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Original Article

# Tumor Suppressive Effect of miRNA-205-3p on Hepatocellular Carcinoma *via* Regulating ZDBF2

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## ABSTRACT

**Objectives:** The present study aimed to clarify the functional involvement of microRNA-205-3p (miRNA-205-3p) in Hepatocellular Carcinoma (HCC) progression and to determine its potential clinical relevance in predicting disease behaviour and patient prognosis. **Materials and Methods:** Quantitative real-time PCR analysis was conducted to determine miRNA-205-3p expression in hepatocellular carcinoma specimens and matched adjacent liver tissues. Correlations between miRNA-205-3p levels and clinicopathological variables were subsequently analyzed. Functional experiments were carried out in Hep3B and Huh7 cells following ectopic miRNA-205-3p upregulation to evaluate its influence on proliferative capacity, clonogenicity, migratory behavior, and invasive potential. Cell growth was monitored using CCK-8 and colony formation assays, whereas transwell assays were employed to assess migration and invasion. In addition, the regulatory relationship between miRNA-205-3p and ZDBF2 was confirmed through luciferase reporter experiments. Rescue experiments were subsequently conducted to determine whether ZDBF2 mediated the phenotypic effects induced by miRNA-205-3p. **Results:** A substantial downregulation of miRNA-205-3p was identified in hepatocellular carcinoma tissues relative to matched non-cancerous liver specimens, with metastatic tumors showing the lowest expression levels. Decreased miRNA-205-3p expression was associated with lymph node metastasis, distant metastasis, shorter progression-free survival, and poorer overall survival. Functional restoration of miRNA-205-3p suppressed proliferation, clonogenic growth, migration, and invasion in Hep3B and Huh7 cells. Consistently, miRNA-205-3p overexpression restrained tumor growth in nude mouse xenograft models. Mechanistically, ZDBF2 was identified as a direct downstream target of miRNA-205-3p and showed an inverse expression relationship with miRNA-205-3p. Recovery of ZDBF2 expression counteracted, to some extent, the suppressive influence of miRNA-205-3p on aggressive cellular behaviors. **Conclusion:** MiRNA-205-3p was downregulated in HCC and appeared to suppress malignant progression by directly targeting ZDBF2. These findings suggest that the miRNA-205-3p/ZDBF2 axis may serve as a promising molecular target for HCC prognosis assessment and therapeutic intervention.

**Keywords:** Hepatocellular carcinoma, Invasion, Metastasis, Migration, miRNA-205-3p, Tumor suppression, ZDBF2.

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## INTRODUCTION

Among primary hepatic malignancies, Hepatocellular Carcinoma (HCC) is the most frequently diagnosed pathological type and remains one of the leading causes of cancer-associated death worldwide.[1-3] Despite continuous progress in surgical management and locoregional therapeutic strategies, the long-term prognosis of HCC patients remains unsatisfactory because of high recurrence and metastatic potential.[4,5] Epidemiological studies have demonstrated clear sex- and age-related differences in HCC incidence, with male individuals showing substantially higher susceptibility than females, particularly in regions with elevated disease prevalence.[6,7] Chronic liver injury, cirrhosis, viral hepatitis infection, alcohol consumption, and genetic alterations collectively contribute to the complex molecular pathogenesis of HCC.[8,9]

Current therapeutic approaches for HCC mainly include surgical resection, local ablation, transarterial interventional therapy, and systemic treatment.[10] However, most patients are diagnosed at advanced stages due to the absence of obvious symptoms during early tumor development. Consequently, intrahepatic dissemination and distant metastasis are frequently observed at initial diagnosis, leading to poor clinical outcomes and low long-term survival rates.[11,12] Accordingly, increasing attention has

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been directed toward identifying critical regulatory molecules involved in HCC progression to improve diagnostic accuracy and optimize targeted treatment strategies.[13]

Recent advances in transcriptomic research have highlighted the critical regulatory roles of non-coding RNAs in tumor biology.[14] Among these molecules, microRNAs (miRNAs) are a class of endogenous small non-coding RNAs that negatively regulate gene expression at the post-transcriptional level through complementary binding to target messenger RNAs.[15-17] Aberrant miRNA expression has been implicated associated with tumor initiation and progression, including uncontrolled proliferation, migration, invasion, epithelial-mesenchymal transition, and therapeutic resistance.[18,19] Due to their tissue specificity and functional diversity, miRNAs have attracted increasing attention as potential biomarkers and therapeutic targets in human malignancies.

Abnormal expression patterns of miRNA-205-3p have been identified in multiple human malignancies, including ovarian cancer and breast cancer.[20,21] Nevertheless, its biological role and underlying molecular mechanism in hepatocellular carcinoma remain insufficiently understood. In the present study, expression patterns of miRNA-205-3p in HCC tissues and cell lines were investigated, followed by functional analyses examining its effects on malignant cellular phenotypes. Furthermore, Zinc finger DBF-type containing 2 (ZDBF2) was identified and validated as a downstream regulatory target of miRNA-205-3p through bioinformatic prediction and rescue experiments. These findings provide additional evidence supporting the involvement of the miRNA-205-3p/ZDBF2 regulatory axis in hepatocellular carcinoma progression.

