Article

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# Upregulation of FIGNL1 Mediated by SOX9 Promotes the Proliferation, Migration, Invasion and Cisplatin Resistance of Hepatocellular Carcinoma Cells

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**Background: Fidgetin-like 1 (FIGNL1) is overexpressed in hepatocellular carcinoma (HCC), and its high expression is correlated with the poor prognosis of patients. However, the exact role of FIGNL1 in the progression of HCC remains unclear. The purpose of this research was to investigate the role of FIGNL1 in the malignant biological behaviors of HCC and its potential mechanism.**
**Methods: In this study, the expressions of FIGNL1 and sex determining region Y (SRY)-related High Mobility Group (HMG) box-containing gene 9 (SOX9) were evaluated by quantitative Real-Time polymerase chain reaction (qRT-PCR) and western blot. JASPAR database was applied to predict the binding site between SOX9 and FIGNL1 promoter region. The proliferation, migration and invasion of Huh7 cells were detected by means of Cell Counting Kit-8 (CCK-8), colony formation assay, wound healing and transwell assays. Flow cytometry and western blot were employed to estimate cell apoptosis and cell cycle. With the application of luciferase reporter assay and chromatin immunoprecipitation (ChIP) assay, the combination of SOX9 with FIGNL1 was verified.**
**Results: FIGNL1 level was greatly elevated in HCC tissues ($p < 0.01$, $p < 0.001$) and cells ($p < 0.01$, $p < 0.001$). FIGNL1 knockdown suppressed Huh7 cell proliferation ($p < 0.001$), migration ($p < 0.01$) and invasion ($p < 0.001$) but induced cell cycle arrest ($p < 0.001$). Meanwhile, FIGNL1 silencing promoted cell apoptosis and repressed cisplatin resistance in Huh7 cells ($p < 0.001$). SOX9 expression was abundant in HCC tissues and cells ($p < 0.001$). SOX9 could bind to the FIGNL1 promoter and upregulate FIGNL1 expression. SOX9 elevation counteracted the impacts of FIGNL1 knockdown on Huh7 cell proliferation ($p < 0.001$), migration ($p < 0.01$), invasion ($p < 0.001$), apoptosis ($p < 0.05$, $p < 0.01$, $p < 0.001$), cell cycle arrest and cisplatin resistance ($p < 0.05$, $p < 0.01$, $p < 0.001$).**
**Conclusion: In conclusion, upregulation of FIGNL1 mediated by the transcription factor SOX9 accelerated HCC cell proliferation, metastasis and cisplatin resistance but suppressed cell apoptosis and cell cycle arrest, which might shed novel insights into the targeted therapy for HCC.**

**Keywords:** FIGNL1; hepatocellular carcinoma; SOX9; cisplatin resistance; cell cycle arrest

## Introduction

Liver cancer is a predominant contributor to deaths resulting from cancers [1]. It is believed that liver cancer is more prevalent in men than in women [2]. Hepatocellular carcinoma (HCC) is accountable for 85%–90% of primary liver cancer case [3]. There are various contributors to HCC, such as hepatitis B virus (HBV) and hepatitis C virus (HCV), heavy alcohol drinking and immune imbalance in viral cirrhosis [4]. At present, the effective treatment for patients who are diagnosed early includes surgical excision, local area treatment as well as liver transplantation [5]. Unfortunately, a number of patients are diagnosed with advanced HCC for which only palliative treatment is available [6]. In addition, approximately 90% of HCC patients have underlying cirrhosis with competing morbidity and mortality, making the treatment of HCC challenging [7]. For HCC treatment, the therapeutic effects and the enduring ability of a dysfunctional liver to the interventions should be balanced. Additionally, the 5-year overall survival rate for patients suffering from locally advanced and metastatic disease is approximately 10% and 3%, respectively [8,9]. In view of this, the investigation of biomarkers for disease diagnosis and management is of great urgency.

Fidgetin-like 1 (FIGNL1), which belongs to the AAA-ATPase protein family, affects the depolymerization of multiple protein complexes [10]. AAA-ATPase is involved in a diverse range of cell activities, like bio-synthesis of organelles, along with vesicular transport and cytoskeleton

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maintenance [11]. FIGNL1 has been found to be a critical regulator in hydrolase, ATPase, as well as double-strand break repair by homologous recombination [12]. Additionally, FIGNL1 is reported to be elevated in many human cancers, and FIGNL1 elevation has close association with poor prognosis in patients suffering from renal clear cell carcinoma (KIRP), low-grade glioma (LGG) and HCC [13]. However, the biological role that FIGNL1 plays in HCC remains incompletely understood.

The analysis results of JASPAR database (https://jaspar.genereg.net) showed that sex determining region Y (SRY)-related High Mobility Group (HMG) box-containing gene 9 (SOX9) had the potential to bind to FIGNL1 promoter region SOX9, a member of the SOX transcription factors family, plays a significant role in the organ development and cell-type specific differentiation [14,15]. As a pivotal transcription factor, SOX9 has been demonstrated to be upregulated in a wide range of cancers, such as breast cancer, prostate cancer, ovarian cancer and gastric adenocarcinoma [16–19]. Of note, SOX9 is highly expressed in HCC tissues, and HCC patients with high SOX9 expression are significantly associated with lower 5-year overall survival [20,21]. Particularly, SOX9 facilities the malignant biological behaviors of HCC and participates in the regulation of cisplatin-resistant in cancer cells [22–24]. Therefore, it was speculated that FIGNL1 might be transcriptionally regulated by SOX9 to affect the malignant development of HCC.

In this work, FIGNL1 expression in HCC tissues and cells was evaluated. Then, the impacts of FIGNL1 knockdown on the proliferation, migration, invasion and cisplatin resistance of HCC cells were explored. Further, the potential mechanisms related to SOX9 were analyzed. This study might expose a new therapeutic target for HCC treatment.

## Materials and Methods

### *Bioinformatic Analysis*

FIGNL1 and SOX9 expression and their correlation with prognosis in HCC were analyzed utilizing The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN; http://ualcan.path.uab.edu) database. Immunohistochemical images from normal tissues and HCC tumor tissues were shown in Human Protein Atlas (HPA) database (http://www.proteinatlas.org/). The JASPAR database (https://jaspar.genereg.net) was employed for the prediction of the binding site of SOX9 and FIGNL1 promoter region.

### *Cell Culture and Treatment*

Human HCC cell lines HCCLM3 (BNCC338460), Huh7 (BNCC337690), SNU-449 (BNCC359904), MHCC97H (BNCC359345) (BeNa Culture Collection (Beijing, China)) and human liver epithelial cell line THLE-3 (CRL-11233; American Type Culture Collection, Rockville, MD, USA) were cultivated in Roswell Park Memorial Institute (RPMI)-1640 medium (SH30809.01; Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS; 10099141C; Gibco, Waltham, MA, USA). The following were the requirements for cultivation: 37 °C and 5% $CO_2$. Cisplatin (479306-1G; Sigma-aldrich, St. Louis, MO, USA) was dissolved in double-distilled water. To obtain the drug-resistant phenotype Huh7/cisplatin (DDP), Huh7 cells were subjected to gradually increasing concentrations of DDP for 12 months with minor modifications [25]. An additional 2 μg/mL of DDP was added in the culture media to maintain the resistance phenotype [26]. Prior to the studies, Huh7/DDP cells were placed in medium without drug for 14 days. Cells were authenticated using short tandem repeat (STR) fragment analysis and all cell lines were free of mycoplasma contamination.

### *Cell Transfection*

The collected Huh7 cells were initially inoculated into 6-well plates. For the knockdown of FIGNL1 and SOX9, the small hairpin RNA (shRNA) specific to FIGNL1 (sh-FIGNL1-1, 5′-GCTCTTGCATCAGTGGTAATC-3′; sh-FIGNL1-2, 5′-GGAGCCAAAGATGATTGAACT-3′) or SOX9 (sh-SOX9-1, 5′-ACCTTCGATGTCAACGAGTTT-3′; sh-SOX9-2, 5′-CTCCACCTTCACCTACATGAA-3′) and negative control (NC) shRNA (sh-NC, 5′-TTCTCCGAACGTGTCACGT-3′) were provided by Gene Pharma (Shanghai, China). SOX9-specific pcDNA overexpression (oe) vector (oe-SOX9) and the empty vector (oe-NC) were constructed by Shanghai Genechem Co., Ltd. (Shanghai, China) With Lipofectamine 3000 reagent (L3000015; Invitrogen, Waltham, MA, USA), 100 nm recombinants were transfected into Huh7 cells in light of standard protocol. Following 48 h of transfection, the cells were collected for ensuing studies.

### *Cell Counting Kit-8 (CCK-8) Assay*

CCK-8 method was applied to estimate Huh7 cell viability. In brief, Huh7 cells were initially inoculated into 96-well plates. Following 24, 48, and 72 h of cultivation, 10 μL CCK-8 solution (C0038; Beyotime, Haimen, China) was introduced to cultivate Huh7 cells for additional 2 h. The optical density (OD) value run at 450 nm was recorded utilizing a microplate reader (BioTek ELX800; BioTek instruments, Winooski, VT, USA).

### *Colony Formation Assay*

After indicated treatment, Huh7 and Huh7/DDP cells seeded into six-well plates were cultivated in RPMI-1640 medium with 10% FBS. When the colonies were visible by eye, Huh7 and Huh7/DDP cells were fixed by 4% paraformaldehyde and stained by 0.1% crystal violet (HY-B0324A; MedChemExpress, Shanghai, China). The colonies were counted utilizing a light microscope

(CKX53; Olympus, Tokyo, Japan). The colony formation rate was calculated using ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA).

### *Wound Healing Assay*

After indicated treatment, Huh7 cells plated into a six-well plate and incubated until 90% cell fusion has reached. By virtue of white pipette tips, the wounds were made in cell monolayers. After phosphate buffer solution (PBS) washing, Huh7 cells were cultivated in serum-free medium. Images of the wound areas were pictured using a light microscope (CKX53; Olympus, Tokyo, Japan) and the width of the open area was immediately measured using ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA) to estimate motility.

### *Transwell Assay*

After indicated treatment, the collected cells were suspended in serum-free RPMI-1640 medium. The transwell chambers (3422; Corning Costar, Cambridge, MA, USA) were pretreated by Matrigel (356234; Becton Dickinson, San Jose, CA, USA). Cell suspensions were loaded onto the upper compartment whereas medium equipped with 10% FBS was introduced to the lower compartment. Following 24 h incubation, cotton swabs were adopted to clean the cells on the upper surface while cells in the bottom of the chamber insert was fixed by methanol and stained by 0.1% crystal violet (HY-B0324A; MedChemExpress, Shanghai, China). The images were taken under an inverted light microscope (CKX53; Olympus, Tokyo, Japan) and the invasive capacity was analyzed by using ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA).

### *Flow Cytometry Analysis*

Following indicated treatment, the collected Huh7 cells were incubated and then centrifugated at 300 ×g for 5 min to collect cell precipitates. After that, the pre-chilled PBS-rinsed precipitates were fixed by 70% ethanol. Afterwards, Huh7 cells were centrifugated at 500 ×g for 5 min again, following which was the exposure to a Cell Cycle Detection Kit (KGA512; KeyGene, Nanjing, China) in light of standard specifications. A FC500-MPL flow cytometry (Beckman Coulter, Brea, CA, USA) was adopted to analyze cell cycle distribution.

### *Cell Apoptosis Analysis*

Huh7 cell apoptotic level was appraised utilizing the fluorescein isothiocyanate (FITC) Annexin V/propidium iodide (PI) Apoptosis Detection Kit I (A200-02 100 rxn; Vazyme, Nanjing, China) in light of standard protocol. Briefly, after indicated treatment, cells were rinsed with precooled PBS, following which was the re-suspension in 100 μL binding buffer. After that, Huh7 cells were exposed to Annexin V-FITC and 10 mg/mL PI. Cell apoptosis was detected using a FC500-MPL flow cytometry (Beckman Coulter, Brea, CA, USA). Flowjo V7 software (Tree Star, Ashland, OR, USA) was employed to analyze cell apoptosis.

### *Dual-Luciferase Reporter Assay*

The fragments of FIGNL1 promoter were procured from Ensembl and JASPAR database was adopted to predict the binding of SOX9 and FIGNL1 promoter. The wild-type (WT) and mutated (MUT) fragments of FIGNL1 promoter were amplified utilizing polymerase chain reaction (PCR), which were then cloned into the firefly luciferase reporter plasmid, pGL3-basic vector (PR-E1761; Promega, Madison, WI, USA). Following the transfection, a Dual-Luciferase Reporter Assay Kit (RG028; Beyotime, Shanghai, China) was employed to estimate the luciferase activity. Firefly luciferase activity was normalized to that of Renilla.

### *Chromatin Immunoprecipitation (ChIP) Assay*

Following the cross-linking with 1% formaldehyde, Huh7 cells were quenched utilizing 2.5 M glycine. After cultivation with rabbit SOX9 antibody (Use 4 μg for 30 μg of chromatin; HY-P80335; MedChemExpress, Shanghai, China) or negative control rabbit IgG antibody (1:50; 3900S; Cell Signaling Technology, Boston, MA, USA), the DNA was immunoprecipitated from sonicated cell lysates and subsequently the FIGNL1 binding site was amplified by PCR. The annealing temperature was 60 °C. The input DNA and immunoprecipitated DNA were estimated utilizing PCR analysis.

### *RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)*

RNA that was isolated from sample Huh7 cells employing Trizol reagent (10296010; Invitrogen, Carlsbad, CA, USA) was converted to complementary DNA (cDNA) by virtue of PrimeScript RT Master Mix (RR014A, Takara, Shiga, Japan) in light of the operating manual, following which PCR was amplified using the SYBR Premix Ex Taq™ II kit (RR820B; Takara, Shiga, Japan) in light of recommended specifications on an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA, USA). The relative expression of target genes was assessed with comparative Ct approach. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* served as an internal reference. The sequences of primers used in this study were shown below: *FIGNL1* forward, 5′-GCAGCATCAAGGAGCATTGT-3′, reverse, 5′-GCTGGGCATTCTGAAGGAGT-3′; *SOX9* forward, 5′-GGAAGTCGGTGAAGAACGGG-3′, reverse, 5′-CAAGGTCGAGTGAGCTGTGT-3′; *GAPDH* forward, 5′-ACAACTTTGGTATCGTGGAAGG-3′, reverse, 5′-GCCATCACGCCACAGTTTC-3′.

### *Western Blot Analysis*

The concentration of proteins that were isolated from sample cells employing radioimmunoprecipitation assay ly-

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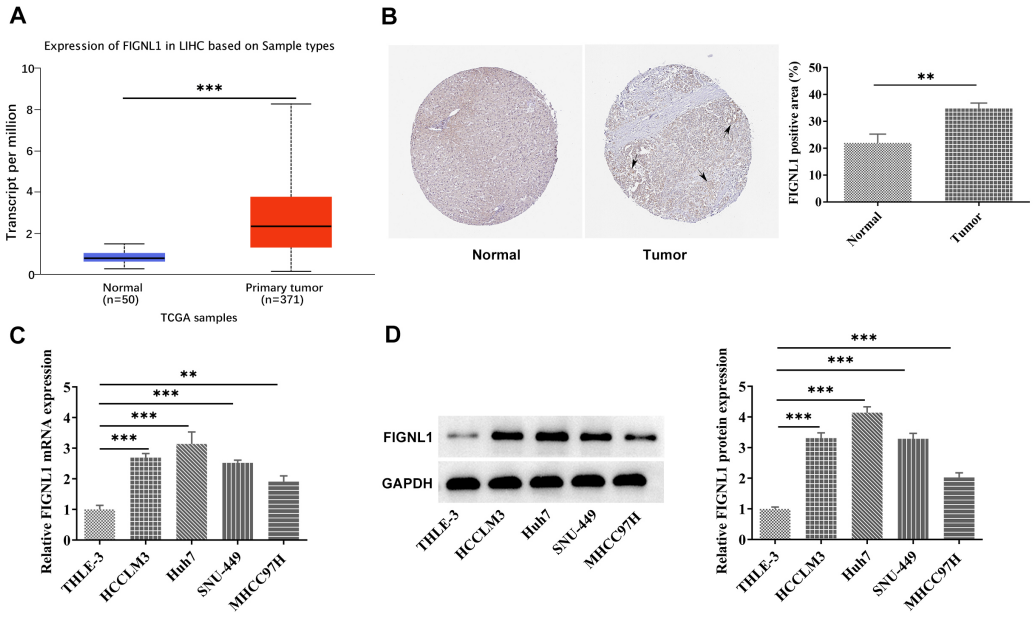


**Fig. 1. Fidgetin-like 1 (FIGNL1) is upregulated in hepatocellular carcinoma (HCC) tissues and cells.** (A) GEPIA database displayed the upregulation of FIGNL1 in HCC. (B) Human Protein Atlas (HPA) database showed immunohistochemical images from normal tissues (https://images.proteinatlas.org/55542/128833_A_7_4.jpg) and HCC tumor tissues (https://images.proteinatlas.org/55542/128834_B_9_5.jpg) of HCC patients. The areas marked by arrows were representative of the areas where FIGNL1 was expressed in HCC tumor tissues of HCC patients. (C) *FIGNL1* mRNA level in HCC cell lines (HCCLM3, Huh7, SNU-449, MHCC97H) and human liver epithelial cell line THLE-3 was detected utilizing quantitative Real-Time polymerase chain reaction (qRT-PCR). (D) FIGNL1 protein expression in HCC cell lines (HCCLM3, Huh7, SNU-449, MHCC97H) and human liver epithelial cell line THLE-3 was examined using western blot. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). $^{**}p < 0.01$, $^{***}p < 0.001$. TCGA, The Cancer Genome Atlas; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LIHC, liver hepatocellular carcinoma.

sis buffer (RIPA) buffer (P0013B; Beyotime, Shanghai, China) was quantified by virtue of bicinchoninic acid assay (BCA) Protein Assay Kits (BCA-01; Dingguo, Beijing, China) in light of recommended instructions. Impeded by 5% bovine serum albumin (BSA; ST023; Beyotime, Shanghai, China), the polyvinylidene fluoride (PVDF) membranes (IPVH0010; Millipore, St. Louis, MO, USA) that were to transfer proteins resolved by 10% sodium dodecyl-sulfate (SDS)-polyacrylamide gels were then immunoblotted with primary antibodies targeting FIGNL1 (ab185674; 1:1000, Rabbit; Abcam, Cambridge, UK), CyclinD1 (55506T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), CyclinD3 (ab183338; 1:100, Rabbit; Abcam, Cambridge, UK), cyclin-dependent kinase 2 (CDK2; 2546T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), cyclin-dependent kinase 4 (CDK4; 12790T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), B cell lymphoma protein-2 (Bcl-2; 3498T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), Bcl-2 associated X (Bax; 2772T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), cleaved poly (ADP-ribose) polymerase (PARP; 9541T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), PARP (9532T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA), SOX9 (HY-P80335; 1:500; Rabbit; MedChemExpress, Shanghai, China) and GAPDH (2118T; 1:1000, Rabbit; Cell Signaling Technology, Boston, MA, USA) overnight at 4 °C, prior to being probed with goat anti-rabbit IgG secondary antibody (ab7090; 1:5000, Rabbit; Abcam, Cambridge, UK). Protein bands were visualized with enhanced chemiluminescence (ECL) detection system (P0018AS; Beyotime, Shanghai, China). ImageJ 1.8.0 software (National Institutes of Health, Bethesda, MD, USA) was applied to calculate signal density.

### *Statistical Analysis*

All experiments were repeated three times independently. Data were displayed in the format of mean ± standard deviation (SD) and subjected to analysis by means of GraphPad Prism 8.0 software (GraphPad software, Inc., San Diego, CA, USA). For the demonstration of differences between two groups, student's *t*-test was employed while comparisons among three or more groups were analyzed utilizing one-way analysis of variance (ANOVA) with a post hoc Bonferroni multiple comparison test. *p* less than 0.05 was an indicative of statistical significance.

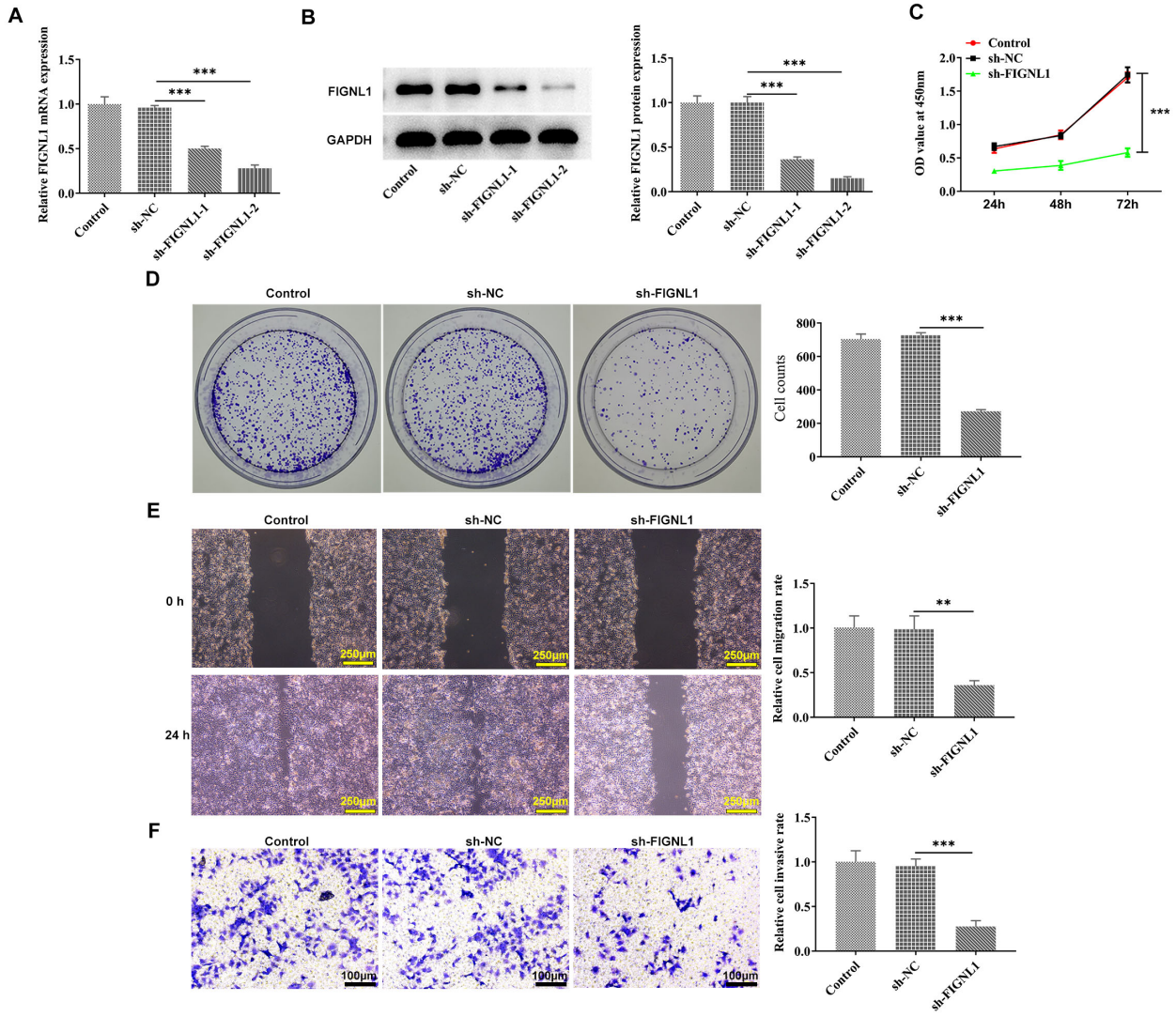


**Fig. 2. FIGNL1 silencing weakens the proliferative, migratory and invasive capabilities of Huh7 cells.** (A) *FIGNL1* mRNA level in Huh7 cells transfected with FIGNL1 interference plasmids was detected utilizing qRT-PCR. (B) FIGNL1 protein expression level in Huh7 cells transfected with FIGNL1 interference plasmids was detected utilizing western blot. (C) Cell proliferative ability was evaluated by Cell Counting Kit-8 (CCK-8) assay. (D) Cell colony-forming ability was evaluated employing colony formation assay. (E) Cell migratory ability was evaluated utilizing wound healing assay. (F) Cell invasive ability was assessed employing transwell assay. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). $^{**}p < 0.01$, $^{***}p < 0.001$. sh-NC, NC shRNA; sh-FIGNL1, shRNA specific to FIGNL1; NC, negative control; shRNA, small hairpin RNA; OD, optical density.

## Results

### *FIGNL1 Expression is Elevated in HCC Tissues and Cells*

FIGNL1 has been implicated in the advancement of malignant tumors. With the purpose of investigating the role that FIGNL1 played in HCC advancement, FIGNL1 expression in HCC patients and cells was initially evaluated. Data obtained from UALCAN database revealed that FIGNL1 expression was elevated in HCC tissues relative to the normal tissues (Fig. 1A, $p < 0.001$). HPA database showed that FIGNL1 expression level in HCC tumor tissues was significantly increased compared with that in the normal tissue (Fig. 1B, $p < 0.01$). Also, results obtained from qRT-PCR and western blot displayed that FIGNL1 expression in HCC cell lines was conspicuously elevated when compared with that in the THLE-3 cells (Fig. 1C,D, $p < 0.01$, $p < 0.001$). Among these HCC cells, FIGNL1 had the highest expression in Huh7 cells, thus we chose Huh7 cell line for the subsequent experiments.

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and Homeostatic Agents

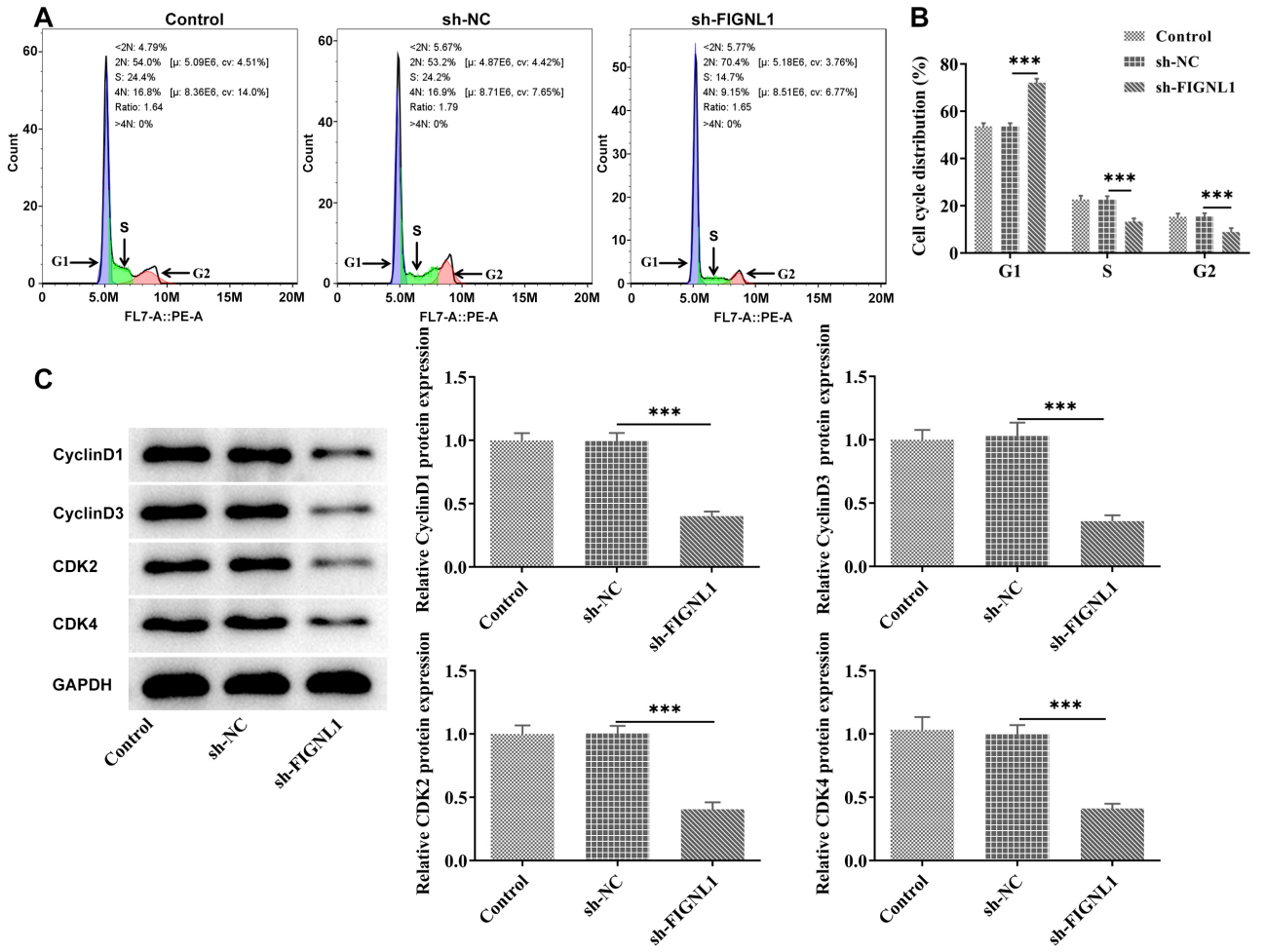


**Fig. 3. FIGNL1 knockdown facilitates cell cycle arrest of Huh7 cells.** (A,B) Cell cycle was appraised employing flow cytometry. (C) The expressions of proteins associated with cell cycle were appraised employing western blot. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). ***$p < 0.001$. CDK2, cyclin-dependent kinase 2; CDK4, cyclin-dependent kinase 4.

### *FIGNL1 Silencing Suppresses the Proliferative, Migratory and Invasive Capabilities of Huh7 Cells*

With the purpose of investigating the role that FIGNL1 played in HCC cell proliferation and metastasis, sh-FIGNL1 was transfected into Huh7 cells and the transfection efficacy was examined with qRT-PCR and western blot. Relative to the sh-NC group, FIGNL1 expression in Huh7 cells was conspicuously reduced by sh-FIGNL1 (Fig. 2A,B, $p < 0.001$). It was noted that sh-FIGNL1-2 contributed to lower expression of FIGNL1 in Huh7 cells, in this way, sh-FIGNL1-2 was selected for follow-up experiments. The impacts that sh-FIGNL1 exerted on cell proliferation, migration and invasion were investigated by CCK-8 assay, colony formation assay, wound healing, along with transwell assay. As Fig. 2C demonstrated, FIGNL1 silencing markedly reduced Huh7 cell proliferation relative to the sh-NC group ($p < 0.001$). Moreover, relative to the sh-NC group, the colony-forming ability of FIGNL1-silenced cells was decreased (Fig. 2D, $p < 0.001$). In addition, the migration and invasion of Huh7 cells were conspicuously repressed after knockdown of FIGNL1 expression (Fig. 2E,F, $p < 0.01$, $p < 0.001$).

### *FIGNL1 Silencing Accelerates Cell Cycle Arrest of Huh7 Cells*

Abnormal progression of cell cycle is a fundamental mechanism underlying tumorigenesis [27]. By means of flow cytometry, the regulatory effects of sh-FIGNL1 in cell cycle was assessed. As Fig. 3A,B depicted, FIGNL1 silencing remarkably elevated the population of cells in G1 phase whereas reduced that in S phase by contrast with the sh-NC group ($p < 0.001$). Moreover, FIGNL1 knockