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Original Article

LncRNA *HCG18* promotes prostate cancer progression by regulating the miR-512-3p/HK-2 axis

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KEYWORDS
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Glycolysis

Abstract *Objective:* Long non-coding RNAs (lncRNAs) play an important role in tumor progression. Numerous studies show that lncRNAs are strongly associated with prostate cancer (PCa) progression. The aim of this study was to investigate the pathway through which lncRNA *HCG18* regulates PCa progression by bioinformatics analysis and experiments.
Methods: We compared *HCG18* expression in PCa versus normal tissue and cells by data and cell lines, followed by comparing the changes in tumor cell proliferation, migration, and invasive ability after knockdown of *HCG18*. Then we searched for its downstream pathway by database and validated the pathway *in vivo* and *in vitro*.
Results: HCG18 was highly expressed in PCa and has the ability to promote tumor proliferation, migration, and invasion; knockdown of *HCG18* led to a decrease in the ability of cells to do so, which can be reversed by knockdown of miR-512-3p or overexpression of hexokinase 2.
Conclusion: Our *in vivo* and *in vitro* experiments suggest that *HCG18* can play a role in promoting PCa progression by blocking the inhibition of hexokinase 2 by miR-512-3p via sponge adsorption.

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1. Introduction

Prostate cancer (PCa) is the second most common cancer in men, with more than 1.2 million new cases diagnosed annually [1]. The prognosis of PCa is depending on the grade of tumor and stage at primary diagnosis [1]. Once distant metastases occur, the 5-year survival rate for PCa is only 30% [2]. Therefore, it is critical to explore the pathogenesis of PCa, and identifying novel therapeutic targets is critical for the development of future effective therapies for PCa.

Long non-coding RNAs (lncRNAs) are a class of RNA transcripts with a length larger than 200 nucleotides which cannot encode protein [3]. However, lncRNAs have been confirmed to be involved in a variety of biological processes [4]. Especially, lncRNAs have been proven to regulate tumor progression [5]. The mechanism of lncRNAs in PCa has been extensively studied. Shang et al. [6] showed that lncRNA *PCAT1* is important in the progression of castration-resistant PCa. Lang et al. [7] demonstrated the involvement of lncRNA *PCAT6* in the bone metastasis process of PCa. LncRNA *HCG18* has been confirmed to be involved in the occurrence and development of a variety of tumors like osteosarcoma, hepatocellular carcinoma, and clear cell renal cell carcinoma through its spongy effect on microRNAs (miRNAs) [8—10]. Bioinformatics studies have shown that lncRNA *HCG18* may be associated with PCa, but the specific mechanism remains unclear [11].

miRNAs are evolutionarily conserved short non-coding single-stranded RNA molecules, with a length of 18—22 nucleotides [12]. A large number of miRNAs have been demonstrated to be involved in the progression of PCa [12]. For example, Wang et al. [13] showed that lncRNA *AFAP1-AS1* promoted proliferation and invasion in PCa via targeting miR-512-3p. Playing a role as a cancer inhibitor, miR-512-3p has been found to be down-regulated in the majority of cancer cells. For example, miR-512-3p was confirmed to enhance chemosensitivity and decrease metastatic potential in breast cancer [14]. Meanwhile, miR-512-3p has also been shown to be regulated by lncRNA *PART1* in ovarian cancer to enhance drug resistance [15]. However, Rao et al. [16] showed that miR-512-3p can promote cell proliferation in PCa. This indicates that the role of miR-512-3p in PCa may still be controversial, suggesting the important significance of exploring it.

Metabolic reprogramming is an important feature of tumor cell development [17]. In contrast to normal cells, tumor cells can obtain energy through anaerobic glycolysis even under oxygen-enriched conditions, a change known as the Warburg effect [18,19]. Glycolysis is a tightly regulated process in which hexokinases (HKs), phosphofructokinases, and pyruvate kinases play a crucial role, and overexpression of HKs (mainly HK-2), as well as enhanced activity, has been found in many types of cancer [20]. HKs are involved in the glycolytic process of tumor cells on the one hand and act on voltage-dependent ion channels to inhibit apoptosis on the other hand [21]. In addition, HKs have also been shown to be involved in tumor progression by affecting tumor vascular functions [22], and Zhang et al. [17] showed that in colorectal cancer, HK-2 affects cellular sensitivity to drugs. Therefore, HK-2 is now considered to be an important target for anti-cancer drugs [19]. It has been shown that HK-2 is highly expressed in PCa cells and is a good marker for high-risk PCa, and closely associated with PCa progression, while its mechanism has not been fully elucidated [23]. Meanwhile, in a variety of tumors, lncRNAs have been shown to regulate HK-2 by targeting miRNAs, thereby affecting tumor progression [24—26].

In this study, we aimed to explore the role of the lncRNA *HCG18*/miR-512-3p/HK-2 axis in the development of PCa, and provide a theoretical basis for the diagnosis and treatment of PCa.

2. Materials and methods

2.1. Bioinformatics analysis

The data of lncRNA *HCG18* expression were obtained from the ENCORI project database. Correlation of lncRNA expression with survival time was examined in the GEPIA 2 database using a Kaplan—Meier curve. miRNA was predicted through the starBase, MicroRNA Target Prediction Database (miRDB), miRWalk, and lncRNASNP2. starBase is a public database which can be used to predict the interaction between lncRNA and miRNA [27]. Further, miRDB [28] and miRWalk [29] also have the same function as well (predicting the correlation between lncRNA and miRNA). In addition, starBase can also predict the connection between miRNA and mRNA. Besides, lncRNASNP2 [30] has the same action as well (finding the association between miRNA and mRNA). The databases used in this study are publicly available and can be reused without restriction through open licenses.

2.2. Cell culture

Six PCa cell lines (PC-3, C4-2, C4-2R, DU145, LNCaP, and 22RV1) and prostate epithelial cell line (RWPE1), were all obtained from American Type Culture Collection (Rockville, MD, USA). Cells were cultured in RPMI-1640 (R8758; Sigma, Darmstadt, Germany) containing 10% fetal bovine serum (FBS; Gibco, Carlsbad, CA, USA) and 1% penicillin-streptomycin (HyClone, Logan, UT, USA), and then preserved in the atmosphere with 5% carbon dioxide (CO_2) at 37 °C.

2.3. Cell transfection

Plasmids, miRNA inhibitors, and mimics were commercially purchased from Youze Biological Corporation, Hunan, China. As per the manufacturer, cell transfection was performed using Lipofectamine 2000 (Invitrogen, Inc., CA, USA).

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

The total RNA was extracted using TRIzol reagent (Sigma, Darmstadt, Germany). An equivalent of 1 μg of total RNA was subjected to reversed transcription into cDNA using PrimeScript™ RT reagent Kit (Takara, Kyoto, Japan) and Mir-X™ miRNA qRT-PCR SYBR® Kit (Takara, Kyoto, Japan).

The mRNA and miRNA expression of the target gene was determined by qRT-PCR conducted on ABI-7900 system with SYBR Green (Takara, Kyoto, Japan). The expression of a target gene was normalized to that of *GAPDH* or *U6*. Primer information can be viewed in Supplementary Table 1.

2.5. Western blot analysis

Cells were harvested and lysed in RIPA buffer (PC101, Epyzime, Shanghai, China) with 1% phosphatase and protease inhibitors (Sangon, Shanghai, China). Protein content was quantified by BCA protein quantification kits (Epyzime, Shanghai, China). We separated 20 μg of proteins and transferred it onto the nitrocellulose filter membrane (Merck, Darmstadt, Germany). After blocking membranes for 1 h at room temperature, the nitrocellulose filter membranes were incubated with dilutions containing specific primary antibodies (HK-2, ab209847, Abcam, Cambridge, UK; GAPDH, ab8245, Abcam, Cambridge, UK) overnight at 4 °C. The next day, the membrane was washed three times with Tris-buffered saline with Tween (1×) and incubated with horseradish peroxidase-conjugated secondary antibodies (A0208, Beyotime, Shanghai, China) for 1 h at room temperature. Finally, the membrane was washed three times again and visualized using the electrochemiluminescence exposure system (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Transwell assay

The transwell chamber (24-well) with Matrigel was applied to determine cell invasion following the manufacturer's instructions. Transfected PC-3 or LNCaP cells (1×10^5) were added in the upper chamber with 200 μL RPMI-1640 medium without FBS. The lower chamber was added with 500 μL of RPMI-1640 medium containing 10% FBS. PC-3 cells were cultured for 12 h and LNCaP for 36 h at 37 °C with 5% CO_2. The cells were fixed with formaldehyde for 15 min and then stained with 0.1% crystal violet for 15 min. The invasive cell number was counted using a Zeiss Microscope (Nikon Corporation, Tokyo, Japan).

2.7. Cell counting kit

Cells were selected in the logarithmic growth phase and seeded in 96-well plates at 2×10^3 cells/well for 0 h, 24 h, 48 h, and 72 h, respectively. We added 10 μL cell counting kit-8 (CCK-8) solution (Dojindo, Tokyo, Japan) in each well and then the plate was incubated at 37 °C for 2 h in the dark. The absorbance was detected at 450 nm using a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.8. Wound healing assay

Cells were plated in 6-well plates and scratched vertical wounds with 10 μL tips after completely adherent. Cells were cultured in serum-free RPMI-1640 and photographed at 0 h and 48 h, respectively.

2.9. RNA pull-down assay

Biotin was attached to miR-512-3p (Bio-miR-512-3p) or miR-negative control (Bio-miR-negative control). Bio-miR-512-3p or Bio-miR-negative control was transfected into LNCaP cells. Cells were collected and lysed after transfection for 48 h. The RNA pull-down assay was conducted according to the protocol. qRT-PCR was conducted to examine the level of *HCG18* in LNCaP cells.

2.10. Luciferase reporter assay

The relative luciferase activity was determined with the Dual-Luciferase® Reporter Assay System based on supplier's instructions (Promega, Beijing, China).

2.11. ^{13}C metabolic flux analysis

^{13}C was used to label glucose, which was subsequently introduced into the bioreaction system and cells were incubated for more than 3 h. Glycolytic metabolites reached isotopic steady state, and tricarboxylic acid cycle products reached isotopic steady state after 24 h. Subsequently, metabolic fluxes were estimated by nuclear magnetic resonance spectroscopy.

2.12. Subcutaneous tumor formation assay

BALB/c nude mice (male; 4–6 week; 18–22 g) were selected from Shanghai Slake Experimental Animal Co., Ltd, Shanghai, China. The animal experiment was approved by the Ethics Committee of the Tongji Hospital, Tongji University of Medicine, Shanghai, China (2021-DW-[001]). Each nude mouse was injected subcutaneously in the left shoulder with 1×10^6 cells. The nude mouse was executed 45 days after the injection; the tumor size and weight were measured.