## MATERIALS AND METHODS

### Patients and Tissue Specimens

A total of 55 paired Hepatocellular Carcinoma (HCC) tissues and adjacent non-tumorous liver tissues were obtained from patients who underwent surgical treatment at Guangdong Provincial People's Hospital. Eligible patients fulfilled the following criteria: (1) histopathologically confirmed HCC; (2) no history of preoperative anti-tumor therapy, including transcatheter arterial chemoembolization or radiofrequency ablation; (3) absence of other malignant diseases; and (4) complete clinicopathological and follow-up information available for analysis.

### Cell Culture and Reagents

Human hepatocellular carcinoma cell lines, including Bel-7402, MHCC88H, SMMC-7721, Huh7, and Hep3B, as well as the normal hepatic LO2 cell line, were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum together with penicillin (100 U/mL) and streptomycin (100 µg/mL). Cell cultures were maintained under standard conditions at 37°C in a humidified incubator supplied with 5% $CO_2$. Subculturing was carried out when cell density reached approximately 80%-90% using trypsin-EDTA digestion. All cellular experiments were performed in triplicate independently. Assessment of migratory and invasive capacities was conducted by investigators who were unaware of the experimental group allocation.

### Cell Transfection

For transient transfection, HCC cells were first plated into six-well culture plates and allowed to adhere overnight. Once cellular confluence approached 70%-80%, transfection was carried out using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the recommended protocol provided by the manufacturer. MiRNA-205-3p mimic, negative control oligonucleotides (miR-NC), pcDNA-ZDBF2 plasmids, and corresponding empty vectors were obtained from GeneCopoeia (Rockville, MD, USA). 48 hr after transfection, cells were collected for subsequent experimental analyses.

### Cell Proliferation Assay

Proliferative activity was determined using the Cell Counting Kit-8 assay (CCK-8; Dojindo Laboratories, Kumamoto, Japan). Transfected cells were inoculated into 96-well plates at a density of $2.5 \times 10^3$ cells per well. Absorbance values were recorded at 450 nm on days 1 through 4 using a microplate spectrophotometer, and proliferation curves were generated accordingly.

### Colony Formation Assay

To evaluate long-term proliferative potential, approximately 200 treated cells were seeded into each well of six-well plates and maintained under standard culture conditions for 14 days. The culture medium was refreshed once during the first week and subsequently replaced twice per week. Following incubation, colonies were rinsed with Phosphate-Buffered Saline (PBS), fixed using methanol, and stained with 0.1% crystal violet solution. Colony numbers were then photographed and quantified.

### Transwell Migration and Invasion Assays

Cell migratory and invasive properties were assessed using transwell chamber systems. For migration experiments, Hep3B and Huh7 cells suspended in serum-free medium were transferred into the upper compartment, whereas medium supplemented with serum was added to the lower chamber to serve as a chemoattractant. After incubation for 24 hr, non-migrated cells remaining on the upper membrane surface were carefully removed. Cells that traversed the membrane were fixed with methanol, stained with crystal violet, and counted under a microscope. For invasion analysis, Matrigel-coated transwell inserts were prepared before cell inoculation.

### Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

RNA samples were extracted from cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After removal of genomic DNA contamination with DNase I treatment, reverse transcription was performed using the PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan) to generate complementary DNA (cDNA). Quantitative real-time PCR analysis was then conducted with SYBR Premix Ex Taq reagents (TaKaRa, Otsu, Shiga, Japan). Relative transcript abundance was determined according to the 2^-ΔΔCt calculation method, using U6 and GAPDH as endogenous normalization references. The primer sequences used in this study were as follows: miRNA-205-3p: Forward: 5'- GAGGATCCCCGGGTACCGGTAGGCCTTT-3'; Reverse: 5'- CACACATTCCACAG GCTGCTACGGTGGTGGCGGCGGGCGGT-3'; U6: Forward: 5'- GCTTCGGCAGCACATATACTAAAAT-3'; Reverse: 5'- CGCTTCACGAATTTGCGTGCAT-3'; ZDBF2: Forward: 5'- TGACCTTTTCTGCTCCAGGTTA-3'; Reverse: 5'-GCACACGGCAATAACTGCAA-3'; GAPDH: Forward: 5'-GCTGCCCAGAACATCATCC-3'; Reverse: 5'- GTCAGATCCACGACGGACAC-3'.

### Western Blot Analysis

Cellular proteins were lysed in RIPA buffer under ice-cold conditions and subsequently clarified by centrifugation at 13,000 ×g for 15 min at 4°C. Protein concentrations were determined using a Bicinchoninic Acid (BCA) protein quantification kit (Beyotime, Shanghai, China). Equivalent amounts of protein lysates were separated by sodium dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and electrotransferred onto Polyvinylidene Difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After nonspecific binding sites were blocked with 5% skim milk, membranes were sequentially exposed to primary and secondary antibodies. Immunoreactive bands were detected and analyzed densitometrically using ImageJ software (NIH, Bethesda, MD, USA).

