Basic Study

Long non-coding ribonucleic acid W5 inhibits progression and predicts favorable prognosis in hepatocellular carcinoma

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Abstract

BACKGROUND
Accumulating evidence has revealed that several long non-coding ribonucleic acids (lncRNAs) are crucial in the progress of hepatocellular carcinoma (HCC).

AIM
To classify a long non-coding RNA, i.e., lncRNA W5, and to determine the clinical significance and potential roles of lncRNA W5 in HCC.

METHODS
The results showed that lncRNA W5 expression was significantly downregulated in HCC cell lines and tissues. Analysis of the association between lncRNA W5 expression levels and clinicopathological features suggested that low lncRNA W5 expression was related to large tumor size ($P < 0.01$), poor histological grade ($P < 0.05$) and serious portal vein tumor thrombosis ($P < 0.05$). Furthermore, Kaplan-Meier survival analysis showed that low expression of lncRNA W5 predicts poor overall survival ($P = 0.016$).

RESULTS
Gain-of-loss function experiments, including cell counting kit8 assays, colony formation assays, and transwell assays, were performed in vitro to investigate the
experiments were conducted with the approval of the Fifth Medical Center of Chinese PLA General Hospital’s Animal Care and Use Committee.

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biological roles of lncRNA W5. In vitro experiments showed that ectopic overexpression of lncRNA W5 suppressed HCC cell proliferation, migration and invasion; conversely, silencing of lncRNA W5 promoted cell proliferation, migration and invasion. In addition, acting as a tumor suppressor gene in HCC, lncRNA W5 inhibited the growth of HCC xenograft tumors in vivo.

CONCLUSION
These results showed that lncRNA W5 is down-regulated in HCC, and it may suppress HCC progression and predict poor clinical outcomes in patients with HCC. LncRNA W5 may serve as a potential HCC prognostic biomarker in addition to a therapeutic target.

Key Words: Hepatocellular carcinoma; Long non-coding ribonucleic acid; Long non-coding ribonucleic acid W5

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INTRODUCTION
Around 90% of primary liver cancer cases are hepatocellular carcinoma (HCC)[1], approximately 60000 deaths occur globally each year, and half of all deaths occur in China[2,3]. In addition to hepatic resection, liver transplantation, chemotherapy and molecular targeted therapy are often carried out to improve outcomes in patients with HCC; however, the 5-year survival rate of patients with HCC still remains poor[4,5]. The specific signaling mechanisms underlying the development and progression of HCC remain to be defined.

The long non-coding ribonucleic acids (lncRNAs) are a class of noncoding RNAs with more than 200 nucleotides which have no protein-coding potential. Several reports have shown that lncRNAs are critical in numerous biological processes, including tumor development, differentiation, and tumorigenesis[6-10]. The expression of lncRNAs is dysregulated in cancer. Of note, specific lncRNAs are related to cancer recurrence, metastasis and prognosis in various cancers, including HCC[11-14]. To date, several lncRNA have been reported to be associated with the growth and advance of HCC, such as HULC[15], H19[16], MEG3[17], ZFAS1[18], P7[19], ATB[20], GAS8-AS1[21], and so on. In our previous study, we profiled the the expression of lncRNAs in influenza virus infected patients and identified panels of uncharacterized lncRNAs. In the current study, we classified the lncRNA W5 (mitochondrial translation optimization 1 homologue; hsa_lncRNA_0007874/hsa_lncRNA_104135) which is notably down-regulated in HCC tissues and strictly associated with the prognosis of HCC patients. Furthermore, we investigated its roles, underlying mechanisms and clinical significance in HCC progression.
MATERIALS AND METHODS

Clinical samples
A total of 86 resected HCC tissues and matched tumor-adjacent tissues were kindly provided by the Department of Hepatobiliary Surgery of the Fifth Medical Center, Chinese PLA General Hospital between October 2013 and June 2018. Tumor tissues and adjacent non-tumor tissue specimens were obtained from the patients after informed consent in accordance with the institutional guidelines of the hospital’s Ethics Committee. Table 1 indicates the clinical and pathological characteristics of HCC patients obtained from clinical records.

Cell lines
All human cell lines were provided by the Experimental Center of the Fifth Medical Center, Chinese PLA General Hospital (Beijing, China), and included the normal non-malignant liver cell line LO2 and HCC cell lines Huh7, MHCC-97L, MHCC-97H, PLC, Hep3B and HCCLM3. All cell lines were maintained in DMEM (Gibco, Beijing, China) and were supplemented with 10% fetal bovine serum (Gibco, Beijing, China) in an incubator at 37°C with 5% CO₂.

RNA extraction and reverse transcription-polymerase chain reaction
TRIzol reagent (Invitrogen, United States) was used to isolate total RNA from HCC cells and tissues, and first strand complementary deoxyribonucleic acid (cDNA) was synthesized by the use of reverse transcriptase. Quantitative real-time polymerase chain reaction (PCR) was conducted using the SYBR Green PCR kit (Thermo Fisher Scientific, United States). All reactions were performed on the ABI 7500 system (Applied Biosystems). The IncRNA W5-specific reverse transcription-polymerase chain reaction (qRT-PCR) primers used were as follows: forward: 5'-AAGGAGAACACAAAACAGGCAT-3', reverse: 5'-TGTGAAGCCCTAGATTTCCCAT-3'; GAPDH forward: 5'-AAGGAGAACACAAAACAGGCAT-3', reverse: 5'-TGTGAAGCCCTAGATTTCCCAT-3'. Human GAPDH gene was amplified as an internal control.

Vector construction
The IncRNA W5 vector was constructed and sub-cloned into the pcDNA3.1 (+) vector at the BamHI and EcoRI sites, which produced pcDNA3.1-IncRNA W5. The primers used were as follows: forward: 5'-GGCGGGATCCCTGACTCTTTTCGTTAAGC-3', reverse: 5'-CGCGCGGAATTCATGTTGACTTAAGTTCAGG-3'. Empty vector pcDNA3.1 (+) was used as a negative control. The IncRNA W5 and control were transfected into HCC cells using Polyplus (Invitrogen) according to the manufacturer’s instructions and cultured on six-well plates, respectively.

Cell proliferation assay
Cell proliferation experiments were performed using the CCK-8 kit (Dojindo Laboratories) according to the manufacturer’s protocol. Briefly, a Huh7 or LM3 cell suspension was adjusted to a final cell concentration of 5 × 10³/mL and then added to a 96-well plate. HCC cells were cultured for the indicated time points, and then 10 μL of CCK-8 (5 mg/mL) was added to each well. The cell culture plate was placed in the incubator for 1 h, and the absorbance was measured at 450 nm per well using a Thermomax plate reader (Thermo Fisher, China).

Colony formation assay
Huh7 or LM3 cells were completely dispersed into individual cells in 6-well plates and incubated at 37°C in DMEM with 10% fetal bovine serum, respectively. After 14 d, the cell colonies were washed with PBS, fixed in 4% paraformaldehyde for 20 min, and stained with crystal violet for 20 min. Photographs were subsequently taken and only colonies containing more than 50 cells were recorded.

Cell migration and invasion assays
A chamber assay with Matrigel (invasion) or without Matrigel (migration) was performed at least in triplicate. Twenty-four-well chambers with 8 μm pore size were used in this experiment. Briefly, cells were added to the top chamber without Matrigel (migration) or with Matrigel (invasion) in the 24-well plate (Corning). The medium with 15% forward-based system was added to the lower chambers. After incubator for 48-72 h, the DMEM medium was removed and the cells were washed with PBS, and
Table 1 Association of long non-coding ribonucleic acid W5 expression with clinicopathologic features in hepatocellular carcinoma patients

| Parameters                  | Group        | Total | W5 expression | P value |
|-----------------------------|--------------|-------|---------------|---------|
|                             |              |       | Low | High |       |
| Gender                      | Male         | 70    | 34 | 36 | 0.791 |
|                             | Female       | 16    | 10 | 6  |       |
| Age (yr)                    | ≤ 60         | 30    | 15 | 15 | 0.579 |
|                             | > 60         | 56    | 28 | 28 |       |
| Tumor size (cm)             | < 3 cm       | 21    | 6  | 15 | 0.007b|
|                             | ≥ 3 cm       | 65    | 38 | 27 |       |
| AFP                         | < 20         | 39    | 21 | 18 | 0.328 |
|                             | ≥ 20         | 47    | 22 | 25 |       |
| Histological grade          | Well/moderate-poor | 52/34 | 5/28 | 47/6 | 0.046a|
| Clinical stage              | I/II         | 50    | 23 | 27 | 0.501 |
| Tumor number                | III          | 36    | 20 | 16 |       |
|                             | Solitary     | 76    | 36 | 40 | 0.312 |
|                             | Multiple     | 10    | 7  | 3  |       |
| Drinking status             | Yes          | 42    | 24 | 18 | 0.276 |
|                             | No           | 44    | 19 | 25 |       |
| Smoking status              | Yes          | 36    | 18 | 18 | 0.584 |
|                             | No           | 50    | 24 | 26 |       |
| PVTT                        | Yes          | 32    | 22 | 10 | 0.024a|
|                             | No           | 54    | 21 | 33 |       |
| Microvascular invasion      | Yes          | 70    | 36 | 34 | 0.568 |
|                             | No           | 16    | 6  | 10 |       |
| Liver cirrhosis             | Absence      | 59    | 30 | 29 | 0.489 |
| HBV                         | Yes          | 55    | 25 | 30 | 0.365 |
|                             | No           | 31    | 18 | 13 |       |

P < 0.05.  
P < 0.01. PVTT: Portal vein tumor thrombosis; HBV: Hepatitis B virus.

carefully removed from the top chamber with a cotton swab. The cells were fixed with 4% paraformaldehyde, stained with crystal violet, and then photographed in five randomly selected microscope fields.

In vivo tumor growth

Athymic BALB/C mice (4-6 wk old) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) and maintained in a SPF facility. Huh7 cells (5 × 10⁶) over-expressing IncRNA W5 were subcutaneously injected into the flanks of nude mice. Tumor length (L) and width (W) were measured using calipers every 3 d up to 6 wk. Tumor volume was estimated using the formula: π × length × width 2/6. After 6 wk, the mice were sacrificed, and tumor volumes and weights were examined. Proliferation progression was examined and quantified using a noninvasive bioluminescence in vivo Imaging System (Xenogen Corporation, Alameda, CA, United States). All animal experiments were conducted with the approval of the Fifth Medical Center of Chinese PLA General Hospital’s Animal Care and Use Committee.
Statistical analysis
All statistical analyses were performed using the SPSS 20.0 software package (Chicago, IL, United States). Data were expressed as the mean ± SD. Kaplan-Meier analysis was used to determine whether there was a correlation between the expression of lncRNA W5 and overall survival rate of HCC patients. P < 0.05 was considered significant.

RESULTS

HCC cancer tissues and cell lines have low expression of lncRNA W5
Initially, to investigate the potential role of lncRNA W5 in HCC tumorigenesis, we determined the expression of lncRNA W5 in 86 sets of HCC tissues and non-tumor tissues by qRT-PCR. As shown in Figure 1, the expression of lncRNA W5 was significantly reduced in HCC tissues compared with adjacent non-tumor tissues (P < 0.001, Figure 1A and B). In addition, we determined the expression of lncRNA W5 in regular liver cells (LO2) and six HCC cancer cell lines (Huh7, MHCC-97L, MHCC-97H, PLC, Hep3B and HCCLM3). The results revealed that lncRNA W5 expression was significantly downregulated in the six HCC cancer cells compared with the regular liver cell line LO2 (Figure 1C). Levels of lncRNA W5 expression were relatively lower in the Huh7 and LM3 HCC cell lines, and were used in subsequent studies. More importantly, a Kaplan-Meier survival analysis indicated that HCC patients with low expression of lncRNA W5 had shorter overall survival than those patients with high expression of lncRNA W5 (P = 0.016) (Figure 1D). Cox survival analysis was then used to further confirm the prognostic value of lncRNA W5 in HCC. Univariate analysis showed that the analyzed variables (lncRNA W5 expression, Pathologic-stage and Pathologic-TMN) were markedly associated with the overall survival time of HCC patients. Furthermore, multivariate analysis revealed that lncRNA W5 expression (P = 0.027), Pathologic-T (P = 0.014) and Pathologic-M (P = 0.005) were promising independent prognostic factors of HCC (Table 2). Thus, lncRNA W5 could be used as an independent prognostic factor. Finally, we also measured the expression of lncRNA W5 in nuclear and cytosolic fractions of Huh7 cells by qRT-PCR. The differential enrichments of GAPDH, β-actin and U1 RNA were used as fractionation indicators. Subcellular fractionation location results showed that lncRNA W5 was mainly located in the nucleus (Figure 1E), thus suggesting that lncRNA W5 might play an essential regulatory function at the transcriptional level.

To investigate the relationship between the expression of lncRNA W5 and clinicopathological characteristics, lncRNA W5 expression was detected in 86 HCC patients and distributed into two groups (high–high expression of lncRNA W5 and low–low expression of lncRNA W5) based on the median lncRNA W5 expression. The correlations between lncRNA W5 expression and clinical parameters were analyzed and it was found that low expression of lncRNA W5 was linked to large tumor size (P < 0.01), poor histological grade (P < 0.05) and serious portal vein tumor thrombosis (P < 0.05). Nevertheless, no significant correlation was observed between the expression of lncRNA W5 and other clinicopathological features, such as age, gender, AFP levels, tumor number and with/without HBV infection.

In vitro effects of lncRNA W5 on HCC cell proliferation
To assess the role of lncRNA W5 in regulating the biological behavior of HCC cells, we used the lncRNA W5 expression vector pcDNA3.1-lncRNA W5 and overexpressed lncRNA W5 in the HCC cell lines Huh7 and LM3. LncRNA W5 overexpression in the two cell lines was verified by RT-qPCR (Figure 2A). CCK-8 assays, which were used to show overexpression of lncRNA W5 in the HCC cell lines Huh7 and LM3, demonstrated a significant reduction in cell proliferation from 48 h to 96 h (Figure 2B). Accordingly, colony formation assays, which showed that Huh7 and LM3 cells transfected with lncRNA W5, resulted in significantly decreased clonogenic survival than empty vector control Huh7 and LM3 cell lines (Figure 2C). In addition, we constructed shRNA-1 and shRNA-2 containing the back-splicing region of lncRNA W5 for silencing. The efficiency of lncRNA W5 silencing was confirmed by qPCR following transfection with lncRNA W5 shRNA-1 or-2 in Huh7 and LM3 cells (Figure 2D). As expected, we found that lncRNA W5 silencing significantly promoted cell proliferation of Huh7 and LM3 cells as indicated by MTS (Figure 2E). Furthermore, a clone formation assay verified that following lncRNA W5 knockdown, the HCC population dependence and proliferation ability were considerably increased (Figure 2F). Overall, these data demonstrated that lncRNA W5 may inhibit HCC cell proliferation in vitro.
### Table 2 Cox Proportional Hazards Model Analysis of Clinicopathologic Features Related to Overall Survival in Terms of Long Non-Coding Ribonucleic Acid W5 Expression in Hepatocellular Carcinoma Patients

| Variables                          | Univariate Analysis |             |             | Multivariate Analysis |             |             |
|------------------------------------|---------------------|-------------|-------------|-----------------------|-------------|-------------|
|                                    | P value             | HR          | 95%CI       | P value               | HR          | 95%CI       |
| Expression (high/low)              | 0.041¹              | 1.672       | 1.032-2.159 | 0.027                 | 1.285       | 0.867-2.155 |
| Pathologic-Stage (I + II/III + IV) | 0.001¹              | 2.584       | 1.934-4.162 | 0.159                 | 2.436       | 0.715-7.667 |
| Pathologic-T (T1 + T2/T3 + T4)    | 0.004¹              | 4.068       | 1.573-8.869 | 0.014                 | 10.638      | 2.314-57.649|
| Pathologic-M (M0/M1)              | 0.007¹              | 5.294       | 3.195-7.657 | 0.005                 | 3.082       | 1.726-5.342 |
| Pathologic-N (N0/N1 + N2 + N3)    | 0.002¹              | 2.413       | 1.519-3.969 | 0.246                 | 0.719       | 0.312-1.911 |
| Age (< 60/ ≥ 60 yrs)              | 0.342               | 1.402       | 0.914-2.357 |                       |             |             |
| Gender (female/male)              | 0.258               | 1.324       | 0.849-1.905 |                       |             |             |

*P < 0.05. HR: Hazard ratio; CI: Confidence interval.

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### In Vitro Effects of IncRNA W5 on HCC Cell Migration and Invasion

To evaluate the potential role of IncRNA W5 in HCC metastasis, and investigate the effect of IncRNA W5 on cell migration and invasion capacity, we performed transwell assays using Huh7 and LM3 cells. The results revealed that the migration and invasion ability of HCC cells that over-expressed IncRNA W5 was significantly decreased compared with the empty vector group (Figure 3A). On the contrary, IncRNA W5 knockdown with shRNA-1 or-2 significantly promoted cell migration and enhanced cell invasion of Huh7 and LM3 cells, respectively (Figure 3B). These results strongly suggest that IncRNA W5 has a critical effect on the inhibition of HCC cell migration and invasion.

### IncRNA W5 Inhibits Tumor Growth in Vivo

To elucidate the in vivo role of IncRNA W5 in tumorigenesis, we subcutaneously injected the flanks of nude mice with Huh7 cells over-expressing IncRNA W5 and stably expressing luciferase and monitored tumor growth every three days. As shown in Figure 4, mice injected with IncRNA W5 over-expressing cells had a significant decrease in tumor growth at 36 d post-injection as compared with mice injected with control cells. Six weeks after injection, the volumes and weights of tumors examined in mice injected with pcDNA3.1-IncRNA W5 were notably smaller than those in mice injected with the control. At 6 wk after injection, bioluminescent signals were weaker in mice with IncRNA W5 over-expression than in control mice, suggesting that IncRNA W5 may inhibit the growth of HCC xenograft tumors in vivo.

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### Discussion

Increasing evidence has shown that aberrant expression of numerous IncRNAs has been discovered in HCC. Previous studies showed that amplification of IncRNA ZFAS1 promotes metastasis in HCC⁹. Sun SH’s group observed in HCC that the IncRNA-activated by TGF-β (IncRNA-ATB) promoted the invasion-metastasis cascade²⁰. Hur K’s group also reported that IncRNA-ATB could have potential as a biomarker for the prognosis of HCC and as a targeted therapy for HCC patients²². Another study showed that the MBNL3 splicing factor promoted HCC by increasing the expression of PXN by the alternative splicing of IncRNA-PXN-AS1²³. The IncRNA lncHDAC2 may drive the self-renewal of liver cancer stem cells through the activation of Hedgehog signaling²⁴. Super-enhancer associated lncRNA HCCL5 is activated by ZEB1 and promotes the malignancy of HCC²⁵. Recently, Huang et al²⁶ identified oncofetal IncRNA Ptn-dt which might promote HCC proliferation by regulating the Ptn receptor²⁶. These results indicate that IncRNAs may have critical roles in HCC progression and development and can be used in clinical applications.

In this study, we reported an uncharacterized low expression of IncRNA W5 in HCC specimens and cell lines, suggesting that IncRNA W5 expression might be related to HCC carcinogenesis. Decreased expression of IncRNA W5 was associated with aggressive clinicopathological features of HCC tissues, including tumor size,
Figure 1  Expression of long non-coding ribonucleic acid W5 is downregulated in hepatocellular carcinoma tissues and cells. A: The expression of long non-coding ribonucleic acid (lncRNA) W5 was detected by reverse transcription-polymerase chain reaction (qRT-PCR) in tumor tissues and non-adjacent normal tissues of hepatocellular carcinoma (HCC) patients ($n = 86$). LncRNA W5 expression was normalized to GAPDH expression; B: The expression of lncRNA W5 was detected by qRT-PCR in tumor tissues and non-adjacent normal tissues of 86 HCC patients; C: The expression levels of lncRNA W5 in a series of HCC cell lines were reduced compared to that in LO2 cells; D: Analysis of overall survival based on lncRNA W5 expression levels is shown in 86 HCC patients; and E: Subcellular localization of lncRNA W5 in Huh7 cells was examined by qRT-PCR. GAPDH, β-actin and U1 were considered as the control markers, respectively. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. HCC: Hepatocellular carcinoma; lncRNA: Long non-coding ribonucleic acid; qRT-PCR: Reverse transcription-polymerase chain reaction.

histological grade and the presence of portal vein tumor thrombosis. Gain-and loss function experiments showed that over-expression of lncRNA W5 in Huh7 and LM3 cells decreased cell proliferation, and impaired cell migration and invasion. Moreover, lncRNA W5 over-expression inhibited tumor growth in HCC xenograft-bearing mice. These results demonstrated that lncRNA W5 is involved in the progression of HCC and may be a potential target for therapy. Furthermore, our studies showed that HCC patients with low expression of lncRNA W5 had shorter overall survival than patients with high expression of lncRNA W5, suggesting that lncRNA W5 might be a potential prognostic predictor. Of course, down-regulation of lncRNA W5 should be validated in more HCC cohorts. The interactions of this protein and downstream pathways also warrant further investigation in subsequent studies. In addition, whether lncRNA W5
Lei GL et al. HCC and IncRNA W5

A

Huh7

Relative expression

pCDNA-3.1  pCDNA-3.1-IncRNA W5

HCCLM3

Relative expression

pCDNA-3.1  pCDNA-3.1-IncRNA W5

B

Huh7

Optical density at 450 nm

Time (h)  24 h  48 h  72 h  96 h

pcDNA3.1  pcDNA3.1-IncRNA W5

HCCLM3

Optical density at 450 nm

Time (h)  24 h  48 h  72 h  96 h

pcDNA3.1  pcDNA3.1-IncRNA W5

C

pcDNA-3.1  pcDNA-IncRNA W5

Huh7

Relative colony formation ability

HCCLM3

Relative colony formation ability

D

Huh7

Relative expression

shControl  shIncRNA W5-1  shIncRNA W5-2

HCCLM3

Relative expression

shControl  shIncRNA W5-1  shIncRNA W5-2
Figure 2 In vitro suppression of long non-coding ribonucleic acid W5 in hepatocellular carcinoma proliferation. A: Increased long non-coding ribonucleic acid (lncRNA) W5 expression in Huh7 and LM3 cells was confirmed after over-expressed lncRNA W5 transfection by reverse transcription-polymerase chain reaction. LncRNA W5 expression was normalized to GAPDH. \( aP < 0.05; bP < 0.01; cP < 0.001 \); B: Cell viability of pCDNA-3.1 LncRNA W5-transfected Huh7 and LM3 cells were detected by CCK-8 assays. Cell number was determined every 24 h up to 96 h. The results are shown as the mean ± SE from three independent experiments. \( aP < 0.05; bP < 0.01; cP < 0.001 \), compared with the control by two-sided t-test; C: Colony-forming assay was used to determine the effect of lncRNA W5 on the proliferation in Huh7 and LM3 cells; D: Decreased lncRNA W5 expression in Huh7 and LM3 cells was confirmed after sh-1 or sh-2 LncRNA W5 transfection by reverse transcription-polymerase chain reaction. LncRNA W5 expression was normalized to GAPDH. \( aP < 0.05; bP < 0.01; cP < 0.001 \); E: Cell viability of sh-1 or sh-2 LncRNA W5-transfected Huh7 and LM3 cells were detected by CCK-8 assays. Cell number was determined every 24 h up to 96 h. The results are shown as the mean ± SE from at least three independent experiments. \( aP < 0.05; bP < 0.01; cP < 0.001 \), compared with the control by two-sided t-test; and F: Colony-forming assay was used to determine the effect of sh-1 or sh-2 LncRNA W5 on the proliferation of Huh7 and LM3 cells.

exists in other solid tumors remains to be elucidated.

CONCLUSION

In conclusion, our results showed that the expression of lncRNA W5 was considerably reduced in HCC tissues, which suppressed proliferation, migration and invasion of tumor cells in vitro. The results also showed that low expression of lncRNA W5 correlated with tumor progression and poor prognosis. Furthermore, manipulation of lncRNA W5 expression impacted the biological behavior of HCC. These results suggest that lncRNA W5 may serve as a tumor suppressor in the development and progression of HCC, and has potential as a diagnostic and therapeutic target in the clinical management of HCC.
**A**

**Huh7**

- pcDNA 3.1
- pcDNA-IncRNA W5

**Migration**

- pcDNA 3.1
- pcDNA-IncRNA W5

**Invasion**

- pcDNA 3.1
- pcDNA-IncRNA W5

**HCCM3**

- pcDNA 3.1
- pcDNA-IncRNA W5

**Migration**

- pcDNA 3.1
- pcDNA-IncRNA W5

**Invasion**

- pcDNA 3.1
- pcDNA-IncRNA W5

**B**

**Huh7**

- shControl
- shIncRNA W5-1
- shIncRNA W5-2

**Migration**

- shControl
- shIncRNA W5-1
- shIncRNA W5-2

**Invasion**

- shControl
- shIncRNA W5-1
- shIncRNA W5-2

**Relative expression**

- Huh7

- shControl shIncRNA W5-1 shIncRNA W5-2

**Overall survival**

- High IncRNA W5 expression
- Low IncRNA W5 expression

*P (long rank) = 0.016*
Figure 3  Effects of long non-coding ribonucleic acid W5 on hepatocellular carcinoma migration and invasion. A: Cell migration and invasion abilities were determined after transfection with pcDNA-3.1 and pcDNA-3.1 long non-coding ribonucleic acid W5 in Huh7 and LM3 cell lines, respectively; B: Cell migration and invasion abilities were determined after transfection with sh-1 or sh-2 LncRNA W5 in Huh7 and LM3 cell lines, respectively. All experiments were performed in triplicate. *P < 0.05; **P < 0.01; ***P < 0.001.
Figure 4 Long non-coding ribonucleic acid W5 inhibits tumor growth in vivo. A: Huh7 cells (5 × 10⁶) stably expressed with long non-coding ribonucleic acid W5 (IncRNA W5) were subcutaneously injected into the left flank of nude mice, and the effect of IncRNA W5 on hepatocellular carcinoma tumor growth was examined every 3 d during the course of the experiment (n = 5); B: A representative image of the xenograft-bearing mice; C: Tumors were isolated from the nude mice after sacrifice. The effects of IncRNA W5 on hepatocellular carcinoma growth were determined by tumor volume and tumor weight; D: LncRNA W5-overexpressing Huh7 cells which stably expressed luciferase were injected into nude mice (n = 5). The bioluminescence photographs of tumor were recorded with the in vivo 200 Imaging System. A representative luciferase signal was recorded from each group at 6 wk after injection. *P < 0.01.
ARTICLE HIGHLIGHTS

**Research background**
Accumulating evidence has revealed that several long non-coding RNAs (lncRNAs) are crucial in the progress of hepatocellular carcinoma (HCC).

**Research motivation**
To determine the clinical significance and potential roles of lncRNA W5 in HCC.

**Research objectives**
We classified the long non-coding RNA, lncRNA W5, and examined its clinical significance and potential roles in HCC.

**Research methods**
Analysis of the association between lncRNA W5 expression levels and clinicopathological features was performed. In addition, overall survival was determined using Kaplan-Meier survival analysis.

**Research results**
The results showed that lncRNA W5 was down-regulated in HCC, and it may suppress HCC progression and predict a poor clinical outcome in patients with HCC.

**Research conclusions**
lncRNA W5 may serve as a potential prognostic biomarker and therapeutic target in HCC.

**Research perspectives**
lncRNA W5 may serve as a tumor suppressor in the development and progression of HCC, and have potential as a diagnostic and therapeutic target in the clinical management of HCC.

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