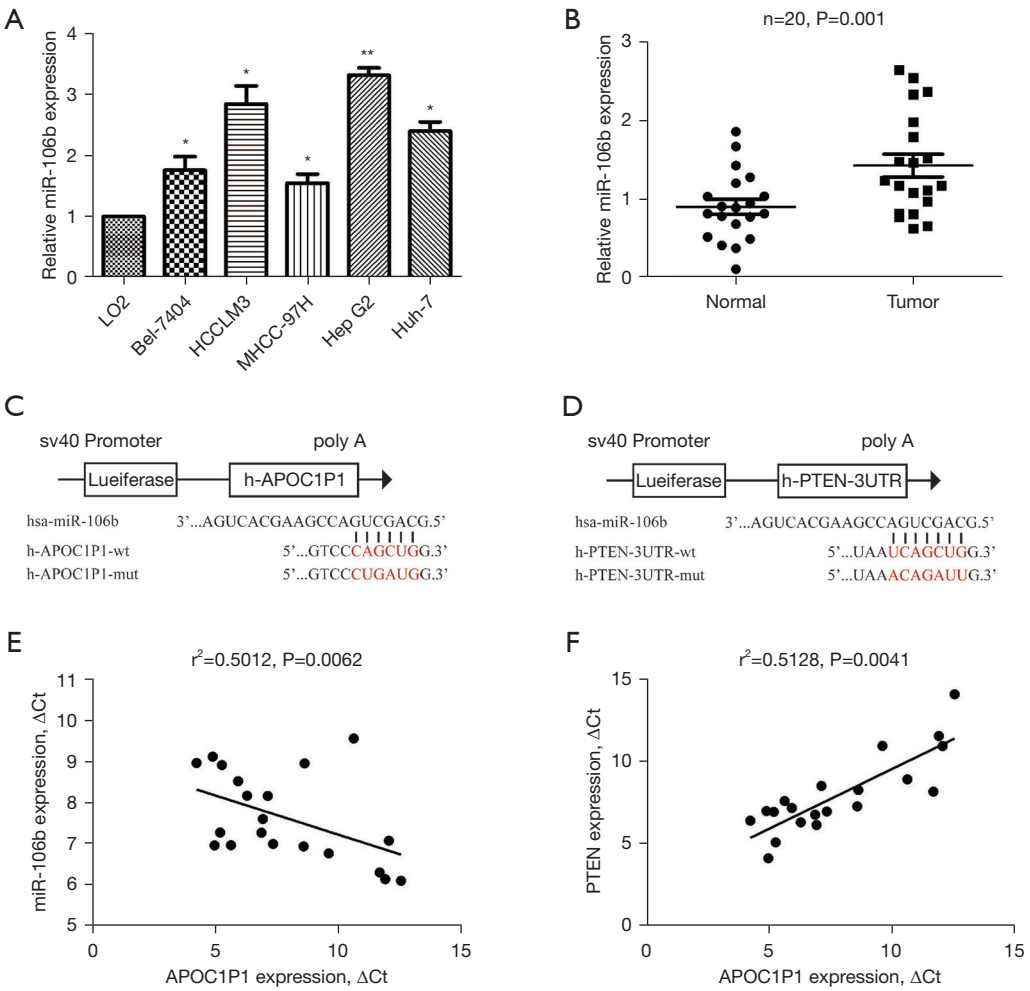

Figure 5 miRNA-106b was the downstream target of APOC1P1. (A) Relative expression of miRNA-106b in HCC tissues and adjacent normal tissues. (B) Relative expression of miRNA-106b in HCC cell lines and normal liver cells. (C,D) The luciferase reporter assay conformed a strong relationship between miR-106b and APOC1P1 as well as that between miR-106b and PTEN. (E,F) Spearman and Pearson correlation analysis for APOC1P1 and miRNA-106b expression as well as the correlation of miR-106b and PTEN expression. *, P<0.05; **, P<0.01. APOC1P1, apolipoprotein C-I pseudogene 1; HCC, hepatocellular carcinoma; PTEN, phosphatase and TENsin homolog deleted on chromosome 10.

the molecular mechanism of *APOC1P1* in HCC. The data indicated that *APOC1P1* was mainly located in the cytoplasm, which has been shown to be the site of ceRNA by numerous studies (18). ceRNA cross-talk as a type of network interaction has also been widely explored, and it has been found that lncRNAs act as molecular sponges in moderating miRNA expression. Evidence also suggests that the ceRNA molecular network is involved in tumor growth (19), with lncRNAs being particularly intriguing within ceRNA cross-talk due to their potential as therapeutic targets. Thus, we speculate that *APOC1P1* acts as ceRNA mechanism in regulating the progression of HCC.

The bioinformatic analysis of GEO data yielded three candidate miRNA targets: miR-106b, miR-222, and miR-221. According to the PCR results, miR-106b was selected to conduct subsequent experiments due to it having the lowest expression in the APOC1P1-overexpressed cells. miR-106b has been demonstrated to be a tumor biomarker that promotes HCC cell proliferation and invasion (20,21). Shen *et al.* (22) reported miR-106b expression to be markedly upregulated in HCC tissues and cell lines, which was consistent with our results. Additionally, our data

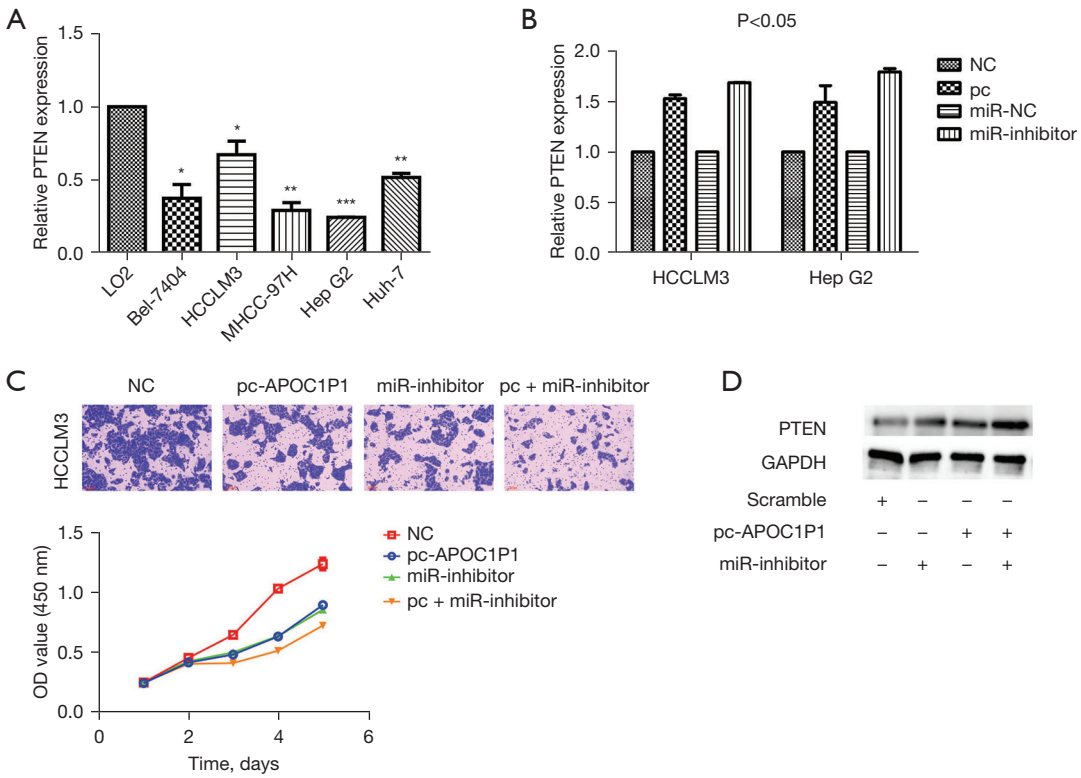

Figure 6 APOC1P1 regulated PTEN by targeting miRNA-106b as a ceRNA. (A) Relative expression of PTEN in HCC cell lines and normal liver cells. (B) Relative expression of PTEN in HCCLM3 cells and Hep G2 cells transfected with pc-APOC1P1 and miRNA inhibitor. (C) CCK-8 and Transwell assay were used to determine the influence of overexpression APOC1P1 and inhibition miRNA106b on the proliferation and invasion of HCCLM3 cells and Hep G2 cells transfected with pc-APOC1P1 and miRNA inhibitor (crystal violet staining). (D) Western blot analysis of PTEN in cells with upregulated APOC1P1 expression and downregulated miRNA-106b expression. *, P<0.05; **, P<0.01; ***, P<0.001. PTEN, phosphatase and TENsin homolog deleted on chromosome 10; NC, negative control; PC, positive control; OD, optical density; APOC1P1, apolipoprotein C-I pseudogene 1; HCC, hepatocellular carcinoma; CCK-8, Cell Counting Kit-8.

revealed that the expressions of miR-106b and *APOC1P1* were negatively correlated. Within the mechanism of lncRNA acting as a ceRNA of miRNA in further regulating the target protein of miRNA, PTEN acts as the target of miR-106b, as indicated by Shi *et al.*'s findings (23) and those of our luciferase reporter assays. PTEN was first discovered to be a tumor suppressor in 1997 and has been studied in depth since then (24,25). In esophageal squamous cell carcinoma, miR-106b contributes to invasion and metastasis by regulating PTEN-mediated epithelial-to-mesenchymal transition (16). Furthermore, miR-106b overexpression directly targets PTEN in medulloblastoma progression (26). In our study, we found that miR-106b was upregulated while PTEN was downregulated in HCC, and the expression of miR-106b and PTEN showed a negative correlation. In addition, *APOC1P1* overexpression and miR-106b inhibition appeared to exert equal effects on cell proliferation and invasion, and APOC1 regulated PTEN expression by targeting miR-106b. Its clinical application still needs to overcome some difficulties in translational medicine research.

Conclusions

In conclusion, *APOC1P1* was overexpressed in HCC, which was associated with good prognosis. Moreover, *APOC1P1* was shown to regulate PTEN expression by targeting miR-106b to suppress HCC progression. Our findings point to the potential role of *APOC1P1* in HCC diagnosis and therapy and offer new directions in HCC research.

APOC1P1 can be used as a potential biomarker for patient risk stratification and local regional metastasis in HCC.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2189/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2189/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2189/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committee of the Second Affiliated Hospital of Nantong University (No. 2020KT014). The study mainly uses biological specimens obtained in previous clinical diagnosis and treatment, and is a secondary use of biological specimens, which is approved by the ethics committee without informed consent.

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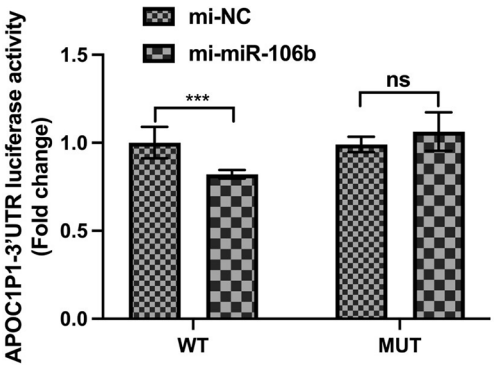

Figure S1 Additional analysis for verification direct binding between APOC1P1 and of miRNA-106b via dual-luciferase reporter assay. WT, pSI-Check2-APOC1P1 wild type; MUT, pSI-Check2-APOC1P1 mutant type. ***, P<0.0001; ns, no significance. Statistical significance was calculated using two-tailed Student's t-test.

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