

Clinical Value of Serum LHPP-associated miR-765 in the Prognosis of Laparoscopic or Open Hepatectomy for Hepatocellular Carcinoma

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Purpose: The current study aims to investigate the effect of tumor suppressor LHPP-associated microRNA (miR)-765 on the prognosis of laparoscopic hepatectomy (LH) or open hepatectomy (OH) for hepatocellular carcinoma (HCC).

Materials and Methods: A total of 160 patients with HCC were enrolled and randomly divided into the LH or OH group. According to the operation time, these patients were followed up for 12 months, and the number of deaths and the corresponding death time during the follow-up period were counted.

Results: The authors found that the *LHPP* gene levels in HCC tissues were lower than that in adjacent normal tissues, whereas *miR-765* was overexpressed in HCC tissue. Overexpression of *miR-765* promoted the epithelial-mesenchymal transition and proliferation and inhibited apoptosis of HCC through directly downregulating LHPP expression. Serum *miR-765* expression level was significantly associated with lymph node metastasis and histologic grading. Survival analysis showed that the overall survival rate in 12 months after the operation was significantly lower in the OH-high *miR-765* group ($P < 0.05$).

Conclusion: For patients with a low *miR-765* level, both LH and OH are available, otherwise, LH is more recommended.

Key Words: microRNA, hepatocellular carcinoma, laparoscopic hepatectomy, open hepatectomy

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Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world.¹ Every year, ~110,000 people die of HCC in China, accounting for 45% of the world.² HCC stem cells not only have infinite proliferation, self-renewal, tolerance to radiotherapy and chemotherapy, and high tumorigenicity but also have stem cell characteristics.³ The proportion of stem cells is

positively correlated to the malignancy of HCC.³ Early patients with HCC can be cured by surgical resection and percutaneous radiofrequency ablation. The 5-year survival rate can reach 60% to 70%.⁴ However, as most patients are in the advanced stage at the time of diagnosis, even after surgical resection or transplantation, the recurrence rate at 5 years is as high as 70% to 80%, and the 5-year survival rate is only 0% to 14%.⁵

Laparoscopic hepatectomy (LH) was first reported by Watanabe et al in 1997,⁶ and now it is widely used in the clinical treatment of liver diseases.⁷ Although laparoscopic surgery has been proven to have many advantages, such as fewer wound and respiratory complications, LH was still criticized for its demanding technique, lack of tactile sensation, and prolonged operating time.⁸ Open hepatectomy (OH) is the earliest operation for liver diseases, and it may ease mastery of laparoscopic techniques with the advantage of tactile sensation.⁹ However, few studies analyzed the long-term outcomes of LH and OH methods for HCC.⁹ Therefore, finding the specific indications for each method, especially the biomarkers that can be detected by noninvasive methods, will be helpful to guide the choice of surgical methods.

In 2018, researchers discovered a new HCC suppressor protein LHPP and found that LHPP expression in HCC tissues was gradually decreased.¹⁰ However, as the above-mentioned experiments are on the basis of the protein quantification, it is not conducive to the spread of cancer screening. MicroRNAs (miRs) are a class of highly conserved, endogenous noncoding RNA molecules with a length of 18 to 23 nucleotides.¹¹ By complementary pairing with the target messenger RNA base, the target gene is modified and regulated at the transcription level to achieve the purpose of silencing gene expression, and then exert the function of an oncogene or a tumor suppressor gene.¹² In the current study, firstly, we identified *miR-765* that was targeted for downregulating LHPP expression through bioinformatics software. Then, we assessed the expression patterns of *miR-765* in HCC tissue, adjacent non-neoplastic tissue, and serum. In addition, the effects of *miR-765* or LHPP on the epithelial-mesenchymal transition (EMT), proliferation, and apoptosis of HCC were determined. Moreover, we analyzed its 12-month survival prognostic evaluation ability for LH/OH treatment by dividing patients into 4 groups according to the median of *miR-765* levels, thus, providing guidance for the choice of surgical methods for HCC.

MATERIALS AND METHODS

The Number of Patients With HCC Required for the Current Study

The formula for calculating the number of patients with HCC required for the current study is as follows: $n = (\mu_{\alpha}^2/\delta)^2$

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$(1-P)$, where α is set to 0.05, μ_α is set to 1.96, δ is set to 0.05, and the $P_{\text{Sensitivity}}$ is set to 90%. After substituting $\mu_\alpha=1.96$, $\delta=0.10$, and $P_{\text{Sensitivity}}=90\%$ into the formula, the number of cases that can be obtained is 130, that is, at least 130 patients with HCC are required to obtain reliable statistical results in our study.

Ethical Approval Statement

This study was approved by the Ethics Committee of The NanHua Hospital, University of South China, Hengyang, Hunan, China (HYLL-20180418001). The acquisition of specimens and clinical information was subject to oral and written informed consent of all subjects. Written informed consent was provided in accordance with the ethical principles of the Declaration of Helsinki.

Inclusion and Exclusion Criteria

The inclusion criteria for patients with HCC are: (1) patients diagnosed with HCC by histopathology; (2) ability to obtain cancer, adjacent tissues, and serum samples from patients; (3) the patient was diagnosed for the first time with HCC and has not yet received chemoradiotherapy; (4) no history of other malignancies; (5) patients who completed follow-up after surgery.

