Int J Clin Exp Med 2023;16(9):193-205
www.ijcem.com /ISSN:1940-5901/IJCEM0151056

Original Article

MiRNA-205-5p promotes development of hepatocellular carcinoma via targeting DLC1

Yan Xu[1,3], Weiming Tang[1,3], Guang Lu[2], Ting Huang[3], Siyi Huang[4], Yin Ji[5], Ying Peng[1], Xiaoming Kong[1]

[1]*Department of Clinical Laboratory, The Liyang Clinical College of Jiangsu Vocational College of Medicine, Liyang 213300, Jiangsu, China;* [2]*Department of Hepatobiliary Surgery, Liyang People's Hospital, Liyang 213300, Jiangsu, China;* [3]*Department of Clinical Laboratory, Liyang People's Hospital, Liyang 213300, Jiangsu, China;* [4]*Department of Immunization, Jiangsu Vocational College of Medicine, Yancheng 224000, Jiangsu, China;* [5]*Department of Pathology, Liyang People's Hospital, Liyang 213300, Jiangsu, China*

Received May 4, 2023; Accepted August 15, 2023; Epub September 15, 2023; Published September 30, 2023

Abstract: Researching biomarkers is of great significance for immunotherapy of hepatocellular carcinoma (HCC). This study aimed to investigate the expression level and specific function of miR-205-5p in HCC and analyze its associated genes. The objective was to establish a theoretical foundation for the treatment of HCC. HCC tissue samples and control specimens were collected from individuals diagnosed with HCC. Human liver immortalized cells (THLE2) and four HCC lines (Huh-7, HepG2, HCCLM3, SK-Hep-1) were used *in vitro*. MiR-205-5p inhibitor, mimic, sh-CD302 Molecule (sh-DCL1), and their corresponding controls were transfected. Starbase and dual-luciferase reporter assays were undertaken for predicting and verifying the binding of miR-205-5p and Deleted in liver cancer 1 protein (DLC1). CCK8, EdU, flow cytometry assays were used to detect cell viability, proliferation, and apoptosis. To assess the metastatic potential, wound healing, and transwell experiments were conducted. Results showed that there was a higher level of miR-205-5p in HCC samples. Knockdown of miR-205-5p inhibited the proliferation and improved apoptosis of Huh-7 and HepG2 cells. Moreover, miR-205-5p-inhibitor decreased the migration and invasion of HCC cells. MiR-205-5p targeted and negatively adjusted DLC1. Interestingly, sh-DLC1 rescued the influence of miR-205-5p-inhibitor. In conclusion, targeting DLC1, which has been investigated as a potential target for HCC therapy, miRNA-205-5p promoted the occurrence and progression of HCC.

Keywords: Hepatocellular carcinoma, miRNA-205-5p, DLC1, proliferation, invasion, apoptosis

Introduction

Primary hepatic carcinoma is a kind of malignant tumor, among which hepatocellular carcinoma (HCC) has a percentage of 85% to 90% [1]. The incidence of primary hepatic carcinoma ranks sixth among malignant tumors [2]. Chronic Hepatitis B virus (HBV) is mainly induced by HCC in China, accounting for 86% [3]. Other causes mainly include chronic hepatitis C virus infection, NAFLD, T2DM, and long-term consumption of aflatoxin-contaminated food [4]. The prevention of HCC can improve the cure rate [5]. HCC treatment comprises surgery, chemotherapy, and targeted drug therapy, yet the 5-year relative survival rate is only 18.1%. Tumor recurrence is frequent post-resection [6, 7].

With continuous in-depth research on the molecular signaling pathways and tumor microenvironment of HCC, targeted therapy has shown obvious advantages and occupies an important position in treatment [8]. The preferred treatment for advanced HCC is targeted therapy [9]. Targeted therapy can accurately identify tumor cells and kill tumor cells through a variety of mechanisms while reducing the damage to normal cells. Therefore, targeted therapy has the characteristics of better efficacy and fewer side effects [10]. Previous studies indicate that miRNAs play a dual role in HCC development. They downregulate tumor suppressor genes, promoting cell transformation while also acting as suppressors to reduce the activity of proto-oncogenes and genes associated with invasion and metastasis. This dual

role of miRNAs helps inhibit the development and spread of liver cancer. Of significant importance, certain miRNAs have been confirmed to play crucial roles in hepatitis virus infection and replication, as well as in modulating the host immune response and antigen presentation processes [11, 12]. For example, miR-122 enhanced the stability of viral genes by binding to related sites in the HCV genome, thereby stimulating HCV viral replication [13, 14]. Furthermore, there is noteworthy evidence that miRNA-205 expression is significantly reduced in various HBV-positive HCC cell lines. This reduced expression is closely associated with the methylation induction of the miRNA-205 promoter by the HBV-X gene [15]. In addition, HCV induces the up-regulated of miR-155 and then improves malignant transformation through the Wnt pathway [16].

Though various targets have been researched and reported, targeted therapy for HCC is a considerable dilemma. That is, the first-line choice of drugs is few. Thereby, a more effective target for HCC is still needed. Furthermore, in recent years, the results of various studies have confirmed that miR-205-5p in a variety of cancer samples is different from that of normal tissues [17, 18]. Additionally, the expression of miR-205-5p varies in different tumors, likely due to variations in tumor sources and biological characteristics [19-21].

Deleted in liver cancer 1 (DLC1) is an adhesion plaque protein that was identified as a putative tumor suppressor in HCC in 1998. Expression of DLC1 inhibits cell proliferation, anchored independent growth, tumorigenicity, and invasiveness of HCC cells [22].

Therefore, we are concerned with the specific role of miR-205-3p in HCC and analyzed related genes DLC1, hoping to lay the research foundation for HCC diagnosis and treatment.

Materials and methods

Patients and tissues

From June 2019 to September 2020, HCC patients who underwent radical surgery in our hospital provided the HCC tissues and the paired adjacent sample. The specimens and corresponding adjacent tissues were from the same HCC patient. A pathologist with more than 5 years of work experience in this hospital examined each tissue sample to confirm the tissue type. HCC was diagnosed according to the European Association for the Study of the Liver (EASL) criteria. Inclusion criteria were patients with confirmed HCC by pathology and those newly diagnosed HCC. The exclusion criteria were patients diagnosed with other conditions and those receiving treatment for ≥ 3 months before the current study. The tumor tissues were immediately frozen for future use at -80°C. Patients approved of the experiments, and the ethics committee approved this study.

Cell grouping and treatment

Human liver immortalized cells (THLE2) and four HCC lines (Huh-7, HepG2, HCCLM3, SK-Hep-1) were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). The Huh-7 and HepG2 cells were cultured in a constant temperature incubator, using DMEM medium supplemented with 10% fetal bovine serum (FBS). When the cell density was close to 70%, the miR-205-5p-inhibitor was transfected with Lipofectamine™ 2000 reagent (Invitrogen, USA). The corresponding experimental operation was completed according to the product manual. According to the different transfection, the cells were divided into the NC-inhibitor group and miR-205-5p-inhibitor groups. For sh-DLC1 transfection, the lentivirus carrying sh-DLC1 and sh-NC was synthesized by Shanghai Genepharma Co., Ltd. (China). The cells were seeded in a confocal dish and allowed to grow until they reached approximately 60% confluency. Subsequently, they were transfected with a lentivirus containing red fluorescent protein (RFP), using a multiplicity of infection (MOI) of approximately 80. The medium was changed 24 hours after transfection, and the culture was continued with DMEM medium (low-glucose), including 10% FBS (Gibco). After 72 hours, the transfection efficiency was observed under the confocal laser microscope (Leica Microsystems, Wetzlar, Germany). The rescue assay included 3 groups (NC-inhibitor+sh-NC, miR-205-5p-inhibitor+sh-NC, and miR-205-5p-inhibitor+sh-DLC1) based on the transfection.

Q-PCR assay

Trizol reagents were applied to extract tissue and RNA in cells in each group. Ultraviolet spec-

trophotometers were applied to determine the concentration and purity of RNA. The reverse transcription process used the Prime Script™ RT kit (Takara, Japan) and operated based on the instructions. The Primer 5.0 software, developed by Primer in Canada, was utilized for primer design in this study. The primers were supplied by Shanghai Sangon Biotech Co., Ltd., which was shown as follows, miR-205-5p, Forward, 5'-TCCACCGGAGTCTGTCTCAT-3', Reverse, 5'-GCTGTCAACGATACGCTACG-3'; DLC1, Forward, 5'-GTTGCCTCAGAGCATCCAG-3', Reverse, 5'-GGGTGTTGAGATGGAAGAGG-3'; U6, Forward, 5'-ATTGGAACGATACAGAGAAGATT-3', Reversed, 5'-GGAACGCTTCACGAATTTG-3'; β-actin, Forward, 5'-AAGTACTCCGTGTGGATCGG-3', Reverse, 5'-ATGCTATCACCTCCCCTGTG-3'. The Sybr Premix Extaq™ kit (Takara, Japan) and Rotor-Gene 3000 systems undertook real-time fluorescence quantitative PCR reactions and measurements. The PCR amplification system includes pre-denaturation at 95°C for 12 min, followed by 95°C for 30 s, 35 cycles, 58°C for 30 s, and final extension at 72°C for 10 min. β-actin was regarded as a reference.

CCK8 assay

The transfected cells were incubated in a plate with 96-wells at 1×10^3 cells per well, and they were examined regularly at 1 d, 2 d, and 3 d. A total of 10 μL of CCK8 solution (Abcam, Cambridge, UK) was supplied in every well and cultured at 37°C for 2 h. An enzyme-linked immunoassay instrument measured the absorbance value at a wavelength of 450 nm of each well was measured on an enzyme-linked immunoassay instrument (Bio-Rad Laboratories, USA). There were 3 parallel replicate wells in all groups.

EdU assay

Proliferation analysis was conducted using the EdU analysis kit (Guangzhou Ruibo Technology Co., Ltd., China) as per the provided instructions. After transfection, the cells were seeded in DMEM with 5 μmol/L EdU and 10% FBS and incubated at 37°C for 2 h. After that, the samples were fixed with 4% formaldehyde at 25°C for 0.5 h and processed with 0.5% Triton X-100 for 20 min for cell membrane permeabilization. Following a 0.5 h incubation of the cells with the 1×Apollo reaction mixture, they were subsequently stained with Hoechst 33342 for another 0.5 h. Finally, the samples were imaged using a fluorescence microscope.

Flow cytometry for measuring apoptosis

The transfected samples were collected and given a PBS rinse after 2 d of transfection. The cells were centrifuged at 4°C for 5 min and then washed with diluted binding buffer. The diluted binding buffer was used to dilute the cell concentration to 10^5-10^6 cells/mL. Five μL of diluted Annexin-V-FITC solution (Wuhan Hualianke Biology Co., Ltd., China) and 5 μL of PI staining solution were added to the sample and mixed well, kept in the refrigerator at 4°C, void of light for 10 min. A flow cytometer tested the rate of apoptosis.

Wound healing assay

When sample density reached 80% to 90% in 24 healthy plates, transfection was performed. After 1 day of transfection, a linear scratch was made using a 200 μL pipette tip. To prevent the cells from entering the proliferation cycle in advance, the cells were incubated in serum-free Opti-DMEM. Scratch healing was photographed at 0, 24, and 48 h. A total of 10 random fields (×200) were selected to calculate the cell migration rate.

Transwell assay for migration and invasion

Fifty μL of the base glue, which had been diluted and dissolved, was tiled into the top layer. After 2 d of culture, the cells were digested and counted. About 5×10^4 cells were resuspended in a 200 μL serum-free DMEM medium and added to the upper chamber. Next, 500 μL of DMEM with 10% PBS was added to the lower chamber and placed in a cell incubator. The upper chamber was obtained, washed 3 times, fixed with 4% paraformaldehyde solution (Sigma) for 0.5 h, and wiped with a cotton swab to clear the excess cells. Then, the cells were stained with crystal violet for 20 min and rinsed with PBS. Finally, an optical microscope (×200) (Leica microscope ICC50HD) was used to count and photograph the cells. For invasion ability, no base glue was used, and the other steps were the same as before.

Bioinformatics prediction

Starbase is a robust database commonly used for lncRNA/circRNA/microRNA research.

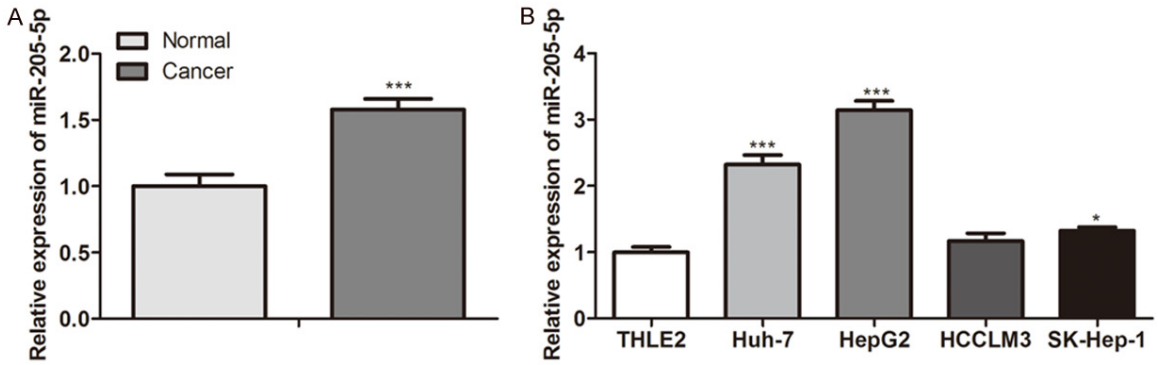


Figure 1. There was a higher expression of miR-205-5p in HCC samples. A. MiR-205-5p was expressed higher in HCC tissues compared with that in normal controls. B. MiR-205-5p was expressed higher in HCC cells. *$P < 0.05$ and ***$P < 0.001$ *vs* Normal group or THLE2 cell group.

The starbase database was employed in this study to address several issues, including identifying non-coding RNAs such as lncRNA and circRNA based on microRNA, screening mRNA targets using microRNA, selecting ceRNA regulatory molecules, and mining RNA binding proteins. Specifically, the starbase database was utilized to predict the target interaction between miR-205-5p and DLC1.

Dual luciferase reporter assay

The wild-type and mutant luciferase reporter plasmids in the 3'UTR region of DLC1 were synthesized by Shanghai Tongke Biotechnology Co., Ltd. First, the 3'UTR region sequence of DLC1 was inserted into the pmirGLO vector (Promega, E1330), which formed the DLC1 wild-type luciferase reporter plasmid after restriction digestion. Then, the sequence mutation that binds to miR-205-5p of DLC1 was inserted into the pmirGLO vector (Promega, E1330) and digested to form a mutant luciferase reporter plasmid for the 3'UTR region of DLC1. Finally, the exact amount of the luciferase mentioned above reporter plasmid and 20 pmol of miR-205-5p mimic were transfected into the cells with lipo2000. After 1 day of culturing, the luciferase detection kit was employed for detection purposes.

Western blot assay

Cells were cultured in a 6-well plate with DMEM for 1 d. The cells were transfected and incubated for 6 h, following which the medium containing the transfection reagent was discarded. After transfection 48 h, the total protein was isolated and dissolved in the lysate buffer, separated by electrophoresis, and transferred to a PVDF membrane (Roche). First, the protein was closed with 2% bovine serum albumin (BSA) at room temperature. Next, the protein was sealed at room temperature with 2% BSA at 25°C and then incubated with antibodies (anti-bax, anti-bcl2, anti-cleaved-caspase 3, anti-DLC1, 1:1000, Santa Cruz) at 4°C. After washing the membrane 3 times, the sample was incubated with the corresponding secondary antibody (1:2000, Santa Cruz) for 1 h. Finally, using an ECL kit (US Millipore), the membrane was developed after being incubated with the ECL reagent.

Statistical analysis

Data analysis was conducted using SPSS 20.0 statistical software (SPSS Software Products). The experimental data were presented as mean ± standard deviation (SD). The unpaired Student t-test test processed a comparison between the two groups. One-way or two-way analysis of variance (one-way or two-way ANOVA) and post-hoc Tukey test were used to analyze the data between multiple groups. $P < 0.05$ represented a difference with significance.

Results

MiR-205-5p expressed higher in HCC samples

The level of miR-205-5p in HCC samples was monitored using a Q-PCR assay. As shown in **Figure 1A**, HCC tissues had higher miR-205-5p expression than normal controls. Similar outcomes were also attained in HCC cells. Huh-7 and HepG2 had the highest levels of miR-205-

5p expression in HCC cells (**Figure 1B**). In the ensuing experiments, Huh-7 and HepG2 were selected for study.

Knockdown of miR-205-5p inhibited the proliferation and improved apoptosis of HCC cells

MiR-205-5p-inhibitor was processed in Huh-7 and HepG2 cell lines to detect the proliferation and apoptosis of HCC cell lines. As a result, the miR-205-5p inhibitor reduced cell activity and proliferation ability (**Figure 2A** and **2B**). Additionally, the miR-205-5p inhibitor group showed a higher rate of apoptosis (**Figure 2C**). In addition, the levels of Bax and cleaved-caspase3 increased, and bcl-2 expression decreased in the miR-205-5p inhibitor group (**Figure 2D**). These pieces of evidence indicated that miR-205-5p inhibitor repressed the proliferation and improved apoptosis of HCC cells.

Knockdown of miR-205-5p inhibited the migration and invasion of HCC cells

Migration and invasion assays were performed to evaluate the impact of miR-205-5p knockdown on the migratory and invasive capabilities of Huh-7 and HepG2 cell lines. As presented in **Figure 3A-C**, the miR-205-5p inhibitor significantly inhibited the migration and invasion ability of HCC cells.

MiR-205-5p targeted and negatively regulated DLC1

Based on the Starbase database, a predicted interaction between miR-205-5p and DLC1 was identified. **Figure 4A** displays the binding between them. To further validate these predictions, a dual luciferase reporter gene assay was conducted. After the miR-205-5p mimic was added to the cells in the DLC1 wild-type group, the fluorescence value decreased ($P < 0.01$). However, after the binding sequence of DLC1 was mutated and transfected with the miR-205-5p mimic, its fluorescence value did not change (**Figure 4B**). Moreover, the miR-205-5p inhibitor significantly up-regulated the level of DLC1 (**Figure 4C** and **4D**). Therefore, miR-205-5p targeted DLC1 in HCC cells.

Sh-DLC1 rescued the influence of miR-205-5p inhibitor

Rescue assays were processed, and the cells were divided into 3 groups, including NC inhibitor+sh-NC, miR-205-5p inhibitor+sh-NC, and miR-205-5p inhibitor+sh-DLC1 group. miR-205-5p inhibitor reduced cell activity and proliferation ability. Moreover, in the miR-205-5p inhibitor group, there was a notable increase in the apoptosis rate. Additionally, the expression levels of Bax and cleaved-caspase 3 were elevated, while the expression of bcl-2 was reduced in the miR-205-5p inhibitor group. Interestingly, sh-DLC1 rescued the influence of miR-205-5p inhibitor on proliferation and apoptosis (**Figure 5A-D**). Interestingly, the miR-205-5p inhibitor significantly repressed the migration and invasion ability of HCC cells, while sh-DLC1 reversed the influence of the miR-205-5p inhibitor (**Figure 6A-C**).

Discussion

Researching biomarkers is critical for the immunotherapy of HCC [23]. In recent years, there has been extensive research on the mechanisms underlying miRNA expression in the development of HCC [24]. In addition, some miRNAs were considered potential targets for prognostic judgment and intervention treatment of HCC [25]. In this study, miR-205-5p was expressed higher in HCC samples than in normal controls. These results indicate that miR-205-5p may play an important role in HCC. Hence, this study aimed to investigate the molecular mechanisms associated with the observed phenomena.

The human miR-205-5p, located in the lq32.2 gene, is in the LOC642587 gene, prone to mutation. At present, various studies are showing that miR-205-5p has a close connection to various tumors and the prognosis of treatment [26, 27]. According to Wang et al.'s gene chip screening, miR-205 was overexpressed in HCC samples (SMMC-7721), and quantitative fluorescence PCR confirmed that it was up-regulated by more than 2.6 times [28]. Besides, it was associated with target ubiquitin-specific peptidase 7 (UPS7) in HCC cells [29]. UPS is the main pathway for selective degradation of intracellular proteins, which is significant for maintaining cell homeostasis [30]. In addition, the hepatitis B virus X gene (HBx) increased the ACSL4 level (the target gene of miR-205 in HCC cells), thereby causing lipid metabolism disorders in HCC models [31]. Furthermore, the knockdown of miR-205-5p inhibited cell proliferation and promoted cell apoptosis in HCC cells (Huh-7 and HepG2). Knockdown of miR-205-5p increased the expression of Bax and

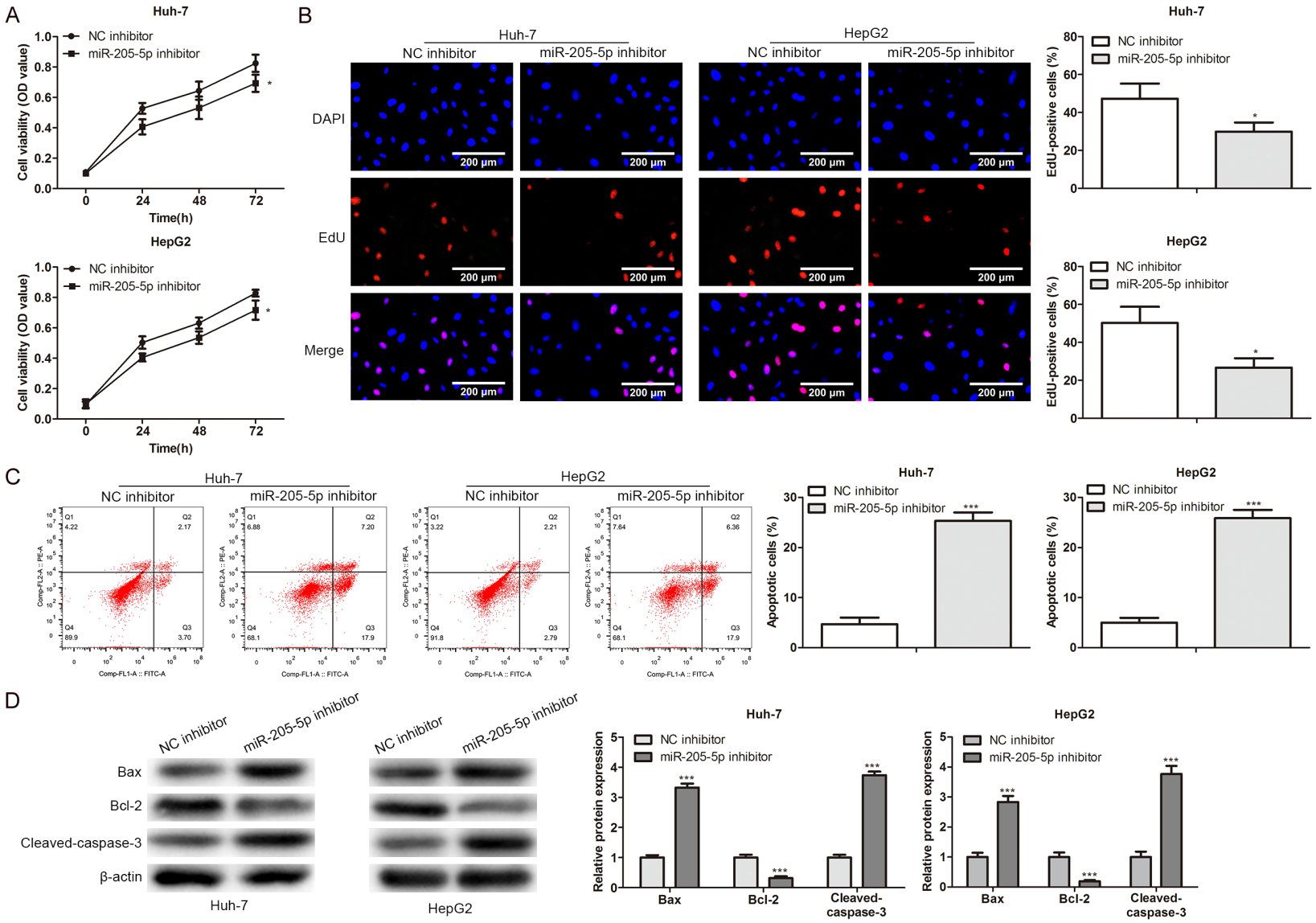


Figure 2. Knockdown of miR-205-5p inhibited the proliferation and apoptosis of HCC cells. A. MiR-205-5p inhibitor reduced cell activity. B. Knockdown of miR-205-5p decreased the proliferation ability of HCC cells. C. Knockdown of miR-205-5p increased apoptosis rate. D. In the miR-205-5p inhibitor group, there was an observed increase in the levels of Bax and cleaved-caspase3, accompanied by a decrease in bcl-2 expression. These findings indicate an improvement in apoptosis within the experimental group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ *vs* NC inhibitor group.

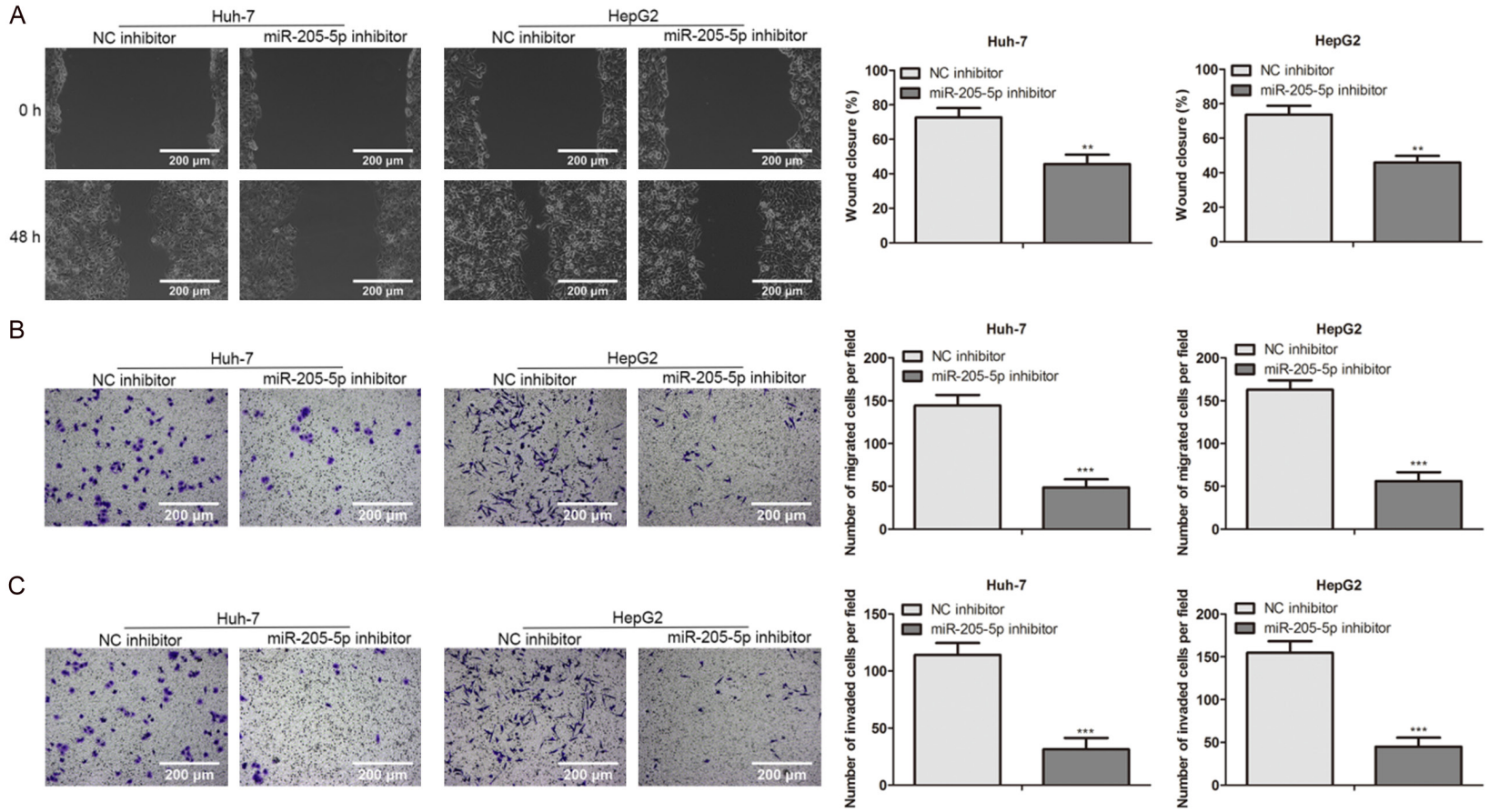


Figure 3. Knockdown of miR-205-5p inhibited the migration and invasion of HCC cells. A. Wound healing assay confirmed that miR-205-5p inhibitor repressed the migration of HCC cells. B. The results of the transwell assay showed that the miR-205-5p inhibitor hindered the migration of HCC cells. C. Knockdown of miR-205-5p inhibited the migration and invasion of Huh-7 and HepG2 cells. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* NC inhibitor group.

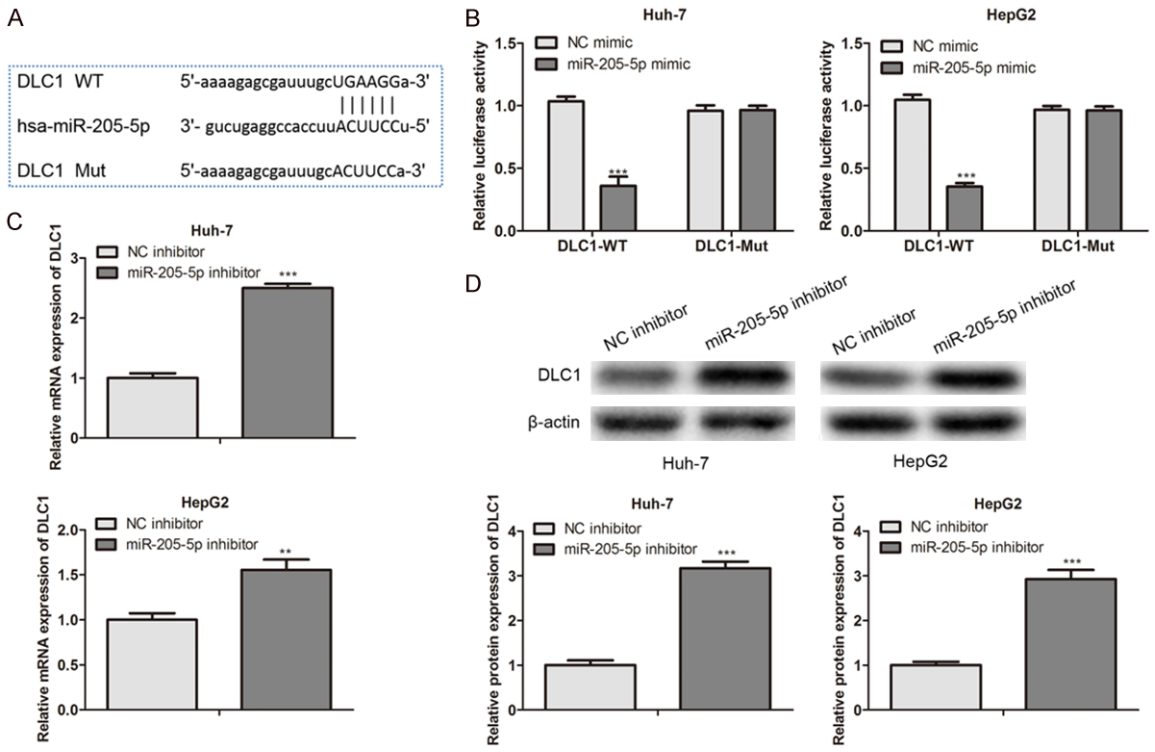


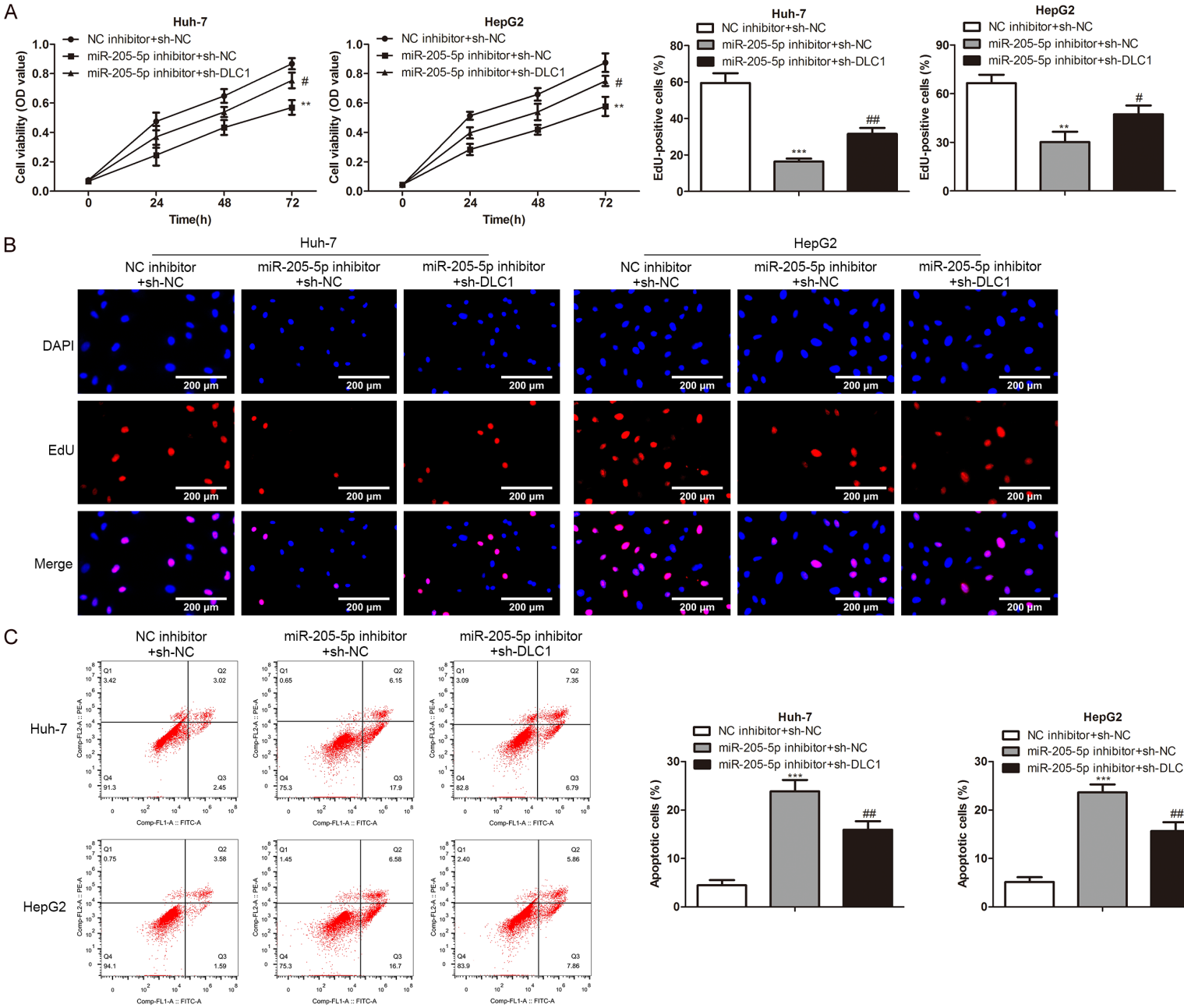
Figure 4. MiR-205-5p targeted and negatively regulated DLC1. A. According to the predictions made by Starbase, a relationship between miR-205-5p and DLC1 was identified. B. MiR-205-5p mimics significantly down-regulated fluorescence value in the DLC1 wild-type group, but no changes were observed in the DLC1 mut-type group. C and D. miR-205-5p inhibitor up-regulated the expression of DLC1. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* NC mimic or NC inhibitor group.

caspase3 and inhibited the level of bcl2. These findings suggested that by controlling the proliferation and apoptosis of HCC cells, miR-205-5p may help to control the development of HCC.

Furthermore, we found that miR-205-5p targeted DLC1. The DLC1 gene is located on 8p21.3-22, and the expression product is RhoA and Cdc42 specific GTPase activating protein (GAP) [32]. It was closely related to the signal transduction pathway that regulated cell proliferation and adhesion and mainly inhibited tumors by down-regulating the activity of Rho [33]. Furthermore, Kim et al. confirmed that DLC1 affected the proliferation, morphology, and invasiveness of HCC cells by acting on RhoGTPase and focal adhesion protein [34]. Interestingly, deletion or low expression of the DLC1 gene is common in primary HCC [35]. In a previous study, Huh-7 and BEL7402 HCC cell lines and 20% of HCC patients had low DLC1 expression.

Moreover, patients with low DLC-1 expression have a decreased median survival time, and are prone to relapse [36]. Furthermore, it was found that the elevated expression of DLC1 resulted in Caspase-3-mediated apoptosis, leading to the inhibition of tumor cell growth, reduced invasiveness of tumor cells, and decreased tumorigenicity in nude mice. So far, different studies have inferred that the DLC1 gene is inhibited or deleted in most tumor tissues, and CpG island methylation modification is one of the main reasons [37]. Moreover, demethylation drugs could effectively prevent the metastasis of certain tumors, which showed that the DLC1 gene had a particular effect on inhibiting tumor invasion and metastasis, and methylation might also be the only reason for its lack of expression. Significantly, sh-DLC1 rescued the influence of miR-205-5p inhibitor in this study.

Although miR-205-5p has been identified as a potential target for HCC treatment, it is important to note that this study has several limitations or shortcomings. Firstly, the number of clinical cases included in this study is small. Secondly, it is worth mentioning that the inves-


A
Huh-7
HepG2
NC inhibitor+sh-NC
miR-205-5p inhibitor+sh-NC
miR-205-5p inhibitor+sh-DLC1
Cell viability (OD value)
Time(h)
EdU-positive cells (%)
B
Huh-7
HepG2
NC inhibitor +sh-NC
miR-205-5p inhibitor +sh-NC
miR-205-5p inhibitor +sh-DLC1
DAPI
EdU
Merge
200 μm
C
Huh-7
HepG2
Apoptotic cells (%)

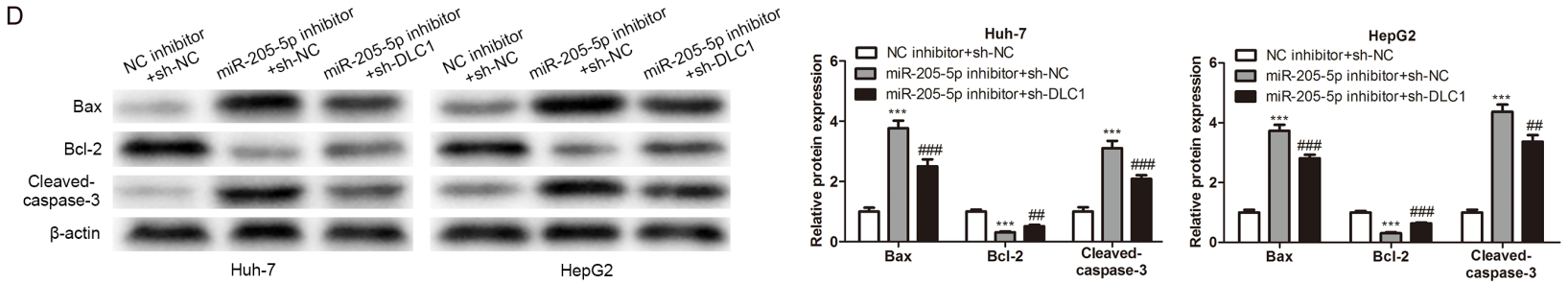
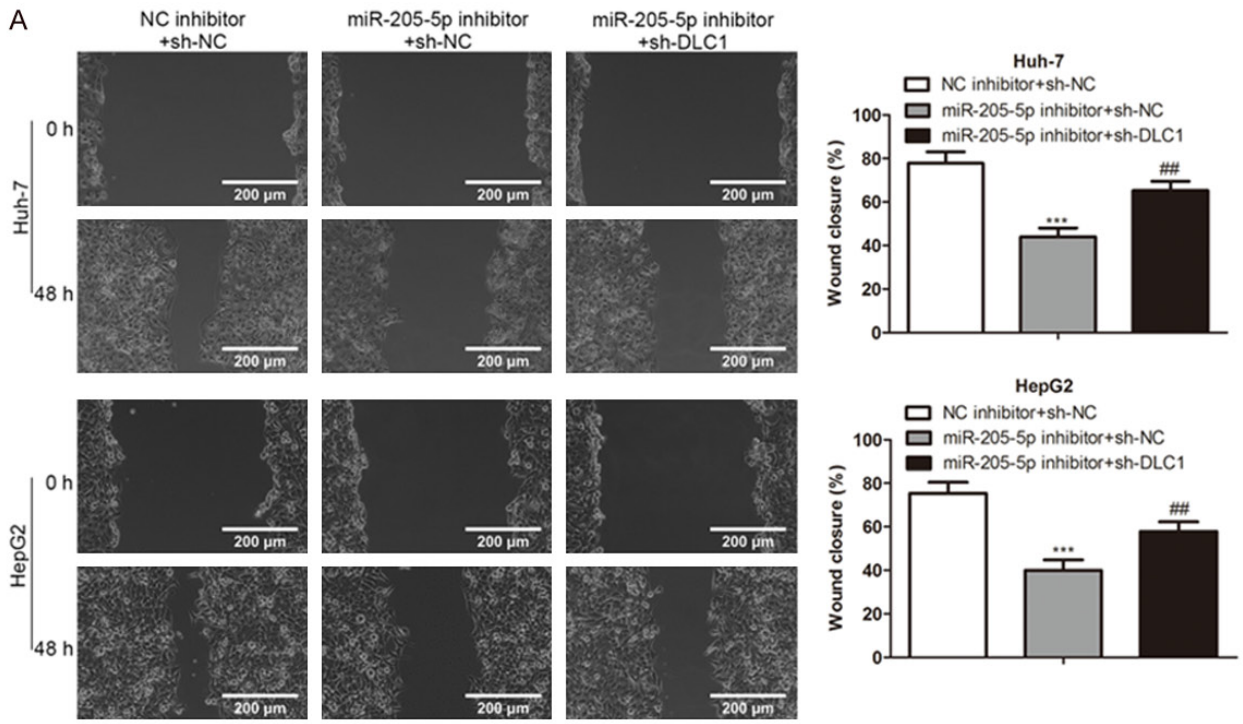


Figure 5. Sh-DLC1 rescued the influence of miR-205-5p inhibitor on proliferation and apoptosis. A. miR-205-5p inhibitor repressed cell activity, while sh-DLC1 rescued the influence of it. B. Knockdown of miR-205-5p inhibited proliferation ability, and sh-DLC1 rescued the effect. C. Apoptosis rate was highest in the miR-205-5p inhibitor+sh-NC group, and sh-DLC1 partly decreased in the miR-205-5p inhibitor+sh-DLC1 group. D. The apoptosis-related proteins were regulated by miR-205-5p inhibitor and sh-DLC1. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* NC inhibitor+sh-NC group; $\#P < 0.05$, $\#\#P < 0.01$ and $\#\#\#P < 0.001$ miR-205-5p inhibitor+sh-NC group.



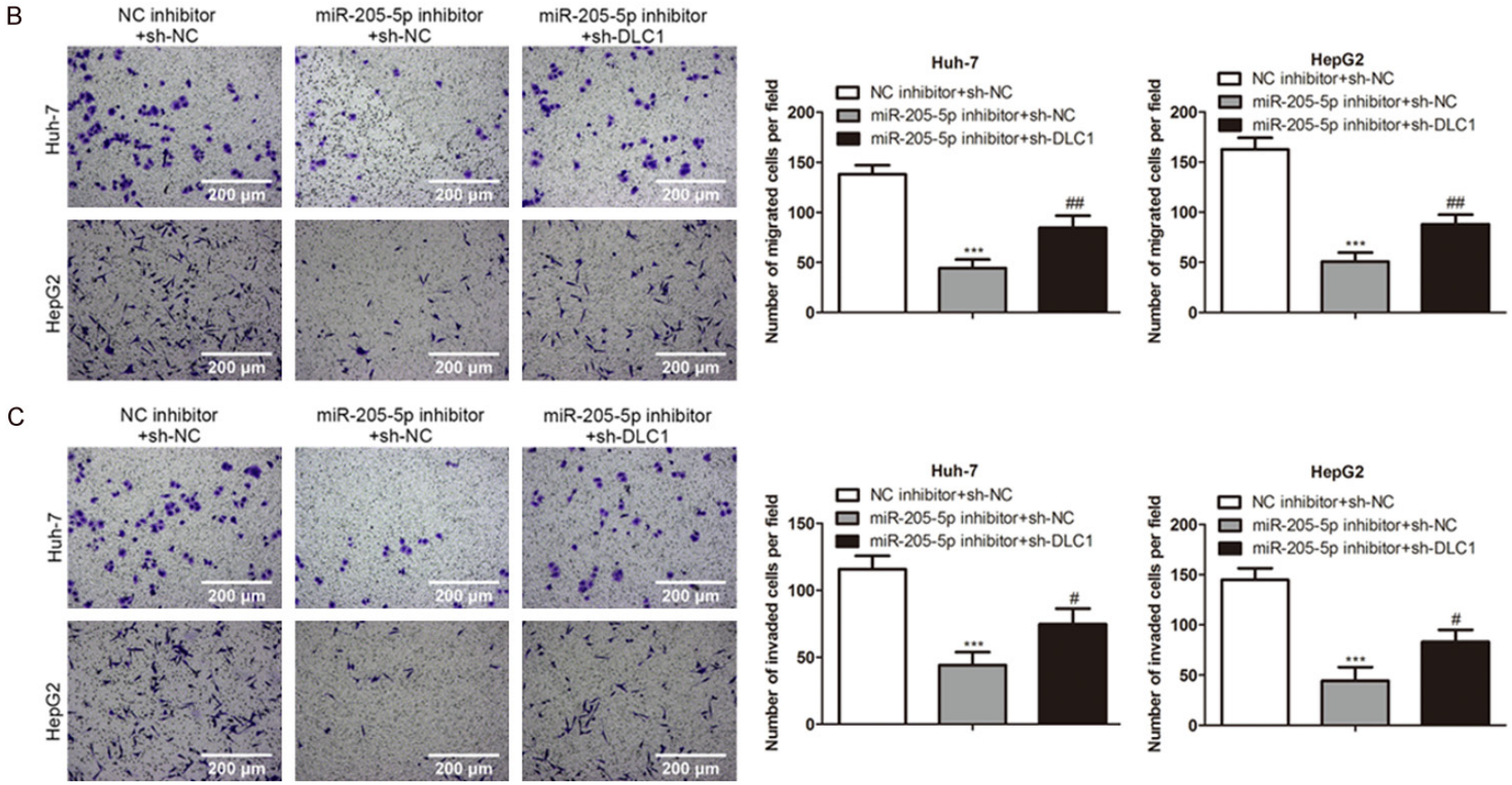


Figure 6. Sh-DLC1 rescued the influence of miR-205-5p inhibitor on migration and invasion. A. Wound healing assay was processed, and the results indicated that the knockdown of miR-205-5p inhibited the migration. B. miR-205-5p inhibitor hindered the migration based on the results of the transwell assay. C. Knockdown of miR-205-5p repressed the migration and invasion. Sh-DLC1 rescued the influence of miR-205-5p inhibitor on migration and invasion. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* NC inhibitor+sh-NC group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ miR-205-5p inhibitor+sh-NC group.

tigation of miR-205-5p and DLC1 was limited to *in vitro* studies only. Therefore, further experiments *in vivo* were needed to be processed. Thirdly, more targets of miR-205-5p will also be further researched.

In conclusion, MiRNA-205-5p promoted the occurrence and development of HCC via targeting DLC1, which provided a potential target for HCC therapy.

Acknowledgements

We deeply appreciate the support of all participants. This work was supported by The Special Funds for Science Development of the Clinical Teaching Hospitals of Jiangsu Vocational College of Medicine (No. 20219107).

Disclosure of conflict of interest

None.

Address correspondence to: Yan Xu, Department of Clinical Laboratory, The Liyang Clinical College of Jiangsu Vocational College of Medicine, No. 70 Jianshe West Road, Licheng Town, Liyang 213300, Jiangsu, China. E-mail: xuyan216012600@163.com

References

[1] Gong D, Feng PC, Ke XF, Kuang HL, Pan LL, Ye Q and Wu JB. Silencing long non-coding RNA LINC01224 inhibits hepatocellular carcinoma progression via microRNA-330-5p-induced inhibition of CHEK1. Mol Ther Nucleic Acids 2020; 19: 482-497.

[2] Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A and Bray F. Cancer statistics for the year 2020: an overview. Int J Cancer 2021; 2021: 1-12.

[3] Ding X, Mizokami M, Yao G, Xu B, Orito E, Ueda R and Nakanishi M. Hepatitis B virus genotype distribution among chronic hepatitis B virus carriers in Shanghai, China. Intervirology 2001; 44: 43-47.

[4] Rao BC, Lou JM, Wang WJ, Li A, Cui GY, Yu ZJ and Ren ZG. Human microbiome is a diagnostic biomarker in hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2020; 19: 109-115.

[5] Jiang JX, Yu C, Li ZP, Xiao J, Zhang H, Chen MY and Sun CY. Insights into significant pathways and gene interaction networks in peripheral blood mononuclear cells for early diagnosis of hepatocellular carcinoma. J Cancer Res Ther 2016; 12: 981-9.

[6] Mandlik DS, Mandlik SK and Choudhary HB. Immunotherapy for hepatocellular carcinoma: current status and future perspectives. World J Gastroenterol 2023; 29: 1054-1075.

[7] Wei F, Yang L, Jiang D, Pan M, Tang G, Huang M and Zhang J. Long noncoding RNA DUXAP8 contributes to the progression of hepatocellular carcinoma via regulating miR-422a/PDK2 axis. Cancer Med 2020; 9: 2480-2490.

[8] Bhutiani N, O'Brien SJ, Priddy EE, Egger ME, Hong YK, Mercer MK, McMasters KM, Martin RCG, Potts MH and Scoggins CR. Correlating serum alpha-fetoprotein in hepatocellular carcinoma with response to Yttrium-90 transarterial radioembolization with glass microspheres (TheraSphere™). HPB (Oxford) 2020; 22: 1330-1338.

[9] Li F and Xue X. Identification and characterization of an ageing-associated 13-lncRNA signature that predicts prognosis and immunotherapy in hepatocellular carcinoma. J Oncol 2023; 2023: 4615297.

[10] Sarkadi B, Saskoi E, Butz H and Patocs A. Genetics of pheochromocytomas and paragangliomas determine the therapeutical approach. Int J Mol Sci 2022; 23: 1450.

[11] Xu W and Liu J. The association between microRNA-323b polymorphism and hepatitis B virus persistent infection - some problems should be addressed. J Viral Hepat 2015; 22: 625.

[12] Hsi E, Huang CF, Dai CY, Juo SH, Chou WW, Huang JF, Yeh ML, Lin ZY, Chen SC, Wang LY, Chuang WL and Yu ML. Peripheral blood mononuclear cells microRNA predicts treatment outcome of hepatitis C virus genotype 1 infection. Antiviral Res 2014; 105: 135-142.

[13] Gebert LFR, Law M and MacRae IJ. A structured RNA motif locks Argonaute2:miR-122 onto the 5' end of the HCV genome. Nat Commun 2021; 12: 6836.

[14] Heyn H, Carmona FJ, Gomez A, Ferreira HJ, Bell JT, Sayols S, Ward K, Stefansson OA, Moran S, Sandoval J, Eyfjord JE, Spector TD and Esteller M. DNA methylation profiling in breast cancer discordant identical twins identifies DOK7 as novel epigenetic biomarker. Carcinogenesis 2013; 34: 102-8.

[15] Zhang XD, Wang Y and Ye LH. Hepatitis B virus X protein accelerates the development of hepatoma. Cancer Biol Med 2014; 11: 182-190.

[16] Zhang Y, Wei W, Cheng N, Wang K, Li B, Jiang X and Sun S. Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. Hepatology 2012; 56: 1631-40.

[17] De Cola A, Volpe S, Budani MC, Ferracin M, Lattanzio R, Turdo A, D'Agostino D, Capone E, Stassi G, Todaro M, Di Ilio C, Sala G, Piantelli M, Negrini M, Veronese A and De Laurenzi V. miR-205-5p-mediated downregulation of

ErbB/HER receptors in breast cancer stem cells results in targeted therapy resistance. Cell Death Dis 2015; 6: e1823.
[18] Zhang J, Zhang J, Pang X, Chen Z, Zhang Z, Lei L, Xu H, Wen L, Zhu J, Jiang Y, Cui Y, Chen G and Wang X. MiR-205-5p suppresses angiogenesis in gastric cancer by downregulating the expression of VEGFA and FGF1. Exp Cell Res 2021; 404: 112579.
[19] Valera VA, Parra-Medina R, Walter BA, Pinto P and Merino MJ. microRNA expression profiling in young prostate cancer patients. J Cancer 2020; 11: 4106-4114.
[20] Jiang M, Zhang P, Hu G, Xiao Z, Xu F, Zhong T, Huang F, Kuang H and Zhang W. Relative expressions of miR-205-5p, miR-205-3p, and miR-21 in tissues and serum of non-small cell lung cancer patients. Mol Cell Biochem 2013; 383: 67-75.
[21] Kimura S, Naganuma S, Susuki D, Hirono Y, Yamaguchi A, Fujieda S, Sano K and Itoh H. Expression of microRNAs in squamous cell carcinoma of human head and neck and the esophagus: miR-205 and miR-21 are specific markers for HNSCC and ESCC. Oncol Rep 2010; 23: 1625-33.
[22] Chai R, Fu H, Zheng Z, Liu T, Ji S and Li G. Resveratrol inhibits proliferation and migration through SIRT1 mediated post-translational modification of PI3K/AKT signaling in hepatocellular carcinoma cells. Mol Med Rep 2017; 16: 8037-8044.
[23] Liao C, Liu H and Luo X. The emerging roles of exosomal miRNAs in nasopharyngeal carcinoma. Am J Cancer Res 2021; 11: 2508-2520.
[24] Komoll RM, Hu Q, Olarewaju O, von Dohlen L, Yuan Q, Xie Y, Tsay HC, Daon J, Qin R, Manns MP, Sharma AD, Goga A, Ott M and Balakrishnan A. MicroRNA-342-3p is a potent tumour suppressor in hepatocellular carcinoma. J Hepatol 2021; 74: 122-134.
[25] Lou W, Liu J, Ding B, Chen D, Xu L, Ding J, Jiang D, Zhou L, Zheng S and Fan W. Identification of potential miRNA-mRNA regulatory network contributing to pathogenesis of HBV-related HCC. J Transl Med 2019; 17: 7.
[26] Yamada Y, Nishikawa R, Kato M, Okato A, Arai T, Kojima S, Yamazaki K, Naya Y, Ichikawa T and Seki N. Regulation of HMGB3 by antitumor miR-205-5p inhibits cancer cell aggressiveness and is involved in prostate cancer pathogenesis. J Hum Genet 2018; 63: 195-205.
[27] Valenti F, Sacconi A, Ganci F, Grasso G, Strano S, Blandino G and Di Agostino S. The miR-205-5p/BRCA1/RAD17 axis promotes genomic instability in head and neck squamous cell carcinomas. Cancers (Basel) 2019; 11: 1347.
[28] Cabiati M, Salvadori C, Basta G, Del Turco S, Aretini P, Cecchettini A and Del Ry S. miRNA and long non-coding RNA transcriptional expression in hepatocellular carcinoma cell line-secreted extracellular vesicles. Clin Exp Med 2022; 22: 245-255.
[29] Zhu L, Liu R, Zhang W, Qian S and Wang JH. MicroRNA-205 regulates ubiquitin specific peptidase 7 protein expression in hepatocellular carcinoma cells. Mol Med Rep 2015; 12: 4652-4656.
[30] Zhou F, Xie F, Jin K, Zhang Z, Clerici M, Gao R, van Dinther M, Sixma TK, Huang H, Zhang L and Ten Dijke P. USP4 inhibits SMAD4 monoubiquitination and promotes activin and BMP signaling. EMBO J 2017; 36: 1623-1639.
[31] Cui M, Xiao Z, Sun B, Wang Y, Zheng M, Ye L and Zhang X. Involvement of cholesterol in hepatitis B virus X protein-induced abnormal lipid metabolism of hepatoma cells via up-regulating miR-205-targeted ACSL4. Biochem Biophys Res Commun 2014; 445: 651-655.
[32] Zhou X, Thorgeirsson SS and Popescu NC. Restoration of DLC-1 gene expression induces apoptosis and inhibits both cell growth and tumorigenicity in human hepatocellular carcinoma cells. Oncogene 2004; 23: 1308-13.
[33] Tripathi V, Zimonjic DB and Popescu NC. Abstract 4017: DLC1 and α-catenin protein interaction enhances DLC1 antioncogenic activity by stabilizing adherens junctions and suppressing NFκB signaling. Cancer Res 2011; 71: 4017.
[34] Kim TY, Healy KD, Der CJ, Sciaky N, Bang YJ and Juliano RL. Effects of structure of Rho GTPase-activating protein DLC-1 on cell morphology and migration. J Biol Chem 2008; 283: 32762-70.
[35] Sjoestroem C, Khosravi S, Cheng Y, Safaee Ardekani G, Martinka M and Li G. DLC1 expression is reduced in human cutaneous melanoma and correlates with patient survival. Mod Pathol 2014; 27: 1203-11.
[36] Song LJ, Liu Q, Meng XR, Li ShL, Wang LX, Fan QX and Xuan XY. DLC-1 is an independent prognostic marker and potential therapeutic target in hepatocellular cancer. Diagn Pathol 2016; 11: 19.
[37] Peng H, Long F, Wu Z, Chu Y, Li J, Kuai R, Zhang J, Kang Z, Zhang X and Guan M. Downregulation of DLC-1 gene by promoter methylation during primary colorectal cancer progression. Biomed Res Int 2013; 2013: 181384.