Exosomal IncRNA H19 promotes the progression of hepatocellular carcinoma treated with Propofol via miR-520a-3p/LIMK1 axis

Dongmei Wang | Na Xing | Tao Yang | Junqi Liu | Huaping Zhao | Juan He | Yanqiu Ai | Jianjun Yang

1Department of Anesthesiology, Pain and Perioperative Medicine, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
2Department of Anesthesiology, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, China
3Department of Radiotherapy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

Correspondence
Jianjun Yang, Department of Anesthesiology, Pain and Perioperative Medicine, The First Affiliated Hospital of Zhengzhou University, No.1, Jianshe East Road, Henan, Zhengzhou 450052, China. Email: agllf@163.com

Funding information
No funding was received.

Abstract
Background: Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths globally. Herein, we explored the underlying mechanism by which Propofol inhibited the development of HCC.

Methods: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to detect the viability and proliferation. Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were performed to detect the expression of long noncoding RNA (lncRNA) H19, microRNA-520a-3p (miR-520a-3p), LIM domain kinase 1 (LIMK1), metastasis-associated markers (Snail, Twist, Vimentin and E-cadherin) and exosome markers (CD9 and CD81). Transmission electron microscopy (TEM) was used to observe the morphology and structure of exosomes. The apoptosis and metastasis were measured by flow cytometry and transwell assays. StarBase software was utilized to predict the targets of H19 and miR-520a-3p. Dual-luciferase reporter assay was performed to confirm the interaction between miR-520a-3p and H19 or LIMK1. Dual-luciferase reporter assay was performed to confirm the interaction between miR-520a-3p and H19 or LIMK1. Nude mice bearing tumors were used to validate the role of exosomal H19.

RESULTS: The high expression of exosomal H19 accelerated the proliferation and motility while hampering the apoptosis of HCC cells. MiR-520a-3p could bind with H19. Exosomal H19 exacerbated HCC through sponging miR-520a-3p. The 3’ untranslated region (3’UTR) of LIMK1 could bind to miR-520a-3p. MiR-520a-3p mimic transfection reversed the inhibitory effect of high expression of exosomal LIMK1 on the apoptosis of HCC cells and the promoting effects on the proliferation and metastasis of HCC cells. The mRNA and protein levels of LIMK1 were regulated by H19/miR-520a-3p signaling. The high level of exosomal H19 promoted the growth of HCC tumors in vivo.

Conclusion: Circulating H19 promoted the proliferation, migration and invasion and inhibited the apoptosis of HCC cells treated with Propofol through upregulating LIMK1 via sponging miR-520a-3p.

KEYWORDS
exosome, H19, hepatocellular carcinoma, LIMK1, miR-520a-3p
1 | INTRODUCTION

The prognosis of HCC patients remains dismal due to the metastasis and recurrence. Propofol is a common intravenous anesthetic agent. The anti-tumor role of Propofol has been reported in former articles. Zhang et al. claimed that Propofol inhibited the development of HCC. Ou et al. proved that Propofol blocked the proliferation and motility of HCC cells via HMGA2 and Wnt/β-catenin signaling. Nevertheless, the potential molecular mechanism by which Propofol exerts its anti-tumor role in HCC remains to be elucidated.

Exosomes are a group of small membrane vesicles containing proteins, DNA and RNA, and they are derived from multivesicular bodies (MVBs) and eventually release to the extracellular environment. Exosomes have been reported to serve as important modulators in the tumor microenvironment to regulate the development of multiple tumors. Long noncoding RNAs (lncRNAs) could act as microRNAs (miRNAs) sponges to function. The oncogenic role of lncRNA H19 has been reported in multiple cancers. For instance, Lv et al. proved that lncRNA H19 accelerated the metastasis of bladder cancer via miR-29b-3p. Yang et al. demonstrated that H19 facilitated the motility of colon cancer cells through sponging miR-138 and upregulating HMG1. Bai et al. reported that Propofol inhibited the motility of breast cancer through declining the expression of H19. The aim of our study was to explore the working mechanism of lncRNA H19 in the progression of HCC.

miRNAs are another group of non-coding RNAs. MiR-520a-3p was a tumor suppressor in a variety of cancers. Li et al. proved that miR-520a-3p blocked the development of breast cancer via CCND1 and CD44. Qu et al. reported that lidocaine could restrain the growth and promote the apoptosis of colorectal cancer cells through elevating the level of miR-520a-3p. Wang et al. proved that dexametomidine hampered the proliferation and motility while accelerated the apoptosis of osteosarcoma cells through upregulating miR-520a-3p. In this study, we intended to uncover the regulatory mechanism between Propofol and miR-520a-3p in HCC cells.