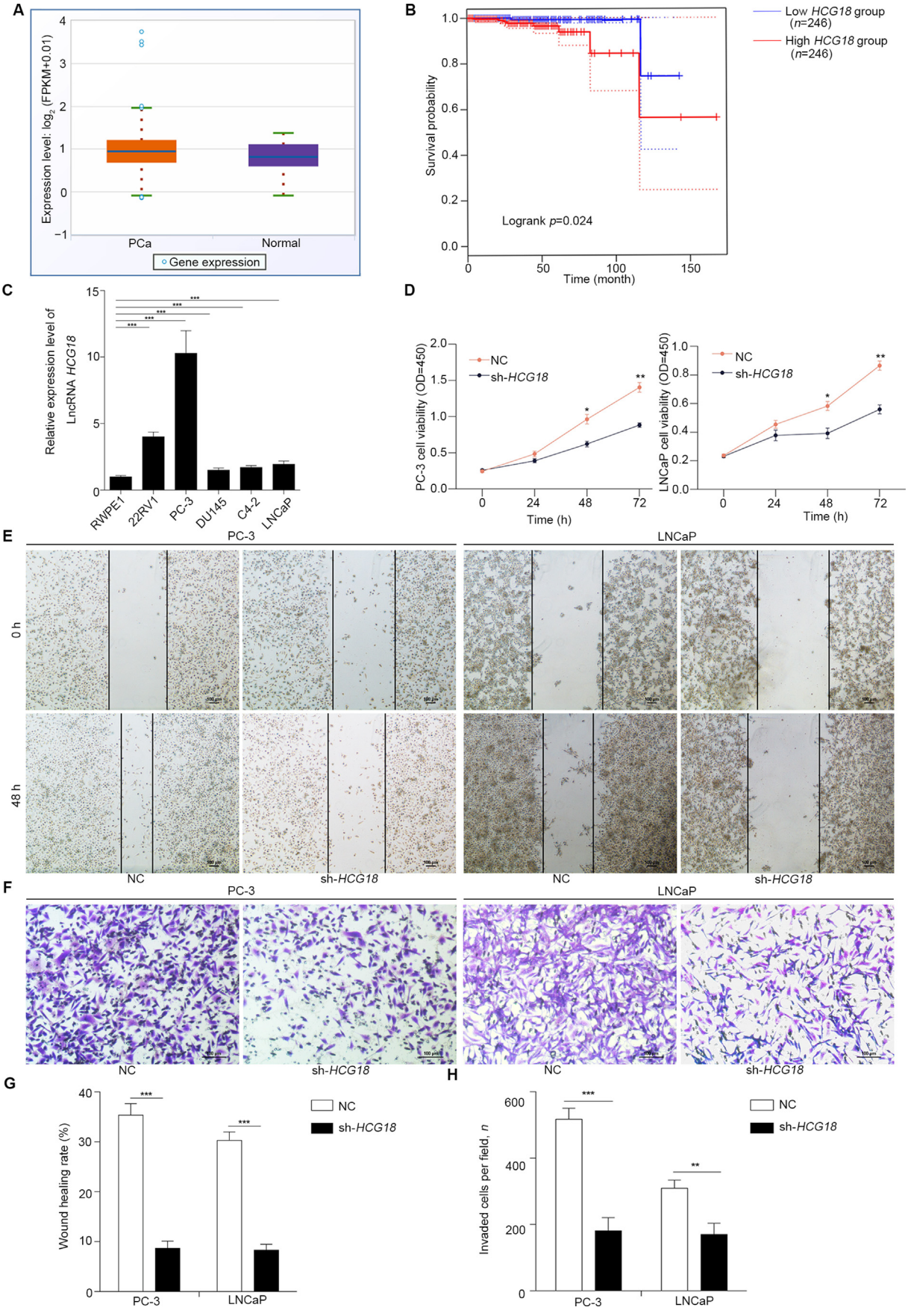
2.13. Statistical analysis

Data were from three independent experiments and presented as the means with standard deviations (SDs) and analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The statistical methods include the ANOVA, Student's *t*-test, Pearson's, and Spearman's correlation analysis for the relationship between variables. A *p*-value of <0.05 shows statistical significance.

3. Results

3.1. LncRNA *HCG18* is markedly increased in PCa and related to cells proliferation, migration, and invasion

To explore the association between *HCG18* and PCa, the data from GEPIA2 database and starBase database were analyzed. The data showed that *HCG18* expression levels were significantly higher in PCa samples than in normal samples (Fig. 1A). In addition, *HCG18* was negatively correlated with the survival of patients; the higher the expression of *HCG18*, the worse the survival of patients (Fig. 1B). Then, we compared the expression level of *HCG18* in several commonly used PCa cell lines (PC-3, C4-2, DU145, LNCaP, and 22RV1) and prostate epithelial cell line (RWPE1)


A
Expression level: log2 (FPKM+0.01)
PCa
Normal
Gene expression
B
Survival probability
Logrank p=0.024
Time (month)
Low HCG18 group (n=246)
High HCG18 group (n=246)
C
Relative expression level of LncRNA HCG18
RWPE1
22RV1
PC-3
DU145
C4-2
LNCaP
D
PC-3 cell viability (OD=450)
LNCaP cell viability (OD=450)
NC
sh-HCG18
Time (h)
E
PC-3
LNCaP
0 h
48 h
NC
sh-HCG18
F
PC-3
LNCaP
NC
sh-HCG18
G
Wound healing rate (%)
PC-3
LNCaP
NC
sh-HCG18
H
Invaded cells per field, n
PC-3
LNCaP
NC
sh-HCG18

by qRT-PCR, and found that *HCG18* in PCa cell lines was significantly higher than that in prostate epithelial cell line (Fig. 1C). To test the role of *HCG18* in PCa, short hairpin RNA (sh-RNA) was transfected into PC-3 cells and LNCaP cells to knock down *HCG18*. Compared with control groups, the proliferation ability of cells decreased significantly in sh-*HCG18* transfected cells (Fig. 1D). Transwell and wound healing assay showed that *HCG18* inhibition remarkably decreased invasion and migration ability in cells (Fig. 1E–H).