Patients with any of the following were excluded: (1) incomplete follow-up after surgery; (2) history of other malignancies; (3) at least one of the following specimens was not obtained: HCC tissue, adjacent tissue or serum; (4) patients with other severe underlying diseases; (5) patients or their families are reluctant to participate in this study.

Postoperative Follow-Up of Patients With HCC

According to the operation time, patients with HCC were followed up for 12 months, and the number of deaths and the corresponding death time during the follow-up period were counted.

Tissue and Serum Samples Collection

HCC cancer and adjacent tissues collection: from August 2018 to December 2018, a total of 160 pairs of HCC cancer and adjacent tissues were collected from The NanHua Hospital, University of South China.

Serum specimen collection: peripheral venous blood was collected from the 160 patients with HCC who fasted for > 8 hours on the admission day.

Reagents and Instruments

The HCC cell lines Huh-7, Hep G2, Hep3B, and human normal liver cell line L-02 were purchased from the Cell Bank of the Chinese Academy of Sciences, China. Fetal bovine serum (FBS) (Lot number: GB101-0021) and trypsin-EDTA digestive kit (Lot number: GB1937021) were purchased from Gibco Company, USA. Dulbecco's modified eagle medium (Lot number: 2018071104) was purchased from HyClone, USA. Dimethyl sulfoxide (Lot number: S16-176354) was purchased from Sigma, USA. LipofectamineTM3000 (Lot number: 191117) and RNA extraction reagent Trizol (Lot number: 180703) were purchased from Invitrogen Company, USA. Reverse transcription (Lot number: 20190613) and quantitative real-time polymerized chain reaction (qRT-PCR; Lot number: 20180923) kits were purchased from Dalian Bao Biological Company, China. MiR-765 mimic, small interfering-LHPP (si-LHPP), miR-765 inhibitor, pcDNA-LHPP plasmid, and negative control (NC) plasmid were purchased from Shanghai Jima Company, China. MiR-765, LHPP and internal reference U6,

and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers were purchased from Tiangenhua Technology Co Ltd, China. Rabbit antihuman LHPP (Lot number: P190013), N-cadherin (Lot number: S190112B), E-cadherin (Lot number: S19017), β -catenin (Lot number: S180902), vimentin (Lot number: 180122), Snail (Lot number: 190226), and GAPDH (Lot number: 171130) polyclonal antibodies were purchased from Abcam, USA.

Cell Culture, Transfection, and Grouping

Huh-7, Hep G2, Hep3B, and L-02 cell lines were cultured in a Dulbecco's modified eagle medium containing 10% FBS in a 37°C, 5% CO₂ incubator. According to the growth of the cells, the culture medium was changed and passaged 1 to 2 days. Hep G2 cells were collected in the logarithmic growth phase, and the cell density was adjusted to about 5×10^3 cells per well. When the degree of cell fusion reached about 80%, Lipofectamine3000 and miR-765 mimic, si-LHPP, miR-765 inhibitor, pcDNA-LHPP, and NC plasmid were mixed and incubated at room temperature for 30 minutes. Cells were divided into 6 groups: (1) NC group; (2) miR-765 mimic group (3) si-LHPP group; (4) miR-765 inhibitor group; (5) pcDNA-LHPP group; (6) miR-765 mimic+pcDNA-LHPP group.

Western Blot

Western blot was used to detect the expression of LHPP, N-cadherin, E-cadherin, β -catenin, vimentin, Snail, and GAPDH. Diquinolincarboxylic acid kit (Lot number: 18101606) was used to detect the protein concentration, and 30 g of protein sample was loaded. Rabbit anti-LHPP, N-cadherin, E-cadherin, β -catenin, vimentin, Snail, and GAPDH antibody solutions were incubated overnight at 4°C. Each protein band was exposed in a gel imaging system, and the band gray value was analyzed. The experiment was repeated 3 times.

qRT-PCR

Cells in the logarithmic growth phase were selected, including Huh-7, Hep G2, Hep3B, L-02 cells, and cells of each group after transfection for 48 hours. Total RNA was extracted from the cells according to the instructions of the Trizol kit, and the purity and concentration of the RNA were detected by a Nanodrop 2000 spectrophotometer. After the total RNA was transcribed, qRT-PCR was performed on a quantitative PCR instrument to detect miR-765 and LHPP gene expression. U6 and GAPDH were used as housekeeping genes. The primers sequences were as follows: miR-765 (forward: 5'-CGATCCGC AGGCCATTCCG-3' and reverse: 5'-CACCTCCGCATTCCG CAGA-3'); LHPP (forward: 5'-CCGTCCGATCCGCCAGC AG-3' and reverse: 5'-GGGACCGCCATGCCGACA-3'); U6 (forward: 5'-CGCTTGCATTATATAC-3' and reverse: 5'-AAG GGGCCACTAATCTT-3'); GAPDH (forward: 5'-CGCTCTA TAGCCTATAC-3' and reverse: 5'-AAGGGGCGCAATGGT-3'). Relative RNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method. The experiment was repeated 3 times.

Immunohistochemistry

Tissue paraffin sections were fixed in an oven at 60°C for 2 hours and were dewaxed with xylene for 20 minutes. After the calf serum was blocked for 30 minutes, rabbit antihuman LHPP (1:2000) was added. The secondary antibody was incubated at 37°C for 30 minutes, rinsed with phosphate-buffered saline (PBS), and developed with diaminobenzidine. After 1 min, the development was stopped, counterstained with hematoxylin, dehydrated with ethanol, cleared with xylene, and mounted. All specimens were confirmed by examination by 2 pathologists.

Flow Cytometry for Apoptosis

Each group of cells was digested with trypsin to prepare a single-cell suspension. After mixed with 5 μ L of Annexin V-fluoresceine isothiocyanate (FITC) fluorescent dye, it was incubated for 15 minutes at room temperature. Then, 5 μ L of propidium iodide fluorescent dye was added, and the apoptosis of each group was observed at 488 nm.

Clone Formation Experiment

Six groups of transfected cells were plated in 96-well plates. After the cells adhered, the transfected cells were cultured for 48 hours. After washing gently with PBS two times, it was fixed with 4% paraformaldehyde for 10 minutes, and stained with Giemsa stain for 15 minutes.

Transwell Assay

Transwell inserts with 8- μ m pore size were coated with 50 μ L Matrigel. Cells were digested by trypsin, and a 1×10^5 cell suspension with no FBS was added into the transwell inserts. Then 200 μ L of medium supplemented with 20% FBS was added into the lower chamber as a chemoattractant. The number of cells from 5 random fields was counted under a light microscope using a $\times 20$ objective. Each experiment was performed in triplicate.

Dual-Luciferase Reporter Gene Assay

MiR-765 mimic and control were transferred to the pmiR-RB-ReportTM reporter gene. Wild type (WT)-LHPP 3' UTR-WT luciferase reporters and mutant type (MUT)-LHPP

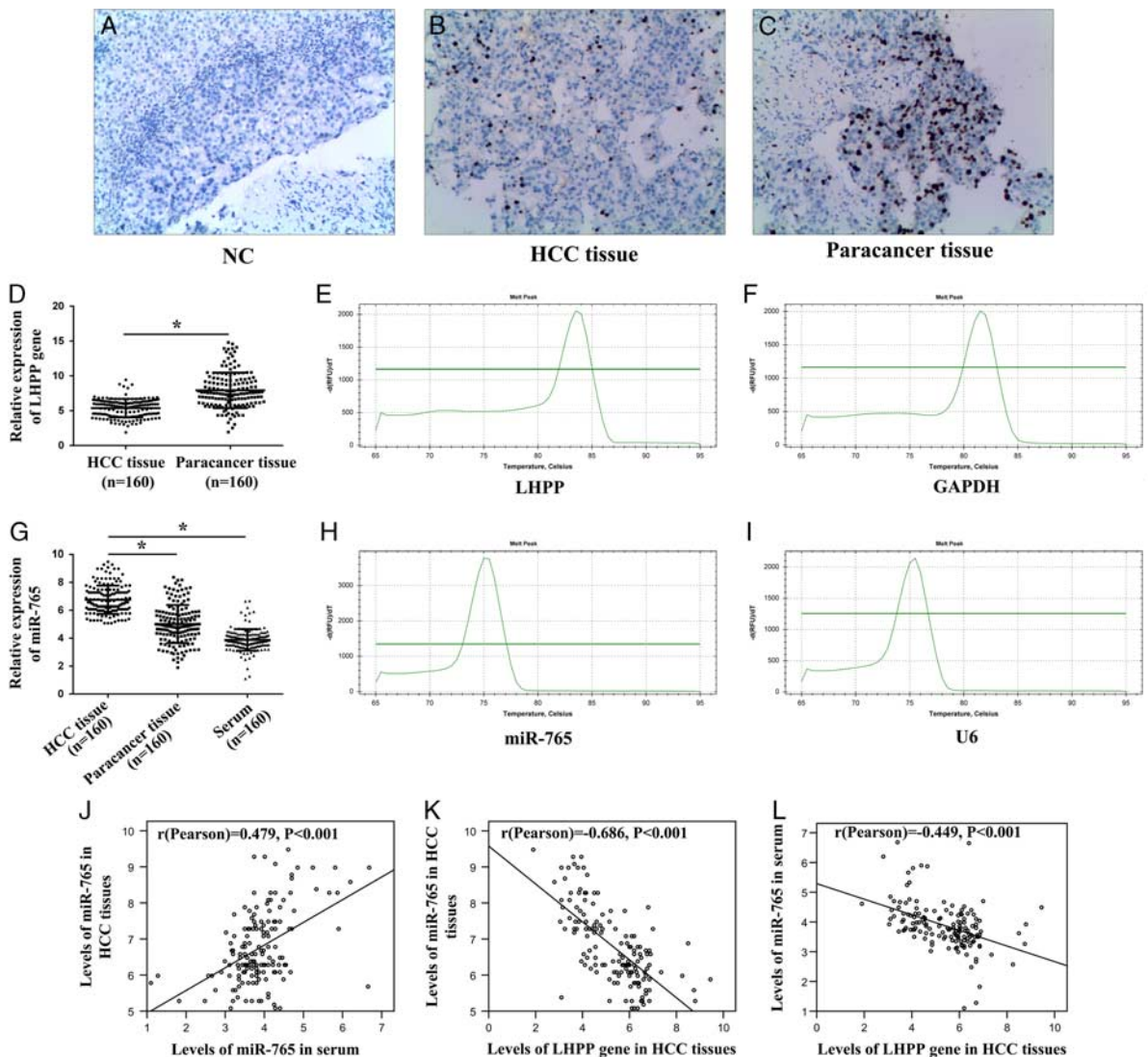


FIGURE 1. LHPP expression in HCC tissue was significantly lower than that in adjacent normal tissue and was negatively correlated to the miR-765 level in HCC tissue and serum. A–C, The results of immunohistochemical staining shown that, compared with adjacent normal tissues, LHPP protein in HCC tissues decreased significantly. D, The *LHPP* gene levels in HCC tissues were lower than that in adjacent normal tissues. E, The melting peak of LHPP. F, The melting peak of GAPDH. G, The levels of miR-765 in tissue and serum of patients with HCC. H, The melting peak of miR-765. I, The melting peak of U6. J, MiR-765 level in HCC serum was significantly correlated to that in HCC tissue. K, MiR-765 level in HCC tissue was significantly negatively correlated to LHPP in HCC tissue. L, MiR-765 level in HCC serum was significantly negatively correlated to LHPP in HCC tissue. * $P < 0.05$. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; HCC, hepatocellular carcinoma; miR, microRNA; NC, negative control. [full color online](#)

3'UTR-MUT luciferase reporters were cotransfected into Hep G2 cells, and luciferase activity was determined 48 hours later.

Statistical Analysis

Statistical analysis was performed using SPSS 20.0. Measurement data are expressed as mean ± standard deviation. Correlation analysis was performed using the Pearson method. Comparison between groups was performed by the *F* test. Survival curve analysis was calculated by the Kaplan-Meier method. *P* < 0.05 was considered statistically significant.

RESULTS

LHPP Expression in HCC Tissue was Significantly Lower Than That in Adjacent Normal Tissue and was Negatively Correlated to miR-765 Level in HCC Tissue and Serum

From August 2018 to December 2018, a total of 160 pairs of HCC cancer and adjacent tissues were collected from The

NanHua Hospital, University of South China. The results of immunohistochemical staining are shown in Figure 1A–C. Compared with adjacent normal tissues (27.6 ± 4.8), LHPP protein in HCC tissues (12.4 ± 3.5) decreased significantly (*P* < 0.05). The immunohistochemical result of using PBS instead of the primary antibody as an NC is shown in Figure 1A. The *LHPP* gene levels in HCC tissues were lower than that in adjacent normal tissues (*P* < 0.05, Fig. 1D). The levels of *miR-765* in tissue and serum of patients with HCC are shown in Figure 1G. The melting peaks of LHPP, *miR-765*, U6, and GAPDH were single, indicating that the primers did not form primer dimers and there was no nonspecific amplification (Figs. 1E, F, H, I). Correlation analysis showed that *miR-765* level in HCC serum was significantly correlated to that in HCC tissue (*r*, 0.479; *P* < 0.001, Fig. 1J), *miR-765* level in HCC tissue was significantly negatively correlated to LHPP in HCC tissue (*r*, -0.686; *P* < 0.001, Fig. 1K), and *miR-765* level in HCC serum was significantly negatively correlated to LHPP in HCC tissue (*r*, -0.449; *P* < 0.001, Fig. 1L).

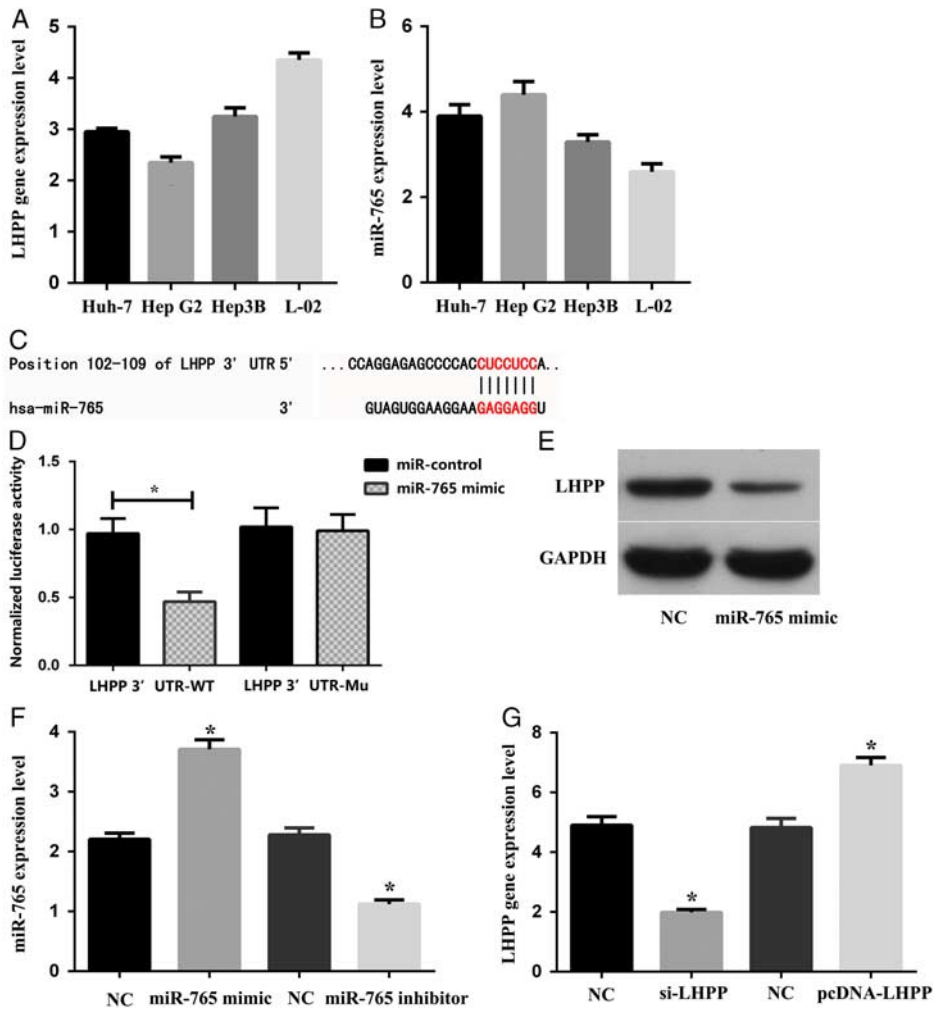


FIGURE 2. LHPP is a direct target of miR-765. A and B, The LHPP level was lowest and the miR-765 level was highest in Hep G2 than that in the other 4 cell lines. C, Schematic representation of LHPP 3'UTRs showing putative miR-765 target site. D, The analysis of the relative luciferase activities of LHPP-WT and LHPP-MUT. E, The protein expressions of LHPP were determined through Western blot assay. F, The level of miR-765 was determined through quantitative polymerase chain reaction assay. G, The level of LHPP was determined through Western blot assay. **P* < 0.05. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; miR, microRNA; MUT, mutant type; NC, negative control; WT, wild type. full color
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MiR-765 Directly Targeted LHPP 3'UTR

We detected the gene expression levels of *miR-765* and LHPP in Huh-7, Hep G2, Hep3B, and L-02 cell lines. Our data found that the *miR-765* level was highest and the LHPP level was lowest in Hep G2 than that in the other 3 cell lines (Figs. 2A, B). Therefore, the Hep G2 cell line was used in the following experiments.

Actually, we found a *miR-765* binding site in the 3' UTR of LHPP by using the TargetScan 7.2 online database (Fig. 2C). Then, we validated LHPP, a critical tumor suppressor gene, which is a direct target of *miR-765* by the luciferase reporter assay. Introduction of *miR-765* significantly suppressed WT LHPP reporter activity but not the activity of the MUT reporter construct in Hep G2 cells, suggesting that *miR-765* could specifically target the LHPP 3'UTR by binding to the seed sequence (Fig. 2D). The upregulation of *miR-765* could obviously reduce LHPP

expression (Fig. 2E). These results suggested that *miR-765* directly targeted LHPP through 3'UTR sequence binding. After transfection with *miR-765* mimic or inhibitor, or pcDNA-LHPP or si-LHPP, the results showed that the *miR-765* or LHPP level was significantly upregulated or down-regulated compared with the NC group (Figs. 2F, G), respectively.

MiR-765 Markedly Promoted the EMT and Proliferation and Inhibited Apoptosis of HCC Cell Through Regulating LHPP Expression

To determine whether *miR-765* regulated the apoptosis, proliferation, and EMT of HCC cell by directly targeting LHPP, Lipofectamine3000, and *miR-765* mimic, si-LHPP, *miR-765* inhibitor, pcDNA-LHPP, and NC plasmid were mixed and incubated at room temperature in Hep G2 cells. The data showed that LHPP overexpression partially reversed the

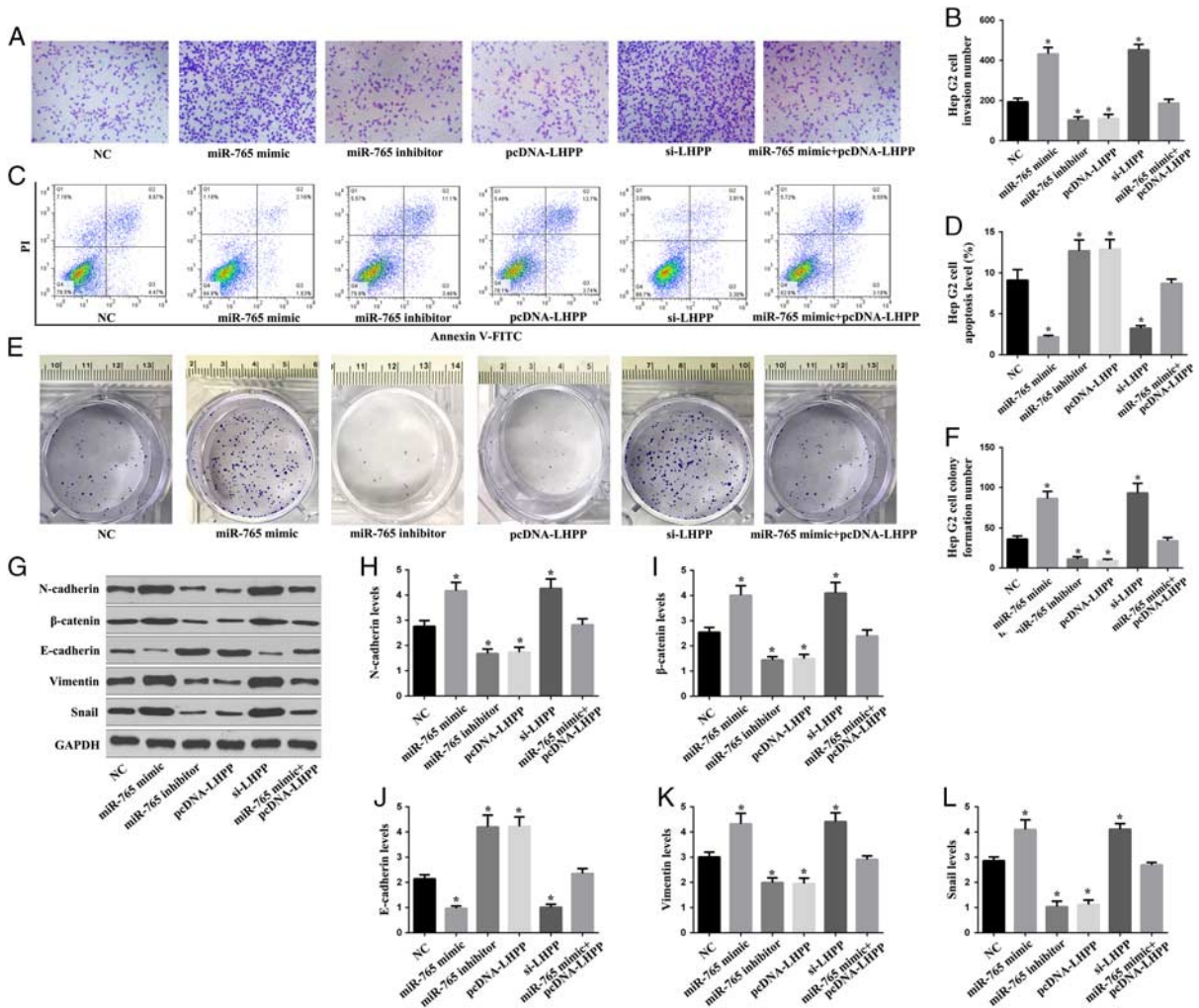


FIGURE 3. MiR-765 markedly promoted the EMT and proliferation and inhibited apoptosis of HCC cells through regulating LHPP expression. A and B, LHPP overexpression partially reversed the invasion of Hep G2 cells promoted by a miR-765 mimic. C and D, Overexpression of LHPP abrogated the inhibited effect of miR-765 mimic on cell apoptosis. E and F, LHPP overexpression partially reversed the proliferation of Hep G2 cells promoted by a miR-765 mimic. G–L, Overexpression of LHPP inhibited miR-765 mimic-induced EMT of Hep G2 cells through increasing E-cadherin expression and decreasing vimentin, β-catenin, N-cadherin, Snail expressions. *P<0.05. EMT indicates epithelial-mesenchymal transition; HCC, hepatocellular carcinoma; miR, microRNA; NC, negative control.

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TABLE 1. Correlations Between Serum miR-765 Expression Level and Clinical Characteristics of Patients With HCC

Clinical Characteristics	n	miR-765 (Mean ± SD)	t	P
Sex			0.463	0.644
Male	107	3.90 ± 0.79		
Female	53	3.84 ± 0.73		
Age (y)			0.585	0.560
≤ 50	38	3.94 ± 0.69		
> 50	122	3.86 ± 0.75		
Body mass index (kg/m ²)			-1.273	0.205
< 24	63	3.79 ± 0.64		
≥ 24	97	3.94 ± 0.78		
Familial history			0.055	0.956
Yes	19	3.89 ± 0.80		
No	141	3.88 ± 0.73		
Smoking			0.516	0.607
Yes	117	3.90 ± 0.75		
No	43	3.83 ± 0.79		
Drinking			0.914	0.362
Yes	122	3.91 ± 0.79		
No	38	3.78 ± 0.68		
T stage			-0.295	0.768
T1-2	124	3.87 ± 0.73		
T3-4	36	3.91 ± 0.66		
N stage			-5.395	< 0.001
N0	101	3.65 ± 0.59		
N1-2	59	4.27 ± 0.86		
Histologic grading			-3.542	< 0.001
Well differentiation	92	3.71 ± 0.68		
Moderate-poor differentiation	68	4.11 ± 0.74		
Tumor size (cm)			-0.521	0.603
< 3	114	3.86 ± 0.78		
≥ 3	46	3.93 ± 0.74		
No. tumors			0.064	0.949
1	132	3.88 ± 0.74		
> 1	28	3.87 ± 0.77		

Bold values indicate significant difference. BMI indicates body mass index; HCC, hepatocellular carcinoma; miR, microRNA; SD, standard deviation.

invasion and proliferation of Hep G2 cells promoted by *miR-765* mimic (Figs. 3A, B, E, and F). Next, the overexpression of LHPP abrogated the inhibited effect of *miR-765* mimic on cell apoptosis (Figs. 3C, D). Moreover, overexpression of LHPP inhibited *miR-765* mimic-induced EMT of Hep G2 cells through increasing E-cadherin expression and decreasing vimentin, β-catenin, N-cadherin, and Snail expressions (Figs. 3G–L). Hence, the effects of *miR-765* mimic were reversed by overexpression of LHPP. Altogether, all the above results suggested that overexpression of *miR-765* promoted the EMT and proliferation, and inhibited apoptosis of Hep G2 cells through directly downregulating LHPP expression.

MiR-765 Expression Level in Serum was Associated With Lymph Node Metastasis and Histologic Grading

We further investigated the relationships between serum *miR-765* expression level and clinical characteristics of patients with HCC, and the results are listed in Table 1. As results show, *miR-765* expression levels had no significant associations with sex, age, body mass index, familial history, smoking, drinking, tumor size, number of tumors, and T stage. However, we found that serum *miR-765* expression level was significantly associated with lymph node metastasis and histologic grading (*P* < 0.05). Patients in the N1-2 stage and patients with moderate-poor differentiated HCC were likely to have higher *miR-765* expression levels in serum.

Serum miR-765 Level Affects the Long-Term Prognosis of Patients With HCC who Underwent OH

To explore the effects of *miR-765* expression level on the efficacy of LH and OH for HCC, 160 patients with HCC were randomly divided into the LH group (n = 80) and the OH group (n = 80). Then patients were further divided into the low *miR-765* group (< 3.96) and the high *miR-765* group (≥ 3.96) according to the median of *miR-765* level in each group. Finally, there were 4 groups—LH-low *miR-765*

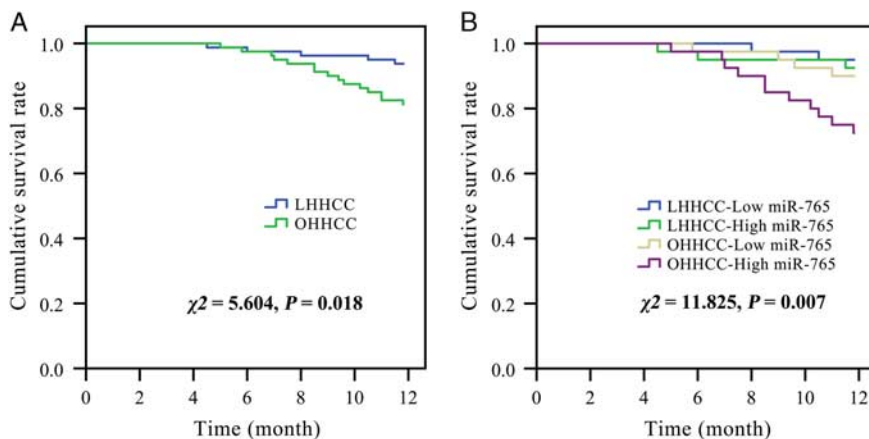


FIGURE 4. Serum miR-765 expression level on the efficacy of LH and OH for HCC. A, Compared with the LH group, patients in the OH group had a significantly lower survival rate. B, The 12-month overall survival rate of patients in the OH-high miR-765 group was the lowest, whereas there was no difference between the other 3 groups. HCC indicates hepatocellular carcinoma; LH, laparoscopic hepatectomy; miR, microRNA; OH, open hepatectomy.

TABLE 2. 12-Month OS and MST of Patients With HCC Receiving LH or OH

Long-term Prognosis	Serum miR-765 Level			
	LH-Low miR-765	LH-High miR-765	OH-Low miR-765	OH-High miR-765
OS (%)	95.00*	92.50*	90.00*	72.50
MST (mo)	11.86 ± 0.67	11.65 ± 1.50	11.69 ± 1.13	11.11 ± 1.80

*Compared with the OH-high miR-765 group, $P < 0.05$.

HCC indicates hepatocellular carcinoma; LH, laparoscopic hepatectomy; miR, microRNA; MST, mean survival time; OH, open hepatectomy; OS, overall survival.

group (n = 40), LH-high miR-765 group (n = 40), OH-low miR-765 group (n = 40), and OH-high miR-765 group (n = 40). Compared with the LH group, patients in the OH group had a significantly lower survival rate (Fig. 4A). The follow-up results showed that among the 4 groups, the 12-month overall survival rate of patients in the OH-high miR-765 group was the lowest, whereas there was no difference between the other 3 groups (Fig. 4B, Table 2). The results of the Cox proportional regression model analysis of risk factors for the death of patients with HCC are shown in Table 3. Sex, age, body mass index, familial history, smoking, drinking, tumor size, number of tumors, and T stage all had no relationship with the poor prognosis of patients in the LH or OH group ($P > 0.05$). N stage, histologic grading, and serum miR-765 level were the risk factors for only patients in the OH group (Table 3).

DISCUSSION

In the present study, we found that the *LHPP* gene levels in HCC tissues were lower than that in adjacent normal tissues, whereas miR-765 was overexpressed in HCC tissue. Correlation analysis showed that miR-765 level in HCC serum was significantly correlated to that in HCC tissue, miR-765 level in HCC tissue was significantly negatively correlated to *LHPP* in HCC tissue, and miR-765 level in HCC serum was significantly negatively correlated to *LHPP* in HCC tissue.

Further cellular mechanism studies showed that overexpression of miR-765 promoted the EMT and proliferation, and inhibited apoptosis of HCC through directly down-regulating *LHPP* expression. Furthermore, we found that serum miR-765 expression level was significantly associated with lymph node metastasis and histologic grading. Patients in the N1-2 stage and patients with moderate-poor differentiated HCC were likely to have higher miR-765 expression levels in serum. Moreover, we found that miR-765 level did affect the long-term prognosis of patients with HCC who underwent OH but had no influence on the long-term prognosis of patients with HCC who underwent LH. For patients with serum miR-765 level lower than 3.96, both LH and OH are available, otherwise, LH is more applicable.

Before our study, several pieces of research had found the effect of miR-765 on cancer metastasis and tumor cell apoptosis and proliferation.¹³⁻¹⁶ MiR-765 was found to be overexpressed in various malignancies such as esophageal squamous cell carcinoma,¹³ HCC,¹⁴ pancreatic cancer,¹⁵ and gastric cancer.¹⁶ However, with respect to HCC, few studies have yet investigated the relationship between miR-765 expression level and the long-term prognosis of patients. In our study, we primarily explored the expression pattern of miR-765 in patients with HCC and found that miR-765 was overexpressed in HCC tissue, which was in line with the study by Xie et al.¹⁴ What is more, for the first time we found that there is a significant positive correlation between miR-765 levels in HCC tissue and serum, which means detecting miR-765 by a noninvasive approach is possible.

To explore the effect of miR-765 on the long-term prognosis of patients with HCC received LH or OH, we first compared the postoperative and oncologic outcomes. The results showed that patients who underwent OH were more likely to have a poor prognosis when they have high levels of miR-765, whereas patients with low miR-765 level had a similar prognosis with patients who underwent LH. In other words, the miR-765 level did not affect the long-term prognosis of LH but did have a significant effect on the long-term prognosis of OH. Considering that miR-765 is correlated to lymph node metastasis and histologic grading, we hypothesized that the above difference was because of the fact that LH can dissect metastatic lymph nodes more

TABLE 3. Cox Proportional Regression Model Analysis of Risk Factors for Death in Patients With HCC

Index	LH (n = 80)						OH (n = 80)					
	β	SE	Wald χ^2	P	HR	95% CI	β	SE	Wald χ^2	P	HR	95% CI
Male	0.109	0.074	2.233	0.139	1.116	0.971-1.282	0.079	0.049	2.782	0.100	1.082	0.991-1.181
Age	0.236	0.127	3.534	0.064	1.266	0.994-1.613	0.116	0.125	1.389	0.698	1.128	0.950-1.385
miR-765	0.079	0.106	0.550	0.461	1.082	0.883-1.326	0.180	0.066	7.737	0.006	1.197	1.056-1.357
Body mass index	0.084	0.167	0.251	0.618	1.088	0.786-1.505	0.116	0.119	1.376	0.681	1.127	0.956-1.380
Familial history	0.231	0.141	2.766	0.102	1.259	0.965-1.642	0.108	0.158	0.443	0.516	1.132	0.834-1.493
Smoking	0.024	0.088	0.054	0.832	1.024	0.873-1.202	0.083	0.112	0.554	0.465	1.086	0.887-1.330
Drinking	0.120	0.129	1.395	0.703	1.138	0.955-1.390	0.093	0.119	0.595	0.452	1.097	0.884-1.361
T stage	0.094	0.120	0.597	0.423	1.095	0.888-1.342	0.237	0.426	0.394	0.590	1.276	0.585-2.709
N stage	0.116	0.148	0.620	0.424	1.124	0.831-1.518	0.389	0.029	20.520	<0.001	1.474	1.249-1.740
Histologic grading	0.227	0.404	0.316	0.572	1.255	0.567-2.777	0.211	0.102	4.466	0.037	1.234	1.019-1.512
Tumor size	0.082	0.165	0.249	0.616	1.086	0.783-1.502	0.171	0.174	0.964	0.314	1.185	0.838-1.675
No. tumors	0.223	0.133	2.758	0.094	1.250	0.956-1.633	0.104	0.133	0.617	0.427	1.110	0.848-1.453

Bold values indicate significant differences.

CI indicates confidence interval; HR, hazard ratio; LH, laparoscopic hepatectomy; miR, microRNA; OH, open hepatectomy; SE, standard error; β , regression coefficients.

widely, this was also in line with the results of Cox proportional regression model analysis that lymph node metastasis and histologic grading were not the risk factors of death in patients with HCC underwent LH, but both were the risk factors of death in patients with HCC who underwent OH.

In conclusion, this is the first study demonstrating the value of *miR-765* to predict long-term outcomes of patients with HCC who underwent LH or OH, which may provide us a noninvasive approach to guide the selection of surgical methods for HCC treatment.

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