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Effects of the miR-199a-5p/DEPDC1B Axis on Hepatocellular Carcinoma Cell Proliferation, Migration, and Wnt/β--Catenin Signaling

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Abstract | **Original Research Article**

Objective: To investigate the effects of miR-199a-5p on the proliferation and migration of hepatocellular carcinoma (HCC) cells and to characterize changes in DEPDC1B and Wnt/β-catenin-related proteins. ***Methods:*** Hep3B and MHCC-97H cells were used to establish miR-199a-5p overexpression and DEPDC1B rescue models. The experimental groups were miR-NC, miR-199a-5p, miR-199a-5p + vector, and miR-199a-5p + DEPDC1B. Expression of miR-199a-5p and DEPDC1B mRNA was measured by quantitative reverse-transcription PCR (qRT-PCR). Cell viability activity was assessed using the CCK-8 assay at 24, 48, 72, 96, and 120 h. Cell migration was evaluated using Transwell assays. Western blotting was used to assess DEPDC1B, E-cadherin, N-cadherin, vimentin, Wnt3a, β-catenin, cyclin D1, and c-Myc protein levels. ***Results:*** Compared with the miR-NC group, transfection with miR-199a-5p significantly increased miR-199a-5p expression and reduced DEPDC1B mRNA and protein levels in both Hep3B and MHCC-97H cells. CCK-8 growth curves showed an overall reduction in OD450 values following miR-199a-5p overexpression, consistent with suppressed cell viability activity. Transwell assays showed fewer migrating cells in the miR-199a-5p and miR-199a-5p + vector groups than in the miR-NC group ($P < 0.01$, as indicated in the figure); cell counts showed a trend toward recovery after DEPDC1B re-expression. Western blotting showed that miR-199a-5p overexpression was accompanied by lower levels of DEPDC1B, Wnt3a, cyclin D1, and c-Myc, as well as reduced levels of the mesenchymal phenotype-associated proteins N-cadherin and vimentin. ***Conclusion:*** miR-199a-5p overexpression suppressed HCC cell proliferation and migration-associated phenotypes, potentially at least in part through downregulation of DEPDC1B and attenuation of Wnt/β-catenin-related signaling. DEPDC1B re-expression partially restored the malignant phenotype, suggesting that the miR-199a-5p–DEPDC1B axis may participate in HCC progression.

Keywords: miR-199a-5p; DEPDC1B; hepatocellular carcinoma; Wnt/β-catenin; epithelial–mesenchymal transition.

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1. INTRODUCTION

Hepatocellular carcinoma is one of the major histological types of primary liver cancer and is associated with substantial risks of recurrence and mortality. The global burden of liver cancer remains considerable and is influenced by multiple factors, including viral hepatitis, alcohol-related liver disease, and metabolic liver disease[1]. Despite advances in locoregional, targeted, and immunotherapies, invasion, metastasis, and treatment resistance remain important determinants of long-term outcomes in HCC[2]. Elucidating the molecular networks that drive malignant behavior and identifying actionable regulatory nodes therefore remain important research priorities.

MicroRNAs (miRNAs) are endogenous non-coding RNAs of approximately 22 nucleotides that can promote target mRNA degradation or repress translation through complementary binding. The role of miR-199a-5p varies across tumor contexts; previous studies in HCC have generally reported reduced expression, and restoration of miR-199a-5p has been shown to suppress cell proliferation, migration, and invasion. FZD7, DDR1, MAP4K3, CLTC, and CDC25A have been reported as functionally relevant targets or mediators[3]. In particular, miR-199a can affect Wnt-pathway downstream molecules, including β-catenin, cyclin D1, and Myc, through FZD7, suggesting a close relationship between miR-199a-5p, HCC cell proliferation, and Wnt/β-catenin signaling[4–8].

Domain-containing protein 1B (DEPDC1B) is associated with cell-cycle regulation, cytoskeletal remodeling, and tumor progression. Previous studies have reported elevated DEPDC1B expression in HCC and an association with poorer prognosis; DEPDC1B

knockdown can inhibit HCC cell proliferation and migration. In other malignancies, including non-small cell lung and breast cancer, DEPDC1B has also been implicated in Wnt/β-catenin signaling and metastasis-associated phenotypes[9,10]. However, the functional relationship between miR-199a-5p and DEPDC1B in HCC has not been adequately characterized experimentally [11,12].

In this study, Hep3B and MHCC-97H cells were used to examine changes in cell proliferation, migration, and Wnt/β-catenin-related proteins following miR-199a-5p overexpression and DEPDC1B rescue. The objective was to assess the potential involvement of the miR-199a-5p–DEPDC1B axis in regulating malignant phenotypes in HCC and to provide a basis for subsequent direct-target validation and mechanistic studies.

2. MATERIALS AND METHODS

Materials

Hep3B and MHCC-97H cells were obtained from Procell Life Science & Technology Co., Ltd. (Wuhan, Hubei, China). The DEPDC1B overexpression plasmid constructs, including the empty vector and overexpression vector, were designed by GeneCopoeia. TRIzol was purchased from Takara (Shanghai, China). RNA reverse-transcription and qRT-PCR kits were obtained from Vazyme Biotech Co., Ltd. A cell-cycle staining kit was purchased from Lianke Biotech. The Cell Counting Kit-8 (CCK-8) was obtained from Dojindo Laboratories, and Lipofectamine 3000 was purchased from Invitrogen.

2.1. Cell Lines and Culture

The human HCC cell lines Hep3B and MHCC-97H were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was changed every 2–3 days, and cells were passaged at a ratio of 1:3.

2.2. Oligonucleotides, Plasmids, and Cell Transfection

Cells were assigned to four groups according to the experimental design: miR-NC, miR-199a-5p, miR-199a-5p + vector, and miR-199a-5p + DEPDC1B. Cells in the miR-199a-5p group were transfected with a miR-199a-5p mimic. For rescue experiments, cells overexpressing miR-199a-5p were co-transfected with either the empty vector or the DEPDC1B overexpression plasmid. Cells were harvested at the prespecified time points for subsequent analyses.

2.3. Quantitative Reverse-Transcription PCR

Total cellular RNA was extracted and reverse-transcribed. Expression of miR-199a-5p and DEPDC1B was measured by real-time quantitative PCR. U6 and GAPDH were used as reference genes for miR-199a-5p and DEPDC1B mRNA, respectively. Primer sequences were as follows: U6 forward, 5′-CTCGCTTCGGCACA-3′; U6 reverse, 5′-AACGCTTCACGAATTTGCGT-3′; miR-199a-5p forward, 5′-TTATTACCCAGGCAGACA CCG-3′; miR-199a-5p reverse, 5′-AGTGCGAACTGT GGCGAT-3′; GAPDH forward, 5′-CAGGAGGCATTG CTGATGAT-3′; GAPDH reverse, 5′-GAAGGCTGG GGCTCATTT-3′; DEPDC1B forward, 5′-AGACCGT GGAGCTTTTTCGTG-3′; and DEPDC1B reverse, 5′-TTCAGGGCCGAAGTTTTGACT-3′. Relative expression was calculated using the 2^−ΔΔCt method.

2.4. CCK-8 Assay for Cell Viability

Transfected Hep3B and MHCC-97H cells were seeded into 96-well plates at 5×10^3 cells per well. CCK-8 working solution was added at 24, 48, 72, 96, and 120 h. After incubation for 2 h, absorbance was measured at 450 nm (OD450), and the values at each time point were used to generate cell growth curves.

2.5. Transwell Assay

Transfected cells were seeded into the upper chambers of Transwell inserts (Corning; 8-μm pore size) at 2×10^4 cells per well. The lower chambers contained RPMI-1640 medium supplemented with 10% FBS. After 24 h of incubation, non-migrating cells on the upper surface of the membrane were removed with a cotton swab. Cells on the lower surface were stained with crystal violet and counted under a microscope.

2.6. Western Blotting

Total protein was extracted using RIPA lysis buffer (Beyotime), and protein concentrations were determined with a BCA protein assay kit (Beyotime). Protein samples were separated by SDS-PAGE and transferred to PVDF membranes (Millipore). After blocking, the membranes were incubated with antibodies against DEPDC1B (1:1,000; OmnimAbs), E-cadherin (1:1,000; Proteintech), N-cadherin (1:1,000; Proteintech), vimentin (1:1,000; Proteintech), Wnt3a (1:1,000; Affinity), β-catenin (1:1,000; Affinity), cyclin D1 (1:1,000; Proteintech), c-Myc (1:5,000; Proteintech), and GAPDH (1:5,000; Proteintech). Signals were visualized using an ECL chemiluminescence kit (Thermo Fisher Scientific).

2.7. Statistical Analysis

Data were analyzed using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS 22.0 (SPSS, Chicago, IL, USA). Results are presented as the mean ± standard deviation (SD). Two-tailed Student's t-tests were used for comparisons between two groups, and one-way analysis of variance (ANOVA) was used for comparisons among multiple groups. The data represent three independent experiments. $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. miR-199a-5p Overexpression Downregulates DEPDC1B in HCC Cells

qRT-PCR showed that, compared with the miR-NC group, transfection with miR-199a-5p significantly

increased relative miR-199a-5p expression in both Hep3B and MHCC-97H cells (Figure 1A and 1C; ***P < 0.001), indicating successful overexpression. DEPDC1B mRNA levels were also significantly reduced in both cell lines (Figure 1B and 1D; ***P < 0.001). Representative Western blots likewise showed reduced DEPDC1B protein following miR-199a-5p overexpression (Figure 4). These findings indicate an inverse association between miR-199a-5p and DEPDC1B expression.

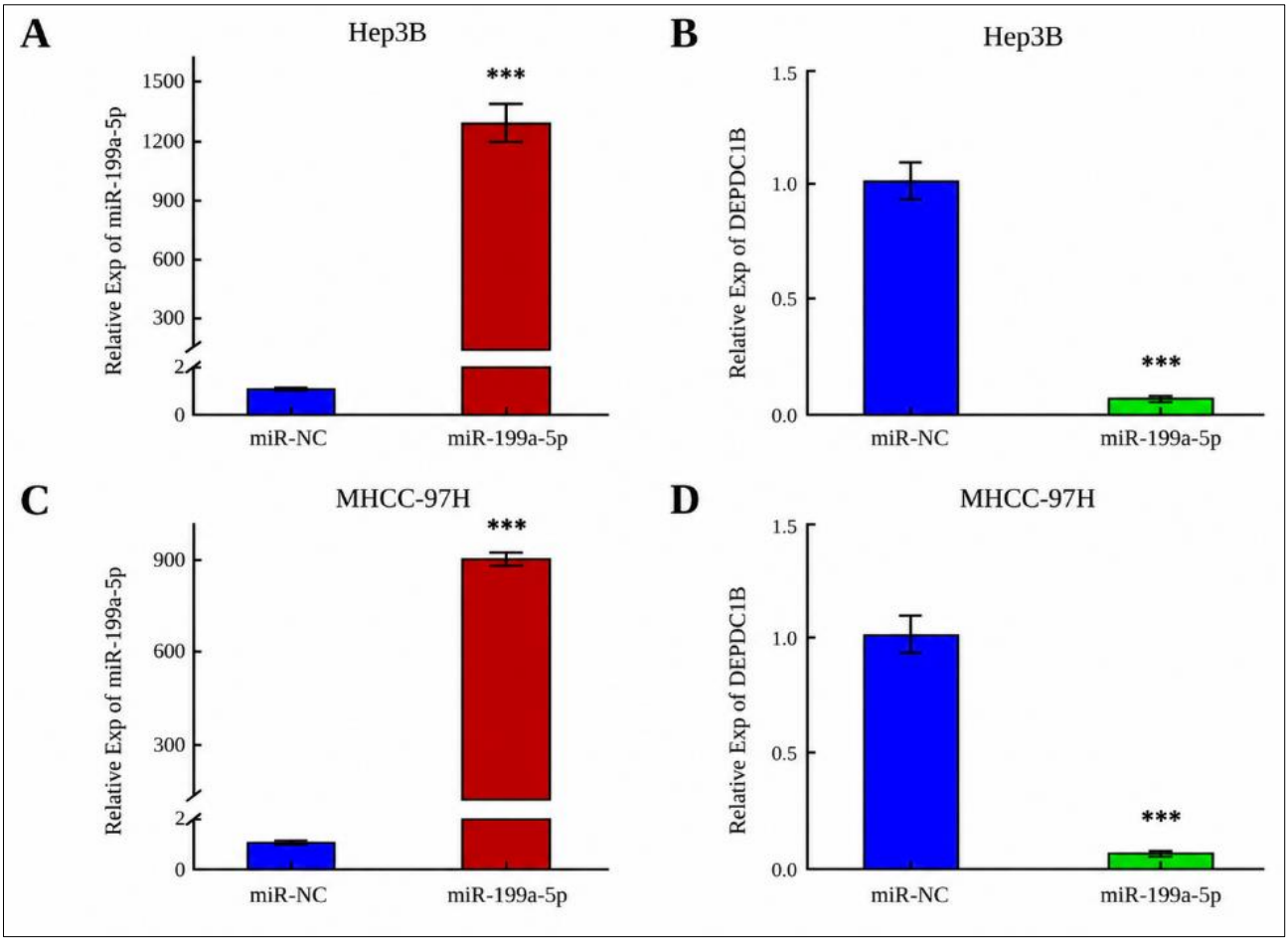


Figure 1. miR-199a-5p over expression and changes in DEPDC1B mRNA expression

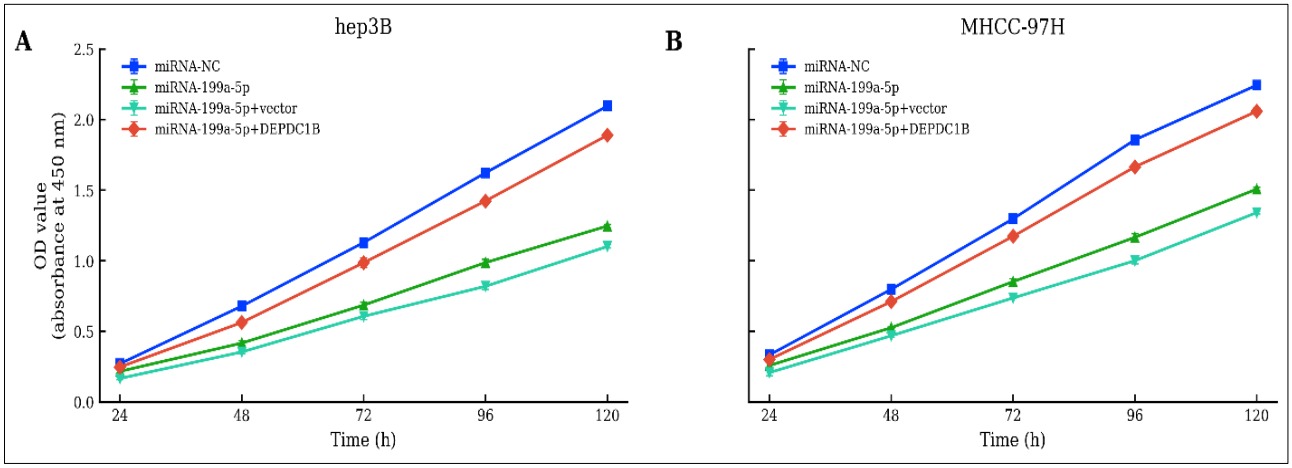


Figure 2: Effects of miR-199a-5p and DEPDC1B re-expression on HCC cell proliferation. (A) Hep3B cells; (B) MHCC-97H cells. OD450 values were measured by CCK-8 assay at 24, 48, 72, 96, and 120 h

3.2. DEPDC1B Re-expression Partially Rescues miR-199a-5p-Associated Suppression of Cell Proliferation

CCK-8 measurements increased over time in all groups of both Hep3B and MHCC-97H cells. Compared with the miR-NC group, the growth curves for the miR-199a-5p and miR-199a-5p + vector groups began to diverge downward from 48 h, with a more apparent difference in the 72–120 h interval (Figure 2A and 2B).

This pattern is consistent with reduced cell viability activity following miR-199a-5p overexpression. Under miR-199a-5p overexpression, DEPDC1B re-expression increased OD450 values in both cell lines relative to the vector group, with values trending toward those in the miR-NC group. These results suggest partial rescue of the miR-199a-5p-associated growth phenotype by DEPDC1B.

3.3. miR-199a-5p Suppresses HCC Cell Migration, with a Trend Toward Recovery After DEPDC1B Re-expression

Representative Transwell images showed fewer crystal-violet-stained cells on the lower membrane surface in the miR-199a-5p and miR-199a-5p + vector groups than in the miR-NC group. Quantification likewise showed lower relative cell counts in both groups than in the miR-NC group (Figure 3; **P < 0.01, as indicated in the figure). The number of migrated cells in the miR-199a-5p + DEPDC1B group was higher than that in the miR-199a-5p and vector groups and approached the control level, suggesting a trend toward recovery of the suppressed migration phenotype after DEPDC1B re-expression.

3.4. The miR-199a-5p/DEPDC1B Axis Is Accompanied by Changes in Wnt/β-Catenin and Migration-Related Protein Profiles

Representative Western blots showed reduced DEPDC1B protein in Hep3B and MHCC-97H cells following miR-199a-5p overexpression. Wnt3a, cyclin D1, and c-Myc, which are associated with Wnt/β-catenin signaling, generally showed a downward trend; several of these proteins showed a trend toward recovery after DEPDC1B re-expression. N-cadherin and vimentin, which are associated with a mesenchymal phenotype, were also generally reduced after miR-199a-5p overexpression and showed a trend toward recovery following DEPDC1B re-expression. Changes in β-catenin and E-cadherin were comparatively limited and inconsistent across the representative blots. Accordingly, the results are more appropriately described as changes in Wnt/β-catenin-related and migration-related protein profiles; without densitometric quantification, stronger claims regarding pathway activation or EMT reversal are not warranted.

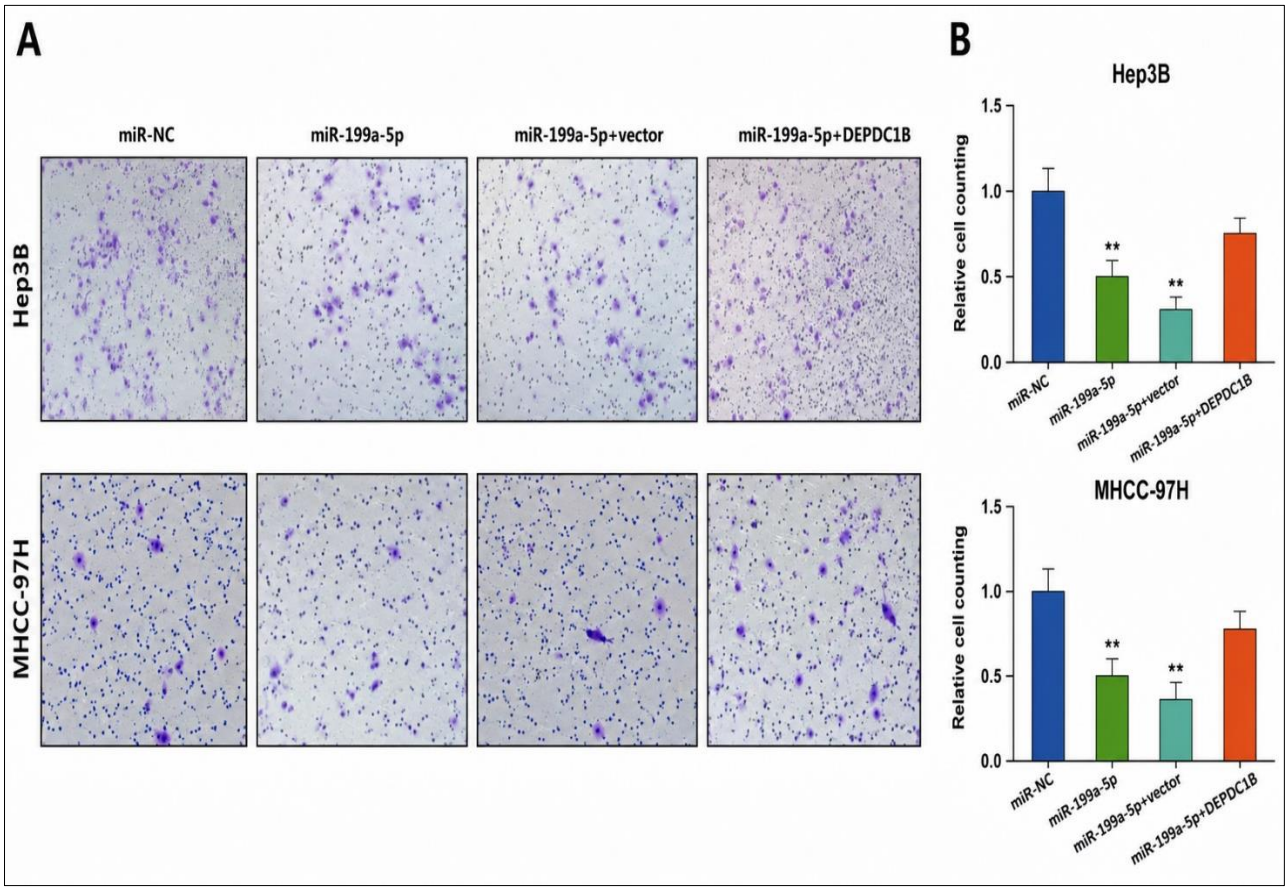
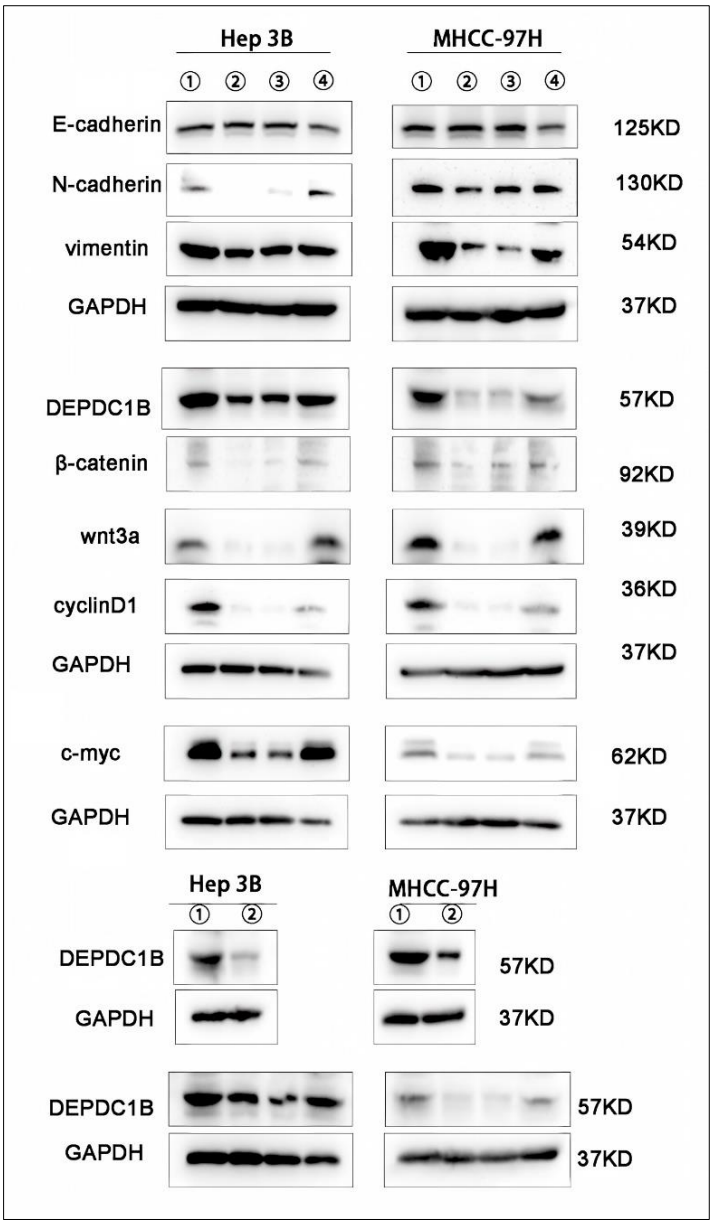


Figure 3: Effects of miR-199a-5p and DEPDC1B re-expression on HCC cell migration. (A) Representative Transwell images for each group in Hep3B and MHCC-97H cells; (B) quantification of relative numbers of migrated cells. **P < 0.01



① miR-NC ②miR-199a-5p ③miR-199a-5p3 + vector ④miR-199a-5p + DEPDC1B

Figure 4. Protein expression associated with the miR-199a-5p/DEPDC1B axis. Western blotting was used to assess DEPDC1B, E-cadherin, N-cadherin, vimentin, Wnt3a, β-catenin, cyclin D1, and c-Myc in Hep3B and MHCC-97H cells, with GAPDH as the loading control.

4. DISCUSSION

This in vitro study examined the relationship between miR-199a-5p, DEPDC1B, and malignant phenotypes in HCC cells. The principal findings demonstrated that miR-199a-5p overexpression significantly reduced DEPDC1B mRNA levels and was accompanied by decreased DEPDC1B protein expression in Hep3B and MHCC-97H cells. Cell growth and Transwell migration were also reduced following miR-199a-5p overexpression. Restoration of DEPDC1B expression in miR-199a-5p-overexpressing cells shifted the proliferation and migration phenotypes toward control levels. These changes were accompanied by alterations in the expression of Wnt3a, cyclin D1, c-Myc, and several migration-related proteins. Taken together, these findings suggest that the miR-199a-5p–DEPDC1B axis may contribute to the regulation of malignant phenotypes in HCC cells.

Previous studies support a tumor-suppressive role for miR-199a-5p in HCC. Previous studies have demonstrated that miR-199a-5p regulates HCC cell invasion through DDR1. Additionally, the miR-199a/FZD7 axis has been shown to suppress HCC cell proliferation and reduce the expression of downstream molecules, including β-catenin, cyclin D1, and Myc[4]. Furthermore, miR-199a-5p has been reported to cooperate with let-7c to inhibit HCC cell migration and invasion[5]. In addition, miR-199a-5p has been reported

to suppress HCC cell growth through molecules such as CLTC and CDC25A[6]. The lower growth curves and reduced Transwell migration observed in both HCC cell lines following miR-199a-5p overexpression in the present study are broadly consistent with these findings[7,8].

DEPDC1B has increasingly been implicated in tumor-promoting processes. Studies of HCC have associated high DEPDC1B expression with tumor progression and have shown that DEPDC1B knockdown can inhibit cell proliferation, migration, and tumor growth in vivo. Studies in other malignancies also suggest that DEPDC1B can promote migration, invasion, and EMT-like phenotypes and may be linked to Rac1/PAK1 or Wnt/β-catenin signaling. In the present study, DEPDC1B levels decreased after miR-199a-5p overexpression, whereas DEPDC1B re-expression partially restored proliferation and migration phenotypes[9,10,13]. These functional rescue findings support DEPDC1B as one downstream effector that may contribute to the suppression of malignant behavior by miR-199a-5p in HCC.

Wnt/β-catenin signaling has an important role in HCC development and progression. Previous studies have shown that Wnt3/FZD7 can activate β-catenin signaling and promote HCC cell proliferation; DEPDC1B has also been reported to enhance Wnt/β-catenin-related signaling in non-small cell lung and breast cancers[14]. In this study, Wnt3a, cyclin D1, and c-Myc protein levels decreased following miR-199a-5p overexpression, while several proteins showed a trend toward recovery after DEPDC1B re-expression. These observations suggest a possible association between the miR-199a-5p–DEPDC1B axis and Wnt/β-catenin-related signaling. However, changes in β-catenin on the current representative blots were not robust, and functional evidence from nuclear fractionation, TOP/FOP reporter assays, or pathway inhibitors is lacking. The present results therefore do not establish that DEPDC1B mediates all observed phenotypes through the Wnt/β-catenin pathway.

Regarding migration-associated proteins, N-cadherin and vimentin generally decreased following miR-199a-5p overexpression and showed a trend toward recovery after DEPDC1B re-expression, broadly consistent with the Transwell findings. E-cadherin changes were not fully consistent between the two cell lines. A cautious interpretation is that the miR-199a-5p/DEPDC1B axis affects proteins associated with migration and cell state; whether these changes represent a complete, canonical reversal of EMT requires confirmation through replicated quantitative analyses, immunofluorescence, and assessment of additional EMT-related transcription factors[15].

This study has several limitations. First, although miR-199a-5p overexpression reduced DEPDC1B mRNA and protein and DEPDC1B re-expression produced a functional rescue, direct binding of miR-199a-5p to DEPDC1B was not demonstrated using binding-site prediction, wild-type and mutant 3′-UTR dual-luciferase assays, or RNA pull-down. Second, the proliferation phenotype was assessed primarily by CCK-8; complementary EdU or colony-formation assays would strengthen the evidence. Transwell analyses should be supported by raw cell counts across multiple fields and independent experiments. Third, densitometric quantification of Western blots is needed, and the reproducibility of β-catenin and E-cadherin measurements should be improved. Fourth, this study was limited to in vitro cell experiments and lacked validation in patient tissues or public datasets and in vivo models. Addressing these limitations would strengthen the evidence and support consideration of the work by journals with more demanding standards.

5. CONCLUSIONS

miR-199a-5p overexpression downregulated DEPDC1B and suppressed proliferation and migration-associated phenotypes in Hep3B and MHCC-97H cells. Re-expression of DEPDC1B partially reversed these effects. These changes were accompanied by alterations in Wnt/β-catenin-related and migration-related protein profiles. The findings suggest that the miR-199a-5p–DEPDC1B axis may represent a functional node in the regulation of malignant progression in HCC. Direct molecular interaction and a causal role for the Wnt/β-catenin pathway remain to be established in further experiments.

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