3.2. HK-2 promotes PCa progression and can be regulated by LncRNA *HCG18*

Many studies have reported that lncRNA regulates HK-2 expression through miRNA to affect tumor progression [25,31,32]. Cellular drug resistance is an important feature of tumor progression. By performing metabolic flux analysis in C4-2 and C4-2R cells, the results showed significantly increased metabolic flow of glycolysis and tricarboxylic acid cycle in drug-resistant C4-2R cells relative to C4-2 cells, indicating an important role of glycolytic process in PCa progression (Fig. 2A). We hypothesized that lncRNA *HCG18* might also be an upstream molecule regulating HK-2 expression. To test this hypothesis, *HCG18* was knocked down in PC-3 and LNCaP cells and Western blotting was performed to compare HK-2 expression. Apparently knockdown of HK-2 reduces HK-2 expression in cells and at the same time, inhibition of *HCG18* significantly reduced HK-2 expression compared with the control group (Fig. 2B and C). After HK-2 knockdown in PC-3 and LNCaP cells, the CCK-8 assay, wound healing assay, and transwell assay were performed. The results showed that HK-2 knockdown significantly reduced the proliferation, migration, and invasion of PC-3 and LNCaP cells compared with the control group (Fig. 2D–H). These results suggest that HK-2 is indeed involved in the progression of PCa and can be regulated by *HCG18*.

3.3. miR-512-3p directly interacts with *HCG18* and HK-2 and inhibits the progression of PCa cells

To explore the intermediate pathway between *HCG18* and HK-2, we searched the databases (starBase, miRDB, miRWalk, and lncRNASNP), and screened out two target molecules, miR-512-3p and miR-676-3p (Fig. 3A). By reviewing the literature, we found that the role of miR-512-3p in PCa has been reported; however, the results obtained from different studies are inconsistent [13,16]; therefore, we think it is more valuable to investigate miR-512-3p. We employed the biotinylated miR-512-3p probe to pull down the lncRNA *HCG18*. Data indicated endogenous lncRNA *HCG18* was enriched specifically in miR-512-3p probe detection compared with the control group, suggesting that miR-512-3p is a direct inhibitory target of *HCG18* (Fig. 3B). Dual luciferase reporter assay showed that luciferase activity was remarkably decreased in cells co-transfected with wild type 3′-UTR of HK-2 and miR-512-3p mimic (Fig. 3C and D). These indicated that miR-512-3p could directly bind to 3′-UTR of HK-2. miR-512-3p significantly impaired cell migratory and invasive capacity in PC-3 and LNCaP cells (Fig. 4A–F). Meanwhile, CCK-8 assay showed a significant decrease in cell proliferation after transfection with miR-512-3p mimic (Fig. 4G and H).

3.4. LncRNA *HCG18* promotes PCa progression via the LncRNA *HCG18*/miR-512-3p/HK-2 axis

Rescue assays were conducted to test the effect of the lncRNA *HCG18*/miR-512-3p/HK-2 axis on the progression of PCa. It was discovered through the CCK-8 assay, wound healing assay, and transwell assay that *HCG18* knockdown suppressed PCa cell proliferation, migration, and invasion; the descending trend was reversed after miR-512-3p inhibitor or HK-2 overexpression plasmids were transfected into cells (Fig. 5A–E). In conclusion, *HCG18* expedited the progression of PCa through sponging miR-512-3p to up-regulate HK-2 expression.

3.5. LncRNA *HCG18*/miR-512-3p/HK-2 axis modulates PCa tumor growth *in vivo*

To verify the role of the lncRNA *HCG18*/miR-512-3p/HK-2 axis *in vivo*, we conducted experiments in nude mice. The results indicated knockdown of *HCG18* or overexpress miR-512-3p could slowdown mice tumor growth, and knockdown of miR-512-3p accelerated tumor growth (Fig. 6A). Meanwhile, the tumor weight of mice in the sh-*HCG18* and sh-miR-512-3p group was smaller than that in the control group, while larger than that in the sh-*HCG18* group and miR-512-3p group (Fig. 6B). The *in vivo* experiments confirmed the oncogenic effect of miR-512-3p and the role of this signaling axis in PCa.

4. Discussion

PCa is the second most common cancer in men. According to the competing endogenous RNA hypotheses, lncRNAs are

Figure 1 LncRNA *HCG18* is markedly increased in PCa and related to cells proliferation, migration, and invasion. (A) LncRNA *HCG18* expression levels in PCa samples ($n=499$) and normal samples ($n=52$); $p=0.039$ (data source: ENCORI project); (B) Kaplan–Meier curves for overall survival of PCa patients with different *HCG18* expression levels, $p=0.024$ (data source: GEPIA 2); (C) Relative expression levels of lncRNA *HCG18* in different PCa cell lines by qRT-PCR; (D) Analysis of PC-3 and LNCaP cell viability using cell counting kit-8 solution (Dojindo, Tokyo, Japan); (E) Analysis of PC-3 and LNCaP cell migratory capacity using wound healing assay; (F) Analysis of PC-3 and LNCaP cell invasive capacity using transwell assay; (G) The statistical chart of healing experiments comparing cell migration capacity; (H) The statistical chart of transwell assay comparing cell invasion capacity. LncRNA, long non-coding RNA; PCa, prostate cancer; qRT-PCR, quantitative real-time polymerase chain reaction; FPKM, fragment per kilobase of transcript per million mapped reads; NC, negative control; sh-RNA, short hairpin RNA; OD, optical density. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Scale bars, 100 μm. The databases we used are publicly available and can be reused without restriction through open licenses.

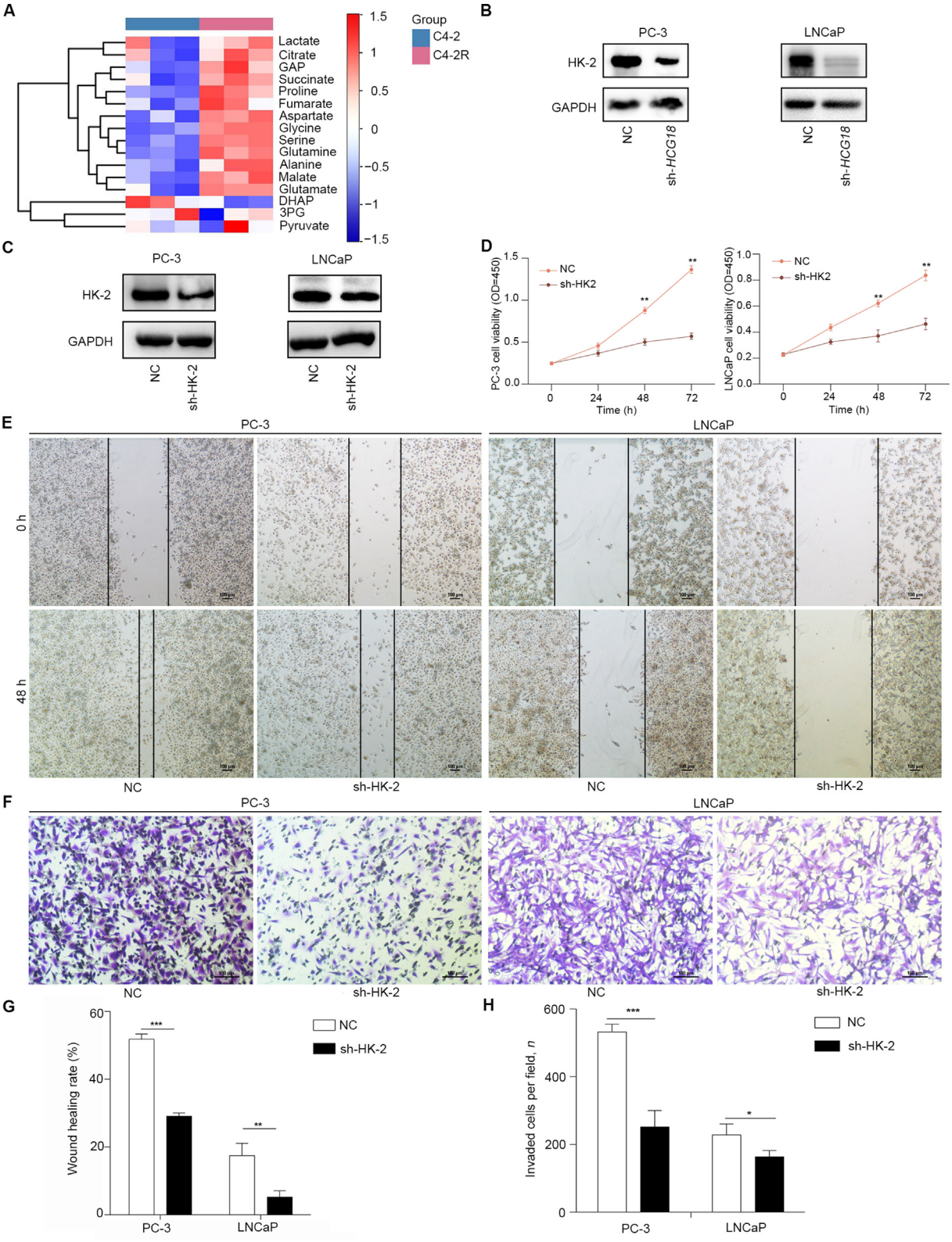


Figure 2 HK-2 promotes PCa progression and can be regulated by LncRNA *HCG18*. (A) Metabolic flux analysis of drug-sensitive (C4-2) and drug-resistant (C4-2R) cells; (B) Western blot comparison of HK-2 expression after knockdown of HCG18; (C) Western blot comparison of HK-2 expression after knockdown of HK-2; (D) Analysis of PC-3 and LNCaP cell viability using cell counting kit-8 solution (Dojindo, Tokyo, Japan); (E) Analysis of PC-3 and LNCaP cell migratory capacity using wound healing assay; (F) Analysis of PC-3 and LNCaP cell invasive capacity using transwell assay; (G) The statistical chart of healing experiments comparing cell migration capacity; (H) The statistical chart of transwell assay comparing cell invasion capacity. GAP, glyceraldehyde 3-phosphate; 3PG, 3-phosphoglycerate; DHAP, dihydroxyacetone phosphate; LncRNA, long non-coding RNA; PCa, prostate cancer; OD, optical density. *$p<0.05$; ** $p<0.01$; *** $p<0.001$. Scale bars, 100 μm.

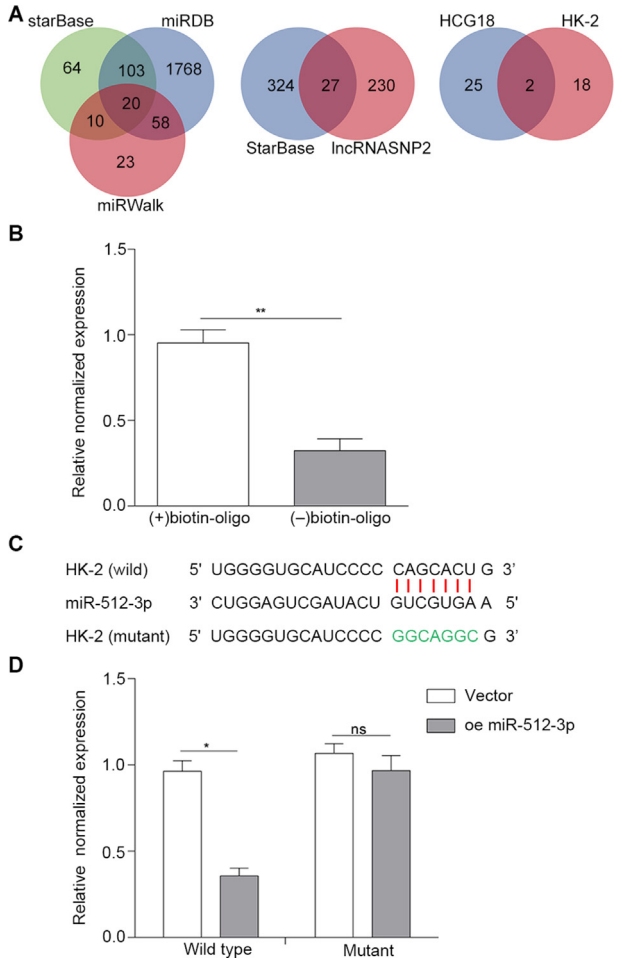


Figure 3 miR-512-3p correlates with *HCG18* and HK-2. (A) Bioinformatic analysis for screening miRNAs; (B) *HCG18* was confirmed to bind to miR-512-3p by RNA pull-down assay; (C) The binding site of HK-2 to miR-512-3p; (D) *HCG18* was confirmed to bind to HK-2 by the luciferase reporter assay. ns, nonsignificant; oe, overexpress. * $p<0.05$; ** $p<0.01$.

capable of regulating gene expression through titrating miRNAs to participate in tumor progression. We focused on the role of the lncRNA *HCG18*/miR-512-3p/HK-2 axis in PCa progression, where *HCG18* regulates HK-2 expression by titrating miR-512-3p, thereby promoting PCa progression.

HCG18 has been shown to have pro-carcinogenic effects in a variety of tumors [8,33]. By bioinformatics analysis and qRT-PCR, we confirmed the high expression of *HCG18* in PCa. By the transwell assay, cell counting kit, and wound healing assay, we confirmed that knockdown of *HCG18* could reduce the proliferation, migration, and invasion ability of PCa cells, suggesting its carcinogenic role. Similarly, Chen et al. [11] speculated that *HCG18* was associated with bone metastasis and poor prognosis of PCa through bioinformatics analysis, which was consistent with our experimental results. *HCG18* affects tumor progression by regulating multiple pathways [34,35]. A recent study showed that in osteosarcoma, *HCG18* can affect osteosarcoma progression by regulating the glycolysis-related enzyme PGK1, suggesting the relevance of *HCG18* to the glycolytic pathway [36]. The Warburg effect is an important feature of tumors, and we found that in PCa cells, drug-resistant progression of tumors correlates with increased levels of glycolysis, and HK-2 is the primary rate-limiting enzyme of glycolysis; therefore, we hypothesized that *HCG18* may affect PCa progression by regulating HK-2. Subsequently, to test the hypothesis that *HCG18* is an upstream gene of HK-2, we knocked down *HCG18* in PCa cells and then compared the changes of HK-2 by Western blot analysis. The results showed that the expression of HK-2 decreased with less *HCG18*. miRNAs together with corresponding proteins form a miRNA induced silencing complex, which inhibits the translation of target mRNAs due to the complementary sequences of nucleotides in miRNAs, whereas lncRNAs can competitively bind miRNAs with mRNAs through sponge adsorption, resulting in the up-regulation of the expression of target molecules of miRNAs [37—39]. We therefore hypothesized that *HCG18* could regulate HK-2 through a certain miRNA. Then, to probe the intermediate factors of *HCG18* and HK-2, we targeted miR-512-3p by the bioinformatics analysis and reading literature. By the luciferase reporter assay and RNA pull-down assay, we confirmed that miR-512-3p has binding sites to both *HCG18* and HK-2 and may be an intermediate factor in the regulation of HK-2 by *HCG18*. However, although a large body of literature has consistently shown that miR-512-3p has a tumor suppressor effect in a variety of tumors, two studies have yielded very different results regarding the role of miR-512-3p in PCa. Wang et al. [13] showed that in PCa, miR-512-3p is tumor suppressive and can be titrated by the tumor-promoting lncRNA *AFAP1-AS1*; however, Rao et al. [16] concluded that miR-512-3p may have a proliferative function in PCa cells using the raw bioinformatics analysis. In our experiments, *in vitro* experiments showed that miR-512-3p mimic could reduce the proliferation, invasion, and migration ability of PCa cells, while inhibitors of miR-512-3p had the opposite effect; *in vivo* experiments also showed that analogs of miR-512-3p could inhibit tumor growth, while inhibitors could promote tumor growth. Our experimental results are consistent with those of Wang et al. [13], but contrary to those of Rao et al. [16]. We believe that this inconsistency may be related to the insufficient number of specimen cases included in the bioinformatics database or the selection of different databases.

Our results combined the lncRNA with the Warburg effect and proposed that *HCG18* could regulate HK-2 via miR-512-3p and thus participate in the mechanism of the Warburg effect affecting tumor progression. In addition, our study validated the role of miR-512-3p with more experiments, providing a basis for the oncogenic effect of miR-512-3p in PCa. Our study also has limitations: this study has not yet included a clinical sample for analysis, and we will further validate the proposed pathway in this study by collecting a large amount of clinical data and a long-term follow-up in a follow-up study. Overall, our study suggests that the lncRNA *HCG18*/miR-512-3p/HK-2 axis is involved in PCa progression and this study may contribute to the development of diagnostic and therapeutic tools for PCa.

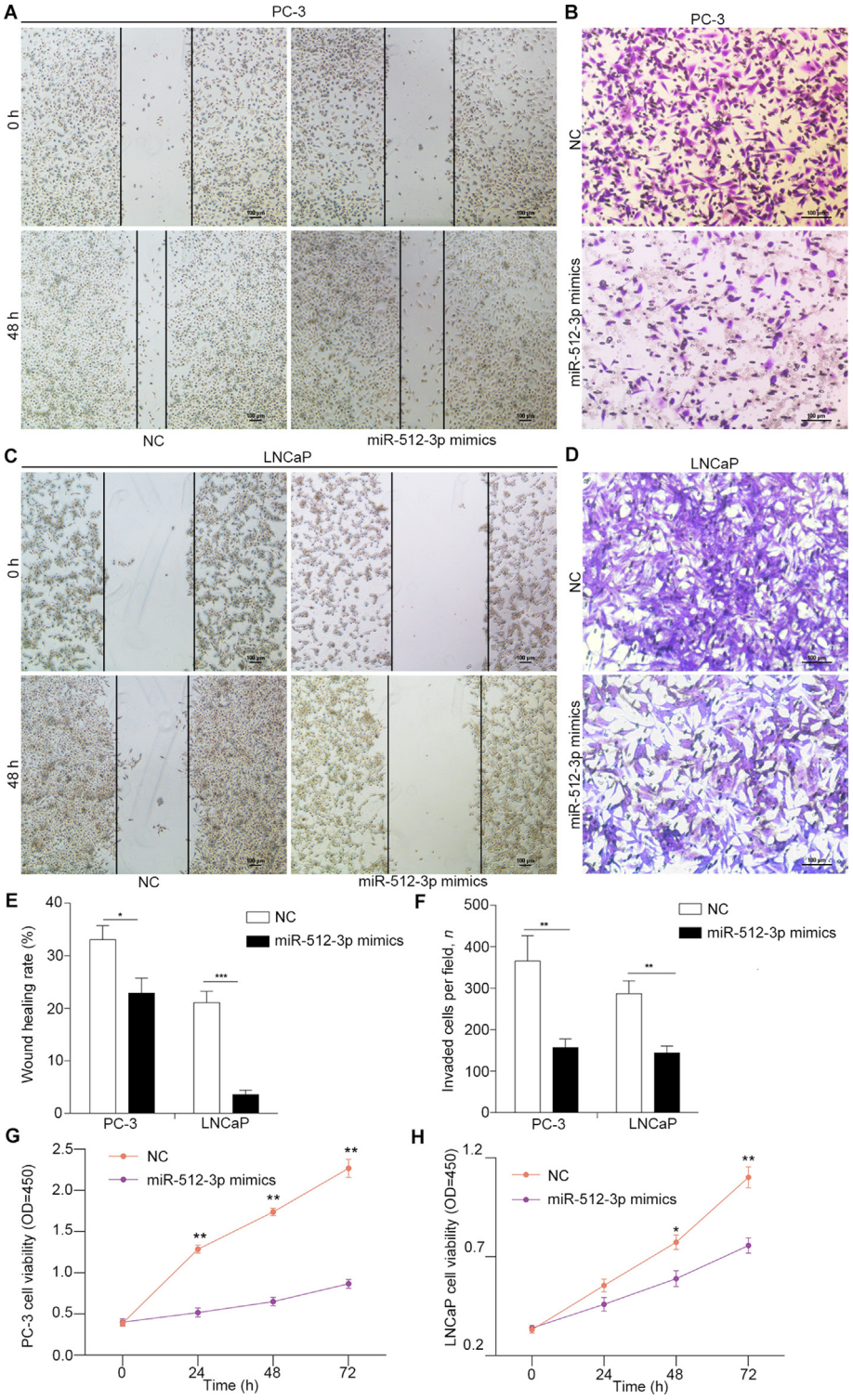


Figure 4 miR-512-3p directly interacts with *HCG18* and HK-2 and inhibits the progression of prostate cancer cells. (A) Analysis of PC-3 cell migratory capacity using wound healing assay; (B) Analysis of PC-3 cell invasive capacity using transwell assay; (C) Analysis of LNCaP cell migratory capacity using wound healing assay; (D) Analysis of LNCaP cell invasive capacity using transwell assay; (E) The statistical chart of healing experiments comparing cell migration capacity; (F) The statistical chart of transwell assay comparing cell invasion capacity; (G and H) Analysis of PC-3 and LNCaP cell viability using cell counting kit-8 solution (Dojindo, Tokyo, Japan). NC, negative control; OD, optical density. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Scale bars, 100 μm.

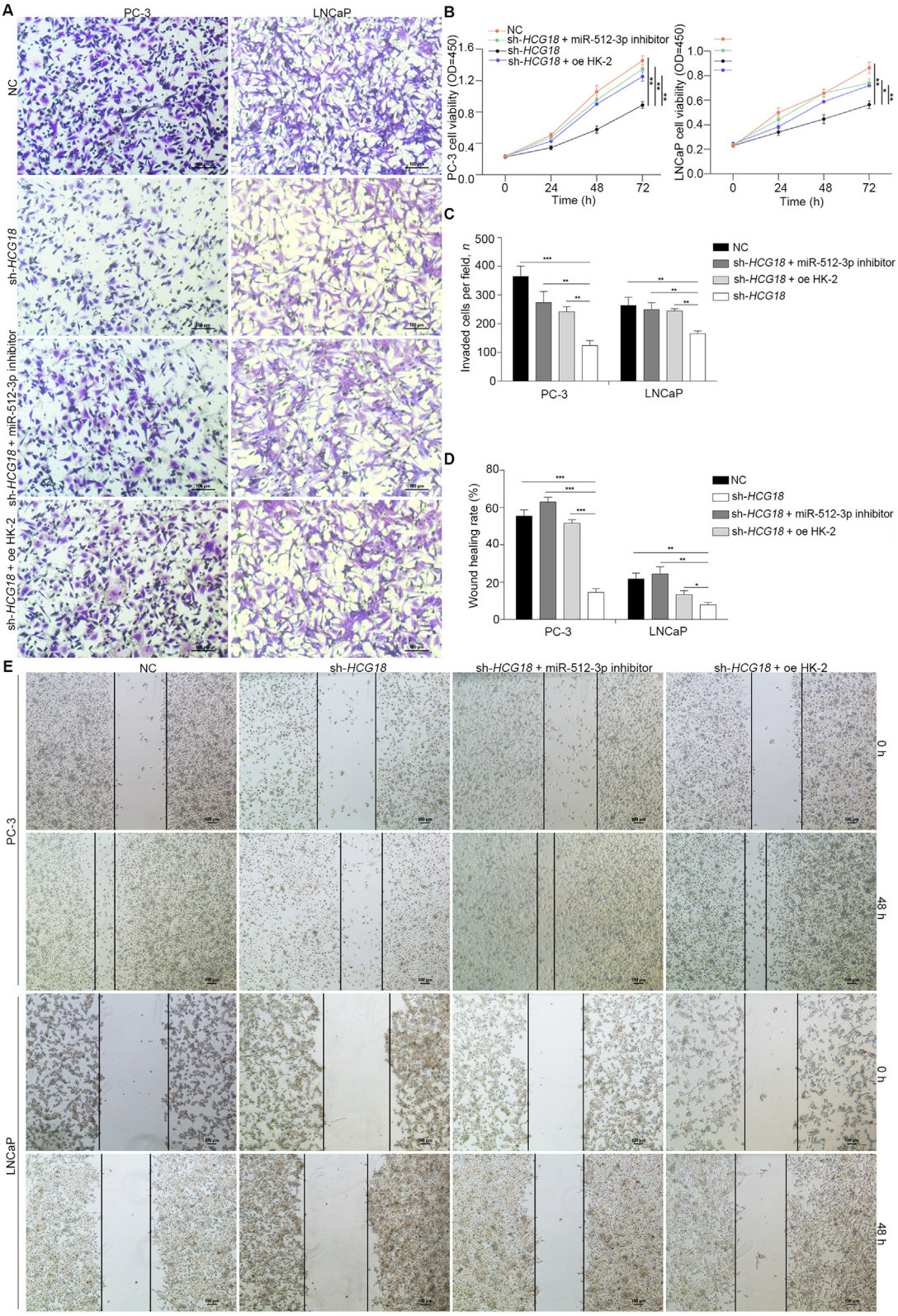


Figure 5 LncRNA *HCG18* promotes PCa progression via the LncRNA *HCG18*/miR-512-3p/HK-2 axis. (A) Analysis of PC-3 and LNCaP cell invasive capacity using the transwell assay; (B) Analysis of PC-3 and LNCaP cell viability using the cell counting kit-8 solution (Dojindo, Tokyo, Japan); (C) The statistical chart of the transwell assay comparing cell invasion capacity; (D) The statistical chart of healing experiments comparing cell migration capacity; (E) Analysis of PC-3 and LNCaP cell migratory capacity using the wound healing assay. OD, optical density; NC, negative control; PCa, prostate cancer; oe, overexpress. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. Scale bars, 100 μm.

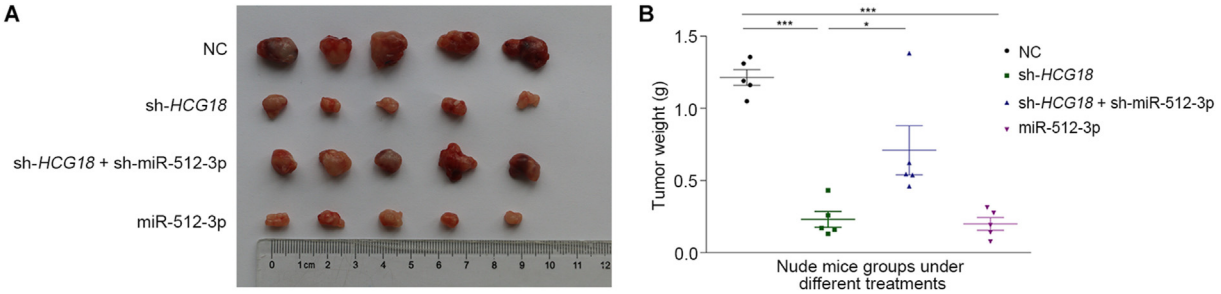


Figure 6 Animal experiments of nude mice groups under different treatments. (A) Tumor visualization. (B) Tumor weight analysis. NC, negative control. * $p<0.05$; *** $p<0.001$.

5. Conclusion

Our *in vivo* and *in vitro* experiments suggest that *HCG18* can play a role in promoting PCa progression by blocking the inhibition of HK-2 by miR-512-3p via sponge adsorption.

Author contributions

Study concept and design: Yaru Zhu, Haopeng Li, Wenhuizi Sun, Xi Chen, Gang Wu.
Data acquisition: Yaru Zhu, Zhijing Wang, Zhen Ren, Tong Zi.
Data analysis: Yaru Zhu, Xin Qin.
Drafting of manuscript: Yaru Zhu.
Critical revision of the manuscript: Gang Wu, Wenhuizi Sun, Xi Chen.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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