### Dual-Luciferase Reporter Assay

Reporter constructs containing either wild-type or mutated miRNA-205-3p binding sequences within ZDBF2 were generated for luciferase activity analysis. Cells cultured in 24-well plates were co-transfected with the reporter vectors together with miRNA-205-3p mimic or miR-NC. Luciferase signals were assessed 48 hr later using a dual-luciferase reporter assay system.

### Xenograft Tumor Assay

All animal-related procedures received approval from the institutional ethics committee. Four-week-old male nude mice were randomly assigned to experimental groups ($n$=5 per group) and subcutaneously inoculated with Hep3B cells transfected with either miRNA-205-3p mimic or miR-NC. Tumor size was monitored weekly, and xenograft volume was estimated according to the formula:

$$\text{volume } (mm^3) = (\text{width}^2 \times \text{length})/2$$

After six weeks, mice were sacrificed and tumor tissues were excised for weight determination as well as subsequent expression analyses of miRNA-205-3p and ZDBF2.

### Statistical Analysis

Data processing and statistical evaluation were performed using GraphPad Prism 9.0 software (GraphPad Software, USA). Experimental results were presented as Mean ± Standard Deviation (SD). Kaplan-Meier survival curves were compared by the log-rank method. Relationships between miRNA-205-3p expression and clinicopathological variables were analyzed using the Chi-square test. Pearson correlation analysis was employed to determine the association between miRNA-205-3p and ZDBF2 expression levels. Differences with a two-tailed $p$ value below 0.05 were considered statistically significant.

## RESULTS

### Expression Profile of miRNA-205-3p in Hepatocellular Carcinoma

Expression analysis was initially performed in 55 paired HCC and adjacent non-tumorous tissue specimens to determine the dysregulation pattern of miRNA-205-3p in hepatocellular carcinoma. Compared with corresponding non-tumorous tissues, miRNA-205-3p expression was significantly decreased in HCC samples (Figure 1A). Consistent findings were further observed in multiple HCC cell lines, all of which exhibited lower miRNA-205-3p expression levels relative to normal hepatocytes (Figure 1B).

Subsequent clinicopathological analysis demonstrated that reduced miRNA-205-3p expression was significantly associated with lymph node metastasis and distant metastatic progression in HCC patients (Table 1). Moreover, metastatic HCC tissues displayed markedly lower miRNA-205-3p expression compared with non-metastatic tumors (Figure 1C). These findings suggested a potential inhibitory role of miRNA-205-3p during HCC progression and metastasis.

### Upregulation of miRNA-205-3p Attenuated Malignant Behaviors of HCC Cells

To further investigate the biological functions of miRNA-205-3p in HCC, gain-of-function experiments were performed in Hep3B and Huh7 cells. Following transfection with miRNA-205-3p mimic, intracellular miRNA-205-3p expression was markedly elevated compared with the control group, confirming successful transfection efficiency (Figure 2A).

Functional assays demonstrated that enforced miRNA-205-3p expression significantly impaired HCC cell growth. CCK-8

**Table 1: Association of miR-205-3p expression with clinicopathologic characteristics of hepatocellular carcinoma.**

| Parameters | Number of cases | miR-205-3p expression | | *p*-value |
|---|---|---|---|---|
| | | High (%) | Low (%) | |
| **Age (years)** | | | | 0.458 |
| <60 | 25 | 15 | 10 | |
| ≥60 | 30 | 15 | 15 | |
| **Gender** | | | | 0.883 |
| Male | 27 | 15 | 12 | |
| Female | 28 | 15 | 13 | |
| **T stage** | | | | 0.396 |
| T1-T2 | 32 | 19 | 13 | |
| T3-T4 | 23 | 11 | 12 | |
| **Tumor size (cm)** | | | | 0.418 |
| <3 | 34 | 20 | 14 | |
| ≥3 | 21 | 10 | 11 | |
| **Lymph node metastasis** | | | | 0.021 |
| No | 27 | 19 | 8 | |
| Yes | 28 | 11 | 17 | |
| **Distance metastasis** | | | | 0.013 |
| No | 36 | 24 | 12 | |
| Yes | 19 | 6 | 13 | |

analysis revealed a substantial reduction in cellular viability at 72 hr and 96 hr after transfection in both Hep3B and Huh7 cells (Figure 2B). Colony formation experiments further demonstrated that enforced miRNA-205-3p expression weakened the clonogenic potential of hepatocellular carcinoma cells (Figure 2C).

In addition to proliferation inhibition, overexpression of miRNA-205-3p also markedly attenuated metastatic phenotypes. Both migratory and invasive cell numbers were significantly decreased in transfected HCC cells compared with controls, indicating that miRNA-205-3p negatively regulated HCC cell motility and invasiveness (Figure 2D).

## MiRNA-205-3p Inhibited Tumor Growth *in vivo*

The suppressive effects of miRNA-205-3p observed *in vitro* prompted further evaluation using an *in vivo* xenograft model. Nude mice were subcutaneously injected with Hep3B cells transfected with either miRNA-205-3p mimic or negative control vectors. Compared with the control group, tumors derived from miRNA-205-3p-overexpressing cells exhibited a markedly slower growth rate, as evidenced by reduced tumor volume and lower tumor weight (Figures 3A and 3B).

After xenograft tissues were harvested, molecular analyses demonstrated significantly elevated miRNA-205-3p expression in tumors derived from the mimic group, accompanied by reduced ZDBF2 protein expression (Figures 3C and 3D). These findings further supported the inhibitory role of miRNA-205-3p in HCC tumor progression *in vivo*.

## Regulatory Interaction Between miRNA-205-3p and ZDBF2

Considering the inhibitory effects of miRNA-205-3p on HCC progression, additional analyses were performed to evaluate its relationship with ZDBF2. Western blot analysis demonstrated that ectopic miRNA-205-3p expression markedly reduced ZDBF2 protein levels in both Hep3B and Huh7 cells (Figure 4A).

In contrast to miRNA-205-3p expression patterns, ZDBF2 was significantly upregulated in HCC tissues compared with adjacent non-tumorous tissues (Figure 4B). Correlation analysis further demonstrated an inverse relationship between miRNA-205-3p and ZDBF2 expression in clinical HCC specimens (Figure 4C).

Dual-luciferase reporter assays were subsequently performed to validate the predicted binding interaction. Overexpression of miRNA-205-3p significantly decreased luciferase activity in cells transfected with the wild-type ZDBF2 reporter construct, whereas no obvious change was observed in the mutant reporter group (Figure 4D). Collectively, the luciferase assay results supported a direct regulatory relationship between miRNA-205-3p and ZDBF2.

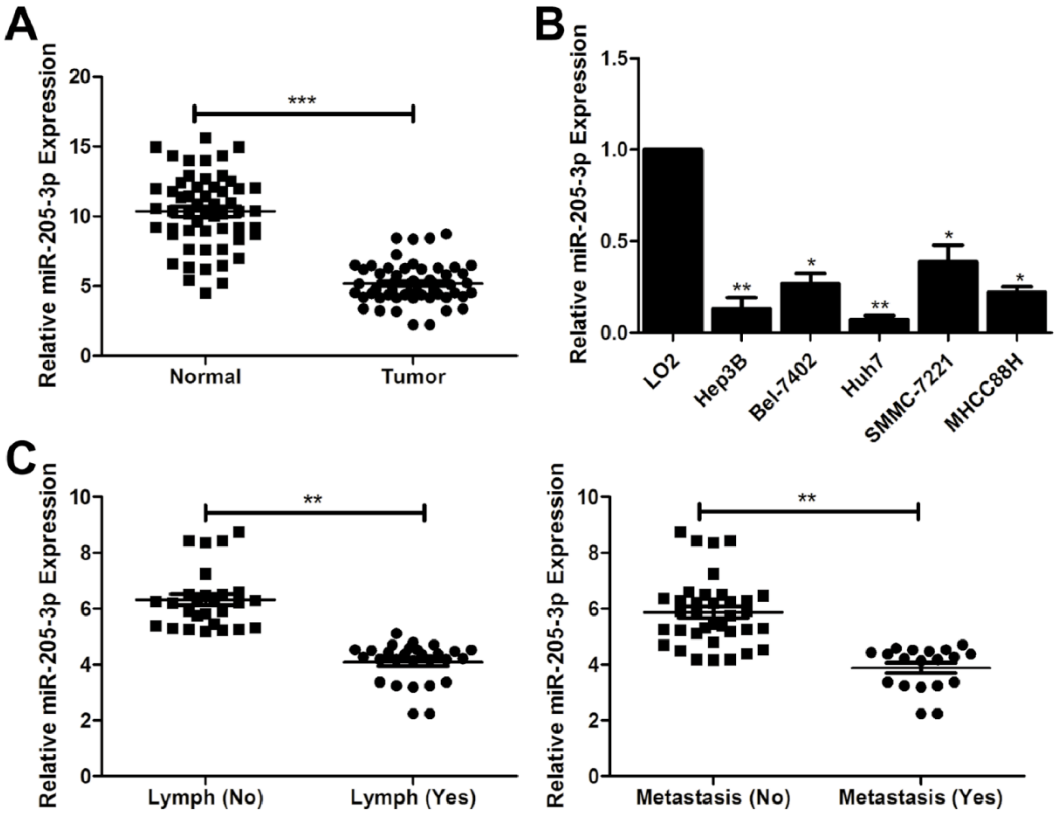


**Figure 1:** Expression level of miRNA-205-3p in HCC. (A) Expression levels of miRNA-205-3p in HCC and non-tumor tissues; (B) Expression levels of miRNA-205-3p in HCC cell lines; (C) Expression levels of miRNA-205-3p in HCC cases either with lymphatic metastasis, distant metastasis or not. Data were expressed as Mean±SD. *$p$<0.05, **$p$<0.01, ***$p$<0.001.