LIM domain kinase 1 (LIMK1) is a member of LIM kinases. LIM kinases could regulate cell motility through modulating actin dynamics. Su et al. reported that down-regulation of LIMK1 suppressed the metastasis of colon cancer. Li et al. demonstrated that miR-200b-3p and miR-429-5p could block the growth and metastasis of breast cancer cells via downregulating LIMK1/CFL1 signaling. Nevertheless, the mechanism of LIMK1 in HCC remains to be revealed.

We found that Propofol-treated Huh7 cells could down-regulate the level of H19 in highly metastatic MHCC97-H and HCCLM3 cells and inhibit the metastasis of the two cells. The underlying mechanism was then explored.

2 | MATERIALS AND METHODS

2.1 | Cell culture and Propofol treatment

Three HCC cell lines, including conventional hepatocellular carcinoma cell line (Huh7) and two highly metastatic HCC cell lines (MHCC97-H and HCCLM3) were acquired from BeNa Culture Collection (Beijing, China). The three cells were grown in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Carlsbad, CA, USA) added with 10% fetal bovine serum (FBS; Gibco), 100 units/mL penicillin/100 μg/mL streptomycin in a 37°C humidified atmosphere with 5% CO2. HCC cells were treated with increased concentrations (0 μmol/L, 5 μmol/L, 25 μmol/L, or 50 μmol/L) of Propofol (Sigma, St. Louis, MO, USA) for 24 hours.

2.1.1 | (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The viability and proliferation of HCC cells were analyzed by MTT assay. Huh7 cells were treated with various concentrations (0 μmol/L, 5 μmol/L, 25 μmol/L, or 50 μmol/L) of Propofol, and MTT reagent (Invitrogen, Carlsbad, CA, USA) was used to analyze the viability of Huh7 cells. MHCC97-H and HCCLM3 cells after exosome treatment and transfection for 0 hours, 24 hours, 48 hours, or 72 hours were mixed with 10 µL MTT reagent (Invitrogen), followed by the detection of the absorbance at 490 nm on a microplate reader (Bio-Rad, Hercules, CA, USA).

2.2 | Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA sample was isolated using RNAiso (Takara, Otsu, Japan). SYBR-Green qPCR Mix (Bio-Rad) was used to amplify template DNA. The abundance of H19, miR-520a-3p and LIMK1 was analyzed using the formula of $2^{-\Delta\Delta Ct}$ and normalized to U6.
small nuclear RNA or glyceraldehyde-3-phosphate dehydroge-
nase (GAPDH). Relevant primers were listed as follows: H19,
Forward (F), 5'-GATGACAGGTGTGGTCAACG-3', Reverse
(R), 5'-CAGACATGAGCTGGGTAGCA-3'; miR-520a-3p,
F, 5'-ACACTCCAGCTGGGAAAGTGCTTCCC-3', R, 5'-
CTCAACTGGTGTCGTGGA-3'; LIMK1, F, 5'-ATGAGGGT
GACGCTACTTTGTTG-3', R, 5'-CTACACTCGCAGCA
CCTGAA-3'; U6, F, 5'-CTCGCTTCGGCAGCACA-3', R,
5'-AACGCTTCACGAATTTGCGT-3'; GAPDH, F, 5'-TGG
GTGTGAACCACGAGAA-3', R, 5'-GGCATGGACTGTG
GTCATGA-3'.

2.3 | Western blot assay

HCC cell lysate was centrifuged, and the protein supernatant was
quantified using BCA Protein Assay kit (Thermo Fisher Scientific,
Waltham, MA, USA). Equal amounts of protein samples (35 μg
for protein detection of cell lysate; 20 μg for the detection of exo-
some markers) were separated using sodium dodecyl sulfate po-
lyacrylamide gel electrophoresis (SDS-PAGE) and transferred
onto polyvinylidene difluoride (PVDF) membranes (Millipore,
Billerica, MA, USA). The membranes were incubated with 5%
nonfat milk to block the nonspecific sites, followed by incuba-
tion with the following primary antibodies at 4°C overnight.
Antibodies against Snail (ab229701; 1:500), Twist (ab175430;
1:1000), Vimentin (ab137321; 1:2000), E-cadherin (ab40772;
1:40000), CD9 (ab195422; 1:1000), CD81 (ab109201; 1:5000),
LIMK1 (ab81046; 1:1000), and GAPDH (ab181602; 1:10000)
were purchased from Abcam (Cambridge, MA, USA). After
washing, the secondary antibody (ab150077, Abcam) was incu-
bated with the membranes. The protein signal was visualized by
the enhanced chemiluminescence (ECL) system (Millipore).

2.4 | Exosome isolation and identification

The culture medium of Huh7 cells was centrifuged at 3000 g
for 15 min, and a 0.22 mm filter (Millipore) was used to fil-
ter the supernatant. The culture medium was then mixed with
Exoquick exosome precipitation solution (System Biosciences,
Beijing, China) for 24 hours. After centrifuging at 1500g for
30 minutes, the supernatant was discarded. Exosomes were
suspended in phosphate buffered saline (PBS) solution for the
following experiments. Exosomes were identified by trans-
mission electron microscopy (TEM) and Western blot assay.

2.5 | Flow cytometry

Annexin-V-fluorescein isothiocyanate (FITC) Apoptosis
Detection Kit (R&D Systems, Abingdon, UK) was used in
this study to detect the apoptosis of HCC cells. HCC cells
were suspended in binding buffer followed by dyeing with
propidium iodide (PI) and Annexin-V-FITC. After washing
using PBS, the samples were analyzed through a flow cytome-
ceter (BD Biosciences, San Jose, CA, USA).

2.6 | Transwell assays

Transwell assays were conducted to detect the metastasis of
HCC cells. For the detection of cell invasion, the porous
membrane was coated with Matrigel (BD Biosciences).
HCC cells suspended in 200 μL serum-free medium were
seeded into the precoated upper chambers. The low cham-
bers were filled with 500 μL culture medium supplemented
with 10% FBS. The invaded HCC cells were dyed using
crystal violet after 24 hours cultivation. The number of in-
vaded HCC cells was counted under a microscope. For the
detection of cell migration, the HCC cells were seeded into
the noncoated upper chambers, and the other methods were
same as above.

2.7 | Cell transfection

Transfection was conducted with lipofectamine 3000
(Invitrogen). H19 ectopic expression plasmid (Over H19),
LIMK1 ectopic expression plasmid (Over LIMK1), empty
vector (Vector), miR-520a-3p mimic and its control
(mimic NC) were obtained from Genepharma (Shanghai,
China).

2.8 | Dual-luciferase reporter assay

The target relationship between miR-520a-3p and H19 or
LIMK1 was validated by dual-luciferase reporter assay. The
sequences of H19, including the mutant or wild-type bind-
ing sites of miR-520a-3p, were cloned into pSI-check2 vec-
tor (Promega, Madison, WI, USA), termed as H19-WT or
H19-MUT. 293T cells were co-transfected with mimic NC
and H19-WT or H19-MUT. Dual-luciferase reporter assay system (Promega) was utilized to
measure the luciferase activity after transfection for 48 hours.

The binding relationship between miR-520a-3p and
LIMK1 was also confirmed using the same experimental
procedure.

2.9 | Animal experiment

This study was performed with the agreement of the Animal
Research Committee of The First Affiliated Hospital of
Zhengzhou University. The right flank of BALB/c nude mice
(Orient Bio Inc, Seongnam, South Korea; n = 12) was injected with $1 \times 10^6$ Huh7 cells. The mice were randomly divided into two groups (n = 6 in each group) when the tumor volume reached about 100 mm$^3$ (Day 10). The two groups were injected with exosomes (30 μg) derived from Huh7 cells treated with Propofol and Vector or Over H19, respectively. Meanwhile, Propofol (35 mg/kg) was intraperitoneally injected to the mice every 2 d. The size of tumors was assessed every 4 d with the formula of volume = $\pi/6 \times \text{length} \times \text{width} \times \text{height}$. The weight of tumors was recorded after inoculation for 34 d. qRT-PCR and Western blot were performed to detect the expression of H19, miR-520a-3p, LIMK1, and metastasis-associated proteins in resected tumor tissues.

2.10 | Statistical analysis

Statistical analysis of the data from three independent experiments was conducted using GraphPad Prism 7. All data were displayed as mean ± standard deviation (SD). P value was analyzed using Student’s t-test or one-way analysis of variance (ANOVA) followed by Tukey’s test. P < 0.05 was indicated as statistically significant.

3 | RESULTS

3.1 | The expression of H19 is downregulated in HCC cells co-cultured with Huh7 cells treated with Propofol

The effect of Propofol on the viability of Huh7 cells was explored by MTT assay. As mentioned in Figure 1A, the viability was markedly decreased in Huh7 cells treated with 25 μmol/L or 50 μmol/L Propofol, while there was no significant change in the viability of Huh7 cells treated with 5 μmol/L Propofol compared with nontreated Huh7 cells. We chose two highly metastatic HCC cell lines (MHCC97-H and HCCLM3 cells) to investigate the influence of Propofol. As indicated in Figure 1B, the expression of H19 was lower in MHCC97-H and HCCLM3 cells co-cultured with Huh7 cells treated with Propofol than that in HCC cells co-cultured with nontreated Huh7 cells. Besides, the cocultivation with Propofol-Huh7 cells reduced the expression of Vimentin, Twist, and Snail in MHCC97-H and HCCLM3 cells, while the level of E-cadherin was up-regulated (Figure 1C). Taken together, Propofol-treated Huh-7 decreased the abundance of H19 in MHCC97-H and HCCLM3 cells.

**FIGURE 1** The expression of H19 is downregulated in HCC cells co-cultured with Huh7 cells treated with Propofol. (A) The viability of Huh7 cells treated with diverse concentrations of Propofol (0 μmol/L, 5 μmol/L, 25 μmol/L, or 50 μmol/L) was detected by MTT assay. (B and C) MHCC97-H and HCCLM3 cells were co-cultured with Huh7 cells treated with Propofol (25 μmol/L) or not. (B) The expression of H19 was measured in MHCC97-H and HCCLM3 cells by qRT-PCR. (C) The abundance of metastasis-associated proteins (Snail, Twist, Vimentin, and E-cadherin) was determined in the two HCC cells by Western blot assay. *P < 0.05
FIGURE 2  Propofol treatment reduces the expression of H19 in the exosomes of Huh7 cells. (A) The exosomes from Huh7 cells treated with Propofol (25 μmol/L) or not were isolated, and then the exosomes were photographed using TEM. (B) The markers of exosomes, including CD9 and CD81, were detected by Western blot assay. (C) The level of H19 was examined in exosomes generated from Huh7 cells treated with Propofol (25 μmol/L) or not by qRT-PCR. *P < .05

FIGURE 3  Exosomes from Huh7 cells treated with Propofol inhibits the proliferation, metastasis, and promotes the apoptosis of HCC cells. MHCC97-H and HCCLM3 cells were incubated with exosomes from Huh7 cells exposed to Propofol (25 μmol/L) or not. (A) MTT assay was performed to examine the proliferation of HCC cells. (B) Flow cytometry was carried out to detect the apoptosis of HCC cells. (C and D) Transwell assays were conducted to detect the migration and invasion of HCC cells. (E) Western blot was conducted to detect the expression of Snail, Twist, Vimentin, and E-cadherin in HCC cells. *P < .05
HCCLM3 cells, and the metastasis of the two HCC cells was also inhibited.

3.2 Propofol treatment reduces the expression of H19 in the exosomes of Huh7 cells

To explore whether exosomes were involved in the modulation of Huh7 cells on MHCC97-H and HCCLM3 cells, we extracted exosomes from the culture medium of Huh7 and Propofol-treated Huh7 cells. The exosomes were characterized by TEM and Western blot assay. The lipid bilayer membrane structure of exosomes from Huh7 and Propofol-treated Huh7 cells was shown in Figure 2A. Western blot results showed that two exosome markers (CD9 and CD81) were detectable in exosomes from Huh7 and Propofol-treated Huh7 cells instead of cell lysate (Figure 2B). There was a notable decrease in the expression of H19 in Propofol-Huh7-exo in comparison with that in Huh7-exo (Figure 2C). These findings implied that exosomal H19 might exert a crucial role in the modulation of MHCC97-H and HCCLM3 cells.

3.3 Exosomes from Huh7 cells treated with Propofol inhibit the proliferation, metastasis and promotes the apoptosis of HCC cells

MHCC97-H and HCCLM3 cells were incubated with Huh7-exo or Propofol-Huh7-exo. As mentioned in Figure 3A, the proliferation of MHCC97-H and HCCLM3 cells was conspicuously restrained in Propofol-Huh7-exo group than that in Huh7-exo group. Moreover, the addition of exosomes generated...
from Propofol-treated Huh7 cells significantly promoted the apoptosis of MHCC97-H and HCCLM3 cells (Figure 3B). The migration and invasion of MHCC97-H and HCCLM3 cells were blocked in Propofol-Huh7-exo group compared with Huh7-exo group (Figure 3C and D). We further examined the protein levels of Snail, Twist, Vimentin, and E-cadherin to confirm the effect of Propofol-Huh7-exo on the metastasis of MHCC97-H and HCCLM3 cells. The enrichment of Snail, Twist, Vimentin, and E-cadherin was significantly decreased in the Propofol-Huh7-exo group compared with the Huh7-exo group. These results suggest that Propofol-Huh7-exo can inhibit the metastasis of HCC cells by downregulating the expression of metastasis-related proteins.

**FIGURE 6** Exosomal H19 from Huh7 cells promotes the proliferation and metastasis and impedes the apoptosis of HCC cells via sponging miR-520a-3p. MHCC97-H and HCCLM3 cells were treated with Huh7-exo, Propofol-Huh7-exo, Vector-Propofol-Huh7-exo, Over H19-Propofol-Huh7-exo, Over H19-Propofol-Huh7-exo + mimic NC or Over H19-Propofol-Huh7-exo + miR-520a-3p mimic. (A) The proliferation of HCC cells was determined through MTT assay. (B) The apoptosis of HCC cells was analyzed by flow cytometry. (C and D) The abilities of migration and invasion of HCC cells were measured through conducting transwell assays. (E) Western blot assay was applied to detect the abundance of metastasis-related proteins in HCC cells. *P < .05.
Twist, and Vimentin was declined while E-cadherin was up-regulated in HCC cells incubated with exosomes derived from Propofol-exposed Huh7 cells (Figure 3E). In summary, Huh7 cells treated with Propofol could inhibit the malignant potential of highly metastatic HCC cells via exosomes.

3.4 | Exosomal H19 from Huh7 cells enhances the malignant potential of HCC cells

We wondered whether H19 in exosomes exerted a pivotal role in the regulation of the behaviors of HCC cells, and rescue experiments were conducted. As indicated in Figure 4A and B, the exosomes from Huh7 cells treated with H19 over-expression plasmid and Propofol could attenuate the inhibitory effect of Propofol-Huh7-exo on the proliferation and the promoting impact on the apoptosis of HCC cells. Also, the migration and invasion of HCC cells were recovered in Over H19-Propofol-Huh7-exo group (Figure 4C and D). The changes in the expression of metastasis-related proteins also suggested that the inhibitory effect of Propofol-Huh7-exo addition on the metastasis of HCC cells was alleviated by the addition of Over H19-Propofol-Huh7-exo (Figure 4E). These findings demonstrated that the depletion of exosomal H19 caused by Propofol treatment in Huh7 cells inhibited the malignant potential of HCC cells.
3.5 | MiR-520a-3p is a target of H19

The expression of miR-520a-3p was markedly enhanced in exosomes generated from Propofol-treated Huh7 cells other than nontreated Huh7 cells (Figure 5A). Furthermore, miR-520a-3p was a candidate target gene of H19 predicted by StarBase software (Figure 5B), and the cotransfection of miR-520a-3p mimic and H19-WT reduced the luciferase activity in 293T cells compared with that in mimic NC and H19-WT group. In summary, miR-520a-3p could directly bind to H19.

3.6 | Exosomal H19 from Huh7 cells promotes the proliferation and metastasis and impedes the apoptosis of HCC cells via sponging miR-520a-3p

MHCC97-H and HCCLM3 cells were treated with Huh7-exo, Propofol-Huh7-exo, Vector-Propofol-Huh7-exo, Over LIMK1-Propofol-Huh7-exo, Over LIMK1-Propofol-Huh7-exo + mimic NC or Over LIMK1-Propofol-Huh7-exo + miR-520a-3p mimic. (A) MTT assay was conducted to detect the proliferation of HCC cells. (B) Flow cytometry was used to detect the apoptosis rate of HCC cells. (C and D) Transwell assays were performed to examine the metastasis of HCC cells. (E) The enrichment of metastasis-related proteins in HCC cells was examined by Western blot assay. *P < .05

3.7 | LIMK1 could bind to miR-520a-3p

Propofol treatment also declined the mRNA and protein expression of LIMK1 in Huh7 exosomes (Figure 7A and
FIGURE 9 Exosomal H19 upregulates the expression of LIMK1 in HCC cells through suppressing miR-520a-3p.
MHCC97-H and HCCLM3 cells were treated with Vector-Propofol-Huh7-exo, Over H19-Propofol-Huh7-exo, Over H19-Propofol-Huh7-exo + mimic NC or Over H19-Propofol-Huh7-exo + miR-520a-3p mimic. (A and B) qRT-PCR and Western blot assay were utilized to detect the levels of LIMK1 mRNA and protein in HCC cells. *P < .05

B). StarBase database predicted that there were binding sites between the 3’ untranslated region (3’UTR) of LIMK1 and miR-520a-3p (Figure 7C), and a notable decrease of the luciferase activity was observed in miR-520a-3p and LIMK1 3’UTR-WT cotransfected group in contrast to that in mimic NC and LIMK1 3’UTR-WT group. Besides, the luciferase activity was unaffected in LIMK1 3’UTR-MUT and mimic NC or miR-520a-3p group. Taken together, miR-520a-3p could bind to the 3’UTR of LIMK1.

3.8 | The accumulation of miR-520a-3p reverses the effects of exosomal LIMK1 on HCC cells

The high expression of LIMK1 in exosomes facilitated the proliferation and restrained the apoptosis of HCC cells, and the co-treatment with miR-520a-3p mimic counteracted the effects caused by the addition of Over LIMK1-Propofol-Huh7-exo (Figure 8A and B). The motility of HCC cells was promoted with the addition of Over LIMK1-Propofol-Huh7-exo, and the addition of miR-520a-3p hampered the motility of HCC cells again (Figure 8C-E). These results suggested that miR-520a-3p inhibited the development of HCC through LIMK1.

3.9 | Exosomal H19 upregulates the expression of LIMK1 in HCC cells through suppressing miR-520a-3p

To further elucidate the regulatory relationship among H19, miR-520a-3p and LIMK1, we treated HCC cells with Over H19-Propofol-Huh7-exo + miR-520a-3p mimic. The addition of Over H19-Propofol-Huh7-exo elevated the mRNA and protein expression of LIMK1 in HCC cells, and the abundance of LIMK1 mRNA and protein was repressed with the transfection of miR-520a-3p mimic (Figure 9A and B). In summary, LIMK1 was modulated by H19/miR-520a-3p axis in HCC cells.

3.10 | Exosomal H19 promotes the growth of HCC tumors treated with Propofol

The volume and weight of HCC tumors were higher in Over H19-Propofol-Huh7-exo + Propofol group than that in Vector-Propofol-Huh7-exo + Propofol group (Figure 10A and B). To verify whether these effects were due to the abnormal expression of H19, miR-520a-3p and LIMK1, qRT-PCR and Western blot were performed. As mentioned in Figure 10C and D, H19 and the mRNA and protein expression of LIMK1 were upregulated with the addition of Over H19-Propofol-Huh7-exo and Propofol, while the change in the
expression of miR-520a-3p revealed a reverse phenomenon. Next, we found the expression of Snail, Twist and Vimentin was enhanced in Over H19-Propofol-Huh7-exo and Propofol group, and a decrease in the expression of E-cadherin was found in this group (Figure 10E). Taken together, high expression of exosomal H19 promotes the growth and metastasis of HCC tumors treated with Propofol.

4 | DISCUSSION

Propofol has been reported to inhibit the progression of HCC.23-25 Besides, Bai et al. reported that Propofol treatment hampered the metastasis of breast cancer cells through reducing the enrichment of H19.16 The expression of IncRNA H19 was declined in MHCC97-H and HCCLM3 cells co-culturing with Propofol-treated Huh7 cells. Meanwhile, the metastasis of MHCC97-H and HCCLM3 cells was blocked. We wondered if exosomes functioned in the above biological possesses.

Exosomes could be released under physiological and pathological conditions. The communication between tumor cells or stromal cells and tumor cells could be achieved through the transferring of exosomes.26-28 The small molecules wrapped in exosomes, including lncRNAs and miRNAs, could alter the expression of their target genes in recipient cells, thus modulating the behaviors of recipient cells.29 The exosomes generated from untreated Huh7 cells or Propofol-exposed Huh7 cells were isolated, and we found that the expression of H19 was lower in the exosomes of Huh7 cells treated with Propofol. To confirm the role of exosomes on the behaviors of MHCC97-H and HCCLM3 cells, we conducted the following experiments. The results revealed that Propofol-Huh7-exo inhibited the proliferation, migration, and invasion while promoted the apoptosis of HCC cells.
We wondered whether lncRNA H19 in exosomes regulated the biological functions of HCC cells, and rescue experiments were performed to verify our hypothesis. The low expression of lncRNA H19 in Propofol-Huh7-exo group inhibited the malignant potential of HCC cells, while the high abundance of H19 in Over H19-Propofol-Huh7-exo group recovered the malignant potential of HCC cells.

StarBase online software was used to search the downstream targets of lncRNA H19, miR-520a-3p was identified as a direct target of lncRNA H19. Yu et al. reported that miR-520a-3p suppressed the progression of nonsmall cell lung cancer. Bi et al. proved that miR-520a-3p blocked the metastasis of papillary thyroid carcinoma cells via JAK/STAT pathway. The relationship between miR-520a-3p and Propofol or lncRNA H19 in HCC has not been reported. We found that the overexpression of miR-520a-3p in MHCC97-H and HCCLM3 cells reversed the effects of the addition of Over H19-Propofol-Huh7-exo, and these findings demonstrated that exosomal H19 exerted its oncogenic role in HCC cells through sponging miR-520a-3p.

LIMK family was important modulator of the actin cytoskeleton, and the abnormal expression of LIMK family was related to the progression of metastatic diseases. LIMK1 was confirmed as a target of miR-520a-3p. Yu et al. demonstrated that sevoflurane treatment mediated learning and memory impairments of rats through upregulating miR-27b and downregulating LIMK1. In the current study, we found that Propofol stimulation decreased the expression of LIMK1 in exosomes. Mechanistically speaking, miR-520a-3p inhibited the progression of HCC through downregulating LIMK1. Moreover, circulating lncRNA H19 enhanced the enrichment of LIMK1 via sponging miR-520a-3p in HCC cells. The results of animal experiment showed that exosomal H19 could promote the growth and metastasis of HCC tumors treated with Propofol in vivo.

5 | CONCLUSION

Taken together, exosomal lncRNA H19 elevated the malignant potential of HCC cells treated with Propofol via miR-520a-3p/LIMK1 axis in vivo and in vitro. The underlying mechanism of LIMK1 on the modulation of behaviors of HCC cells needs further exploration.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

All the patients involved in this study signed informed consents. This study was supported by the ethics committee of The First Affiliated Hospital of Zhengzhou University.

PATIENT CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The analyzed datasets generated during the present study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS

Not applicable.

AUTHORS’ CONTRIBUTION

Conceptualization and Methodology: Tao Yang and Junqi Liu; Formal analysis and Data curation: Huaping Zhao, Na Xing and Juan He; Validation and Investigation: Dongmei Wang, Yanqiu Ai and Jianjun Yang; Writing - original draft preparation and Writing - review and editing: Dongmei Wang, Tao Yang and Junqi Liu; Approval of final manuscript: all authors.

ORCID

Dongmei Wang https://orcid.org/0000-0001-9532-6203
Na Xing https://orcid.org/0000-0003-2638-9045
Tao Yang https://orcid.org/0000-0002-5287-4733
Junqi Liu https://orcid.org/0000-0002-3285-0777
Huaping Zhao https://orcid.org/0000-0001-7113-176X
Juan He https://orcid.org/0000-0002-3887-0082
Yanqiu Ai https://orcid.org/0000-0002-3657-6728
Jianjun Yang https://orcid.org/0000-0002-0781-941X

REFERENCES

1. Mammoto T, Mukai M, Mammoto A, et al. Intravenous anesthetic, propofol inhibits invasion of cancer cells. Cancer Lett. 2002;184(2):165–170.
2. Miao Y, Zhang Y, Han W, Chen L, Wang F. GABA-receptor agonist, propofol inhibits invasion of colon carcinoma cells. Biomed Pharmacother. 2010;64(9):583–588.
3. Wang ZT, Gong HY, Zheng F, Liu DJ, Dong TL. Propofol suppresses proliferation and invasion of pancreatic cancer cells by upregulating microRNA-199a expression. Genet Mol Res. 2015;14(3):7529–7537.
4. Wang ZT, Gong HY, Zheng F, Liu DJ, Yue XQ. Propofol suppresses proliferation and invasion of gastric cancer cells via downregulation of microRNA-221 expression. Genet Mol Res. 2015;14(3):8117–8124.
5. Zhang J, Zhang D, Wu GQ, Feng ZY, Zhu SM. Propofol inhibits the adhesion of hepatocellular carcinoma cells by upregulating microRNA-199a and downregulating MMP-9 expression. Hepatobiliary Pancreat Dis Int. 2013;12(3):305–309.
6. Zhang J, Wu GQ, Zhang Y, Feng ZY, Zhu SM. Propofol induces apoptosis of hepatocellular carcinoma cells by upregulating microRNA-199a expression. Cell Biol Int. 2013;37(3):227–232.
7. Ou W, Lv J, Zou X et al Propofol inhibits hepatocellular carcinoma growth and invasion through the HMGA2-mediated Wnt/beta-catenin pathway. Exp Ther Med. 2017;13(5):2501–2506.
8. Simons M, Raposo G. Exosomes–vesicular carriers for intercellular communication. Curr Opin Cell Biol. 2009;21(4):575–581.
9. Thery C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol.* 2009;9(8):581–593.

10. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol.* 2007;9(6):654–659.

11. Kalluri R. The biology and function of exosomes in cancer. *J Clin Invest.* 2016;126(4):1208–1215.

12. Ruivo CF, Adem B, Silva M, Melo SA. The biology of cancer exosomes: insights and new perspectives. *Cancer Res.* 2017;77(23):6480–6488.

13. Paraskevopoulou MD, Hatzigeorgiou AG. Analyzing MiRNA-LncRNA Interactions. *Methods Mol Biol.* 2016;1402:271–286.

14. Lv M, Zhong Z, Huang M, Tian Q, Jiang R, Chen J. IncRNA H19 regulates epithelial-mesenchymal transition and metastasis of bladder cancer by miR-29b-3p as competing endogenous RNA. *Biochim Biophys Acta Mol Cell Res.* 2017;1864(10):1887–1899.

15. Yang Q, Wang X, Tang C, Chen X, He J. H19 promotes the migration and invasion of colon cancer by sponging miR-138 to upregulate the expression of HMGA1. *Int J Oncol.* 2017;50(5):1801–1809.

16. Bai JJ, Lin CS, Ye HJ, Guo PP, Wang W. Propofol suppresses migration and invasion of breast cancer MDA-MB-231 cells by down-regulating H19. *Nan Fang Yi Ke Da Xue Xue Bao.* 2016;36(9):1255–1259.

17. Li J, Wei J, Mei Z et al Suppressing role of miR-520a-3p in breast cancer through CCND1 and CD44. *Am J Transl Res.* 2017;9(1):146–154.

18. Qu X, Yang L, Shi Q, Wang X, Wang D, Wu G. Lidocaine inhibits proliferation and induces apoptosis in colorectal cancer cells by upregulating mir-520a-3p and targeting EGFR. *Pathol Res Pract.* 2018;214(12):1974–1979.

19. Wang X, Xu Y, Chen X, Xiao J. Dexmedetomidine Inhibits Osteosarcoma Cell Proliferation and Migration, and Promotes Apoptosis by Regulating miR-520a-3p. *Oncol Res.* 2018;26(3):495–502.

20. Scott RW, Olson MF. LIM kinases: function, regulation and association with human disease. *J Mol Med (Berl).* 2007;85(6):555–568.

21. Su J, Zhou Y, Pan Z, et al. Downregulation of LIMK1-ADF/cofilin by DADS inhibits the migration and invasion of colon cancer. *Sci Rep.* 2017;7:45624.

22. Li D, Wang H, Song H et al The microRNAs miR-200b-3p and miR-429-5p target the LIMK1/CFL1 pathway to inhibit growth and motility of breast cancer cells. *Oncotarget.* 2017;8(49):85276–85289.

23. Gong T, Ning X, Deng Z et al Propofol-induced miR-219-5p inhibits growth and invasion of hepatocellular carcinoma through suppression of GPC3-mediated Wnt/beta-catenin signalling activation. *J Cell Biochem.* 2019;120(10):16934–16945.

24. Liu SQ, Zhang JL, Li ZW, Hu ZH, Liu Z, Li Y. Propofol Inhibits Proliferation, Migration, and Promotes Apoptosis Through Down-Regulating miR-374a in Hepatocarcinoma Cell Lines. *Cell Physiol Biochem.* 2018;49(6):2099–2110.

25. Zhang J, Shan WF, Jin TT et al Propofol exerts anti-hepatocellular carcinoma by microvesicle-mediated transfer of miR-142-3p from macrophage to cancer cells. *J Transl Med.* 2014;12:279.

26. Aucher A, Rudnicka D, Davis DM. MicroRNAs transfer from human macrophages to hepato-carcinoma cells and inhibit proliferation. *J Immunol.* 2013;191(12):6250–6260.

27. Challagundla KB, Wise PM, Nevianni P, et al. Exosome-mediated transfer of microRNAs within the tumor microenvironment and neoblasteroma resistance to chemotherapy. *J Natl Cancer Inst.* 2015;107.

28. Yang M, Chen J, Su F et al Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol Cancer.* 2011;10:117.

29. Desrochers LM, Antonyak MA, Cerione RA. Extracellular vesicles: satellites of information transfer in cancer and stem cell biology. *Dev Cell.* 2016;37(4):301–309.

30. Yu J, Tan Q, Deng B, Fang C, Qi D, Wang R. The microRNA-520a-3p inhibits proliferation, apoptosis and metastasis by targeting MAP3K2 in non-small cell lung cancer. *Am J Cancer Res.* 2015;5(2):802–811.

31. Li CL, Zhang YQ, Li B, Guo M, Fu YL. MicroRNA-520a-3p suppresses epithelial-mesenchymal transition, invasion, and migration of papillary thyroid carcinoma cells via the JAK1-mediated JAK/STAT signaling pathway. *J Cell Physiol.* 2019;234(4):4054–4067.

32. Bernard O. Lim kinases, regulators of actin dynamics. *Int J Biochem Cell Biol.* 2007;39(6):1071–1076.

33. Li R, Doherty J, Antonipillai J et al LIM kinase inhibition reduces breast cancer growth and invasiveness but systemic inhibition does not reduce metastasis in mice. *Clin Exp Metastasis.* 2013;30(4):483–495.

34. Yu Y, Zhang P, Yan J, et al. Sevoflurane induces cognitive impairments via the MiR-27b/LIMK1-signaling pathway in developing rats. *Inhal Toxicol.* 2016;28(14):731–738.

How to cite this article: Wang D, Xing N, Yang T, et al. Exosomal IncRNA H19 promotes the progression of hepatocellular carcinoma treated with Propofol via miR-520a-3p/LIMK1 axis. *Cancer Med.* 2020;9:7218–7230. [https://doi.org/10.1002/cam4.3313](https://doi.org/10.1002/cam4.3313)