### MiRNA-205-3p Attenuated Malignant Phenotypes of HCC Through ZDBF2 Regulation

Based on the validated regulatory relationship between miRNA-205-3p and ZDBF2, rescue experiments were further conducted to determine whether ZDBF2 mediated the biological effects induced by miRNA-205-3p. As shown in Figure 4E, reintroduction of ZDBF2 markedly restored its protein expression in HCC cells overexpressing miRNA-205-3p.

Functional analyses demonstrated that forced ZDBF2 expression partially reversed the inhibitory effects caused by miRNA-205-3p restoration. Specifically, the suppressed proliferative activity observed in miRNA-205-3p-overexpressing cells was significantly rescued following ZDBF2 overexpression (Figure 4F). Likewise, migratory and invasive capacities that had been attenuated by miRNA-205-3p were partially restored after ZDBF2 re-expression (Figure 4G).

Collectively, these findings suggested that miRNA-205-3p exerted tumor-suppressive effects in hepatocellular carcinoma, at least in part, through negative regulation of ZDBF2.

## DISCUSSION

Hepatocellular carcinoma remains a highly aggressive malignancy characterized by frequent recurrence, metastatic dissemination, and unfavorable long-term survival despite continuous advances in clinical management.[1-3] Early-stage HCC is often clinically asymptomatic, resulting in delayed diagnosis and limited therapeutic opportunities for a substantial proportion of patients. Consequently, increasing attention has been directed toward identifying novel molecular biomarkers associated with tumor progression and prognosis.[8,9] In recent years, dysregulated microRNA expression has emerged as an important regulatory mechanism contributing to hepatocarcinogenesis and metastatic evolution.[14-19] Numerous studies have demonstrated that miRNAs participate extensively in tumor-related biological processes through post-transcriptional modulation of oncogenic or tumor-suppressive signaling pathways.[15-18]

Analysis of clinical specimens and cultured cells revealed obvious downregulation of miRNA-205-3p in hepatocellular carcinoma. Lower miRNA-205-3p expression was further linked to lymphatic dissemination, distant metastasis, and poor prognosis in affected patients. These observations suggested that miRNA-205-3p may participate in malignant progression and could potentially serve as a prognostic biomarker in hepatocellular carcinoma.

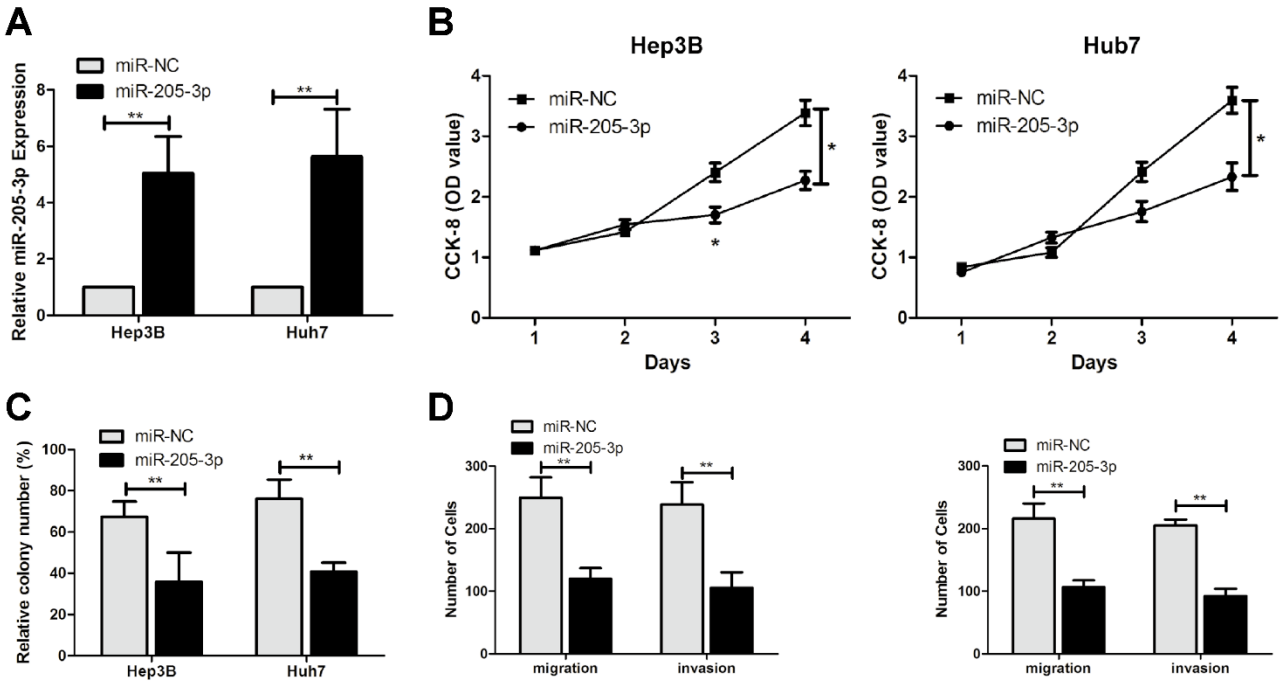


**Figure 2:** MiRNA-205-3p attenuated proliferative, migratory and invasive capacities in HCC. (A) Transfection efficacy of miRNA-205-3p mimic in Hep3B and Huh7 cells; (B) Viability in Hep3B and Huh7 cells overexpressing miRNA-205-3p at day 1, 2, 3 and 4; (C) Relative colony number in Hep3B and Huh7 cells overexpressing miRNA-205-3p (magnification: 10×); (D) Numbers of migratory and invasive cells in Hep3B and Huh7 cells overexpressing miRNA-205-3p (magnification: 40×). Data were expressed as Mean±SD. *$p$<0.05, **$p$<0.01.

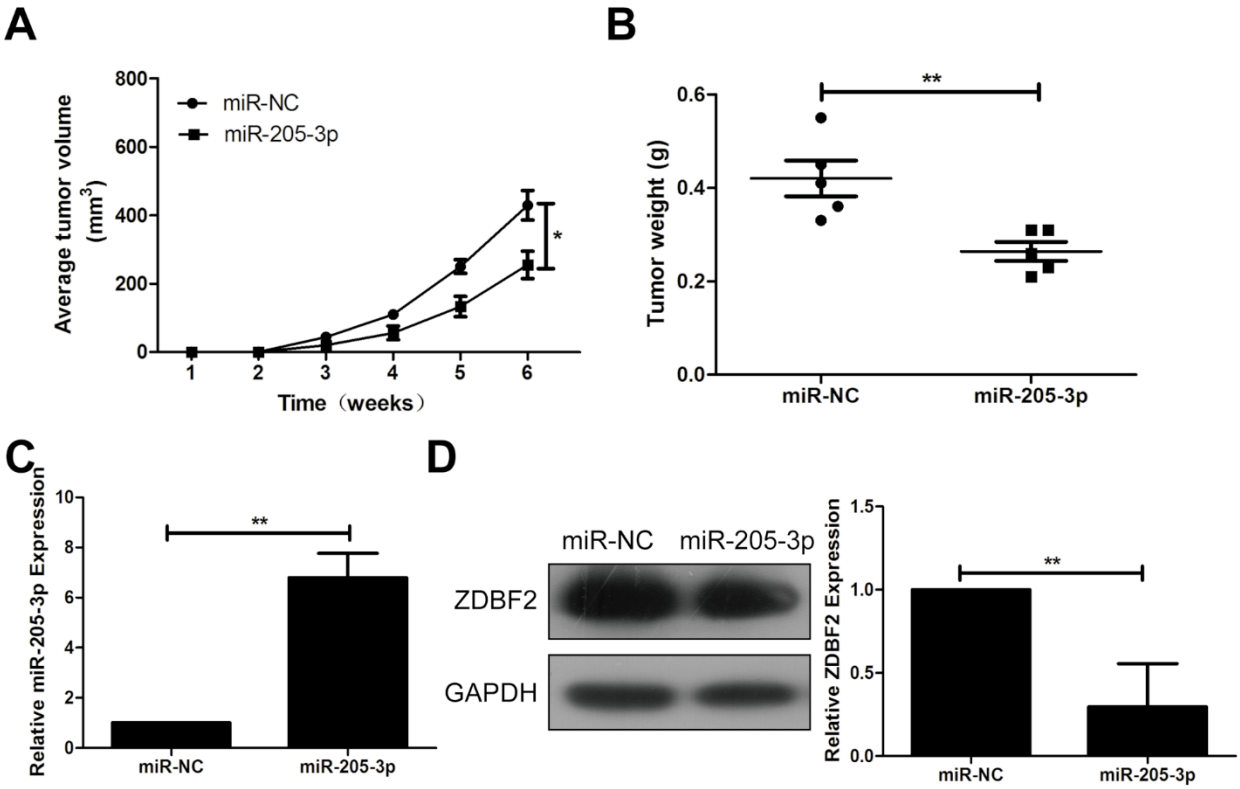


**Figure 3:** MiRNA-205-3p slowed down in vivo growth of HCC. (A) Average tumor volume in nude mice administrated with Hep3B cells transfected with miR-NC or miRNA-205-3p mimic from the 1st week to the 6th week; (B) Tumor weight in nude mice administrated with Hep3B cells transfected with miR-NC or miRNA-205-3p mimic; (C) Expression levels of miRNA-205-3p in HCC tissues harvested from nude mice administrated with Hep3B cells transfected with miR-NC or miRNA-205-3p mimic; (D) Protein level of ZDBF2 in nude mice administrated with Hep3B cells transfected with miR-NC or miRNA-205-3p mimic. Data were expressed as Mean±SD. *$p$<0.05, **$p$<0.01.

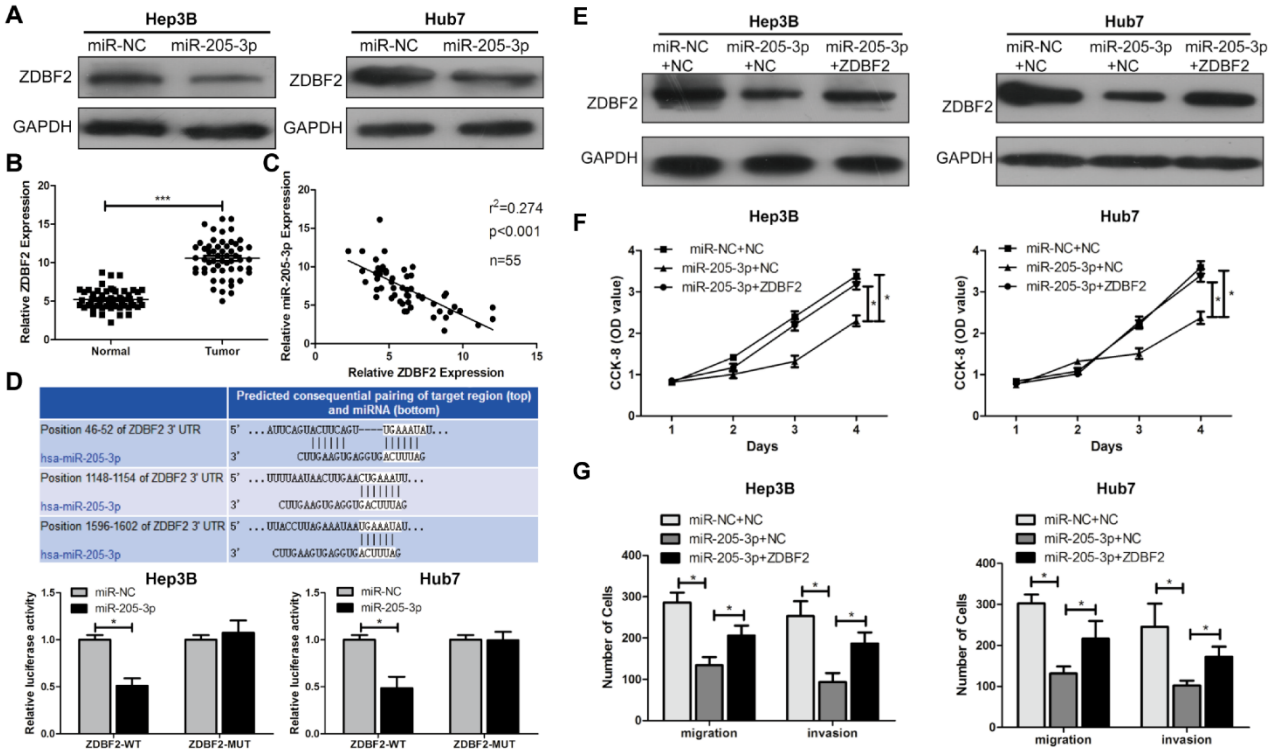


**Figure 4:** MiRNA-205-3p alleviated malignant progression of HCC by competitively binding ZDBF2. (A) Protein level of ZDBF2 in Hep3B and Huh7 cells overexpressing miRNA-205-3p; (B) Expression levels of ZDBF2 in HCC and non-tumor tissues; (C) A negative correlation between miRNA-205-3p and ZDBF2 in HCC tissues; (D) Luciferase activity in Hep3B and Hub7 cells. Data were expressed as Mean±SD. (E) Protein levels of ZDBF2 in Hep3B and Hub7 cells co-transfected with miR-NC+NC, miRNA-205-3p mimic+NC or miRNA-205-3p mimic+pcDNA-ZDBF2; (F) Viability in Hep3B and Hub7 cells co-transfected with miR-NC+NC, miRNA-205-3p mimic+NC or miRNA-205-3p mimic+pcDNA-ZDBF2 at day 1, 2, 3 and 4; (G) Numbers of migratory and invasive cells in Hep3B and Hub7 cells co-transfected with miR-NC+NC, miRNA-205-3p mimic+NC or miRNA-205-3p mimic+pcDNA-ZDBF2. Data were expressed as Mean±SD. *$p$<0.05, ***$p$<0.001.

Functional analyses further demonstrated that restoration of miRNA-205-3p expression markedly suppressed proliferative activity, colony formation, invasion and migration in Hep3B and Huh7 cells. Consistent inhibitory results were also observed in xenograft tumor models, in which miRNA-205-3p overexpression significantly restrained *in vivo* tumor growth. These findings collectively support a tumor-suppressive role for miRNA-205-3p during HCC progression. Given that uncontrolled proliferation and enhanced metastatic potential are critical biological hallmarks of malignant tumors, the inhibitory effects mediated by miRNA-205-3p may have important implications for limiting HCC aggressiveness.[22,23]

Mechanistically, ZDBF2 was identified as a direct downstream target of miRNA-205-3p through bioinformatic prediction and dual-luciferase reporter validation. ZDBF2 expression was elevated in HCC tissues and displayed a significant inverse correlation with miRNA-205-3p expression. Importantly, restoration of ZDBF2 expression partially reversed the suppressive effects induced by miRNA-205-3p overexpression, indicating that the biological functions of miRNA-205-3p were at least partly mediated through negative regulation of ZDBF2. Although the precise oncogenic mechanisms of ZDBF2 in hepatocellular carcinoma remain insufficiently characterized, the present findings suggest that aberrant activation of ZDBF2 may contribute to HCC progression and metastatic behavior.

Several limitations should also be considered when interpreting the present results. First, the sample size of the clinical cohort was relatively limited and derived from a single institution, which may restrict the generalizability of the findings. Despite the anti-tumor effects observed in both cellular and xenograft models, additional studies are still needed to clarify the downstream molecular events regulated by the miRNA-205-3p/ZDBF2 pathway. Furthermore, validation using multicenter clinical cohorts with larger sample sizes will be important for determining the prognostic and therapeutic relevance of miRNA-205-3p in hepatocellular carcinoma.

## CONCLUSION

Collectively, the present findings revealed pronounced downregulation of miRNA-205-3p in hepatocellular carcinoma, and reduced expression levels were associated with metastatic progression as well as poor patient prognosis. Functional experiments demonstrated that miRNA-205-3p inhibited HCC cell proliferation, migration, invasion, and tumor growth through direct targeting of ZDBF2. Collectively, these findings indicate that the miRNA-205-3p/ZDBF2 regulatory axis may represent

a promising biomarker and potential therapeutic target for hepatocellular carcinoma management.

## ACKNOWLEDGEMENT

Not applicable.

## ETHICAL STATEMENT

The study protocol was approved by the Ethics Committee of Guangdong Provincial People's Hospital and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before sample collection.

## ABBREVIATIONS

**HCC:** Hepatocellular carcinoma; **qRT-PCR:** Quantitative real-time polymerase chain reaction; **CCK-8:** Cell counting kit-8; **TNM:** Tumor node metastasis; **UICC:** Union for International Cancer Control; **ATCC:** American Type Culture Collection; **DMEM:** Dulbecco's Modified Eagle's Medium; **FBS:** Fetal bovine serum; **EDTA:** Ethylenediaminetetraacetic acid; **PBS:** Phosphate buffered saline; **cDNA:** Complementary deoxyribose nucleic acid; **GAPDH:** Glyceraldehyde 3-phosphate dehydrogenase; **BCA:** Bicinchoninic acid; **SDS-PAGE:** Sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **PVDF:** Polyvinylidene difluoride; **ANOVA:** Analysis of variance; **SD:** Standard deviation; **UTR:** Untranslated region.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## FUNDING

This study did not receive any funding in any form.

## AUTHORS' CONTRIBUTIONS

# Xin Lu, Wenting Mi and Jianming Zheng contributed equally to this work. Xin Lu, Wenting Mi and Jianming Zheng: Conceptualization, methodology, writing original draft preparation. Meishi Tang and Haoxiang Ou: Investigation, software, statistical analysis. Yubin Liu: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

## SUMMARY

This study investigated the tumor-suppressive role of miRNA-205-3p in Hepatocellular Carcinoma (HCC) and elucidated its underlying molecular mechanism. Quantitative real-time PCR analysis demonstrated that miRNA-205-3p was significantly downregulated in HCC tissues and cell lines compared with non-tumor controls. Reduced miRNA-205-3p expression was closely associated with lymphatic metastasis, distant metastasis, and poor progression-free and overall survival, indicating its clinical relevance. Functional assays revealed that overexpression of miRNA-205-3p markedly inhibited proliferation, colony formation, migration, and invasion in Hep3B and Huh7 cells. *In vivo* xenograft experiments further confirmed that miRNA-205-3p suppressed tumor growth. Mechanistically, dual-luciferase reporter assays identified ZDBF2 as a direct downstream target of miRNA-205-3p. ZDBF2 expression was elevated in HCC tissues and negatively correlated with miRNA-205-3p levels. Rescue experiments demonstrated that ZDBF2 overexpression reversed the inhibitory effects of miRNA-205-3p on malignant phenotypes. Collectively, these findings suggest that miRNA-205-3p functions as a tumor suppressor in HCC by targeting ZDBF2 and may represent a potential therapeutic target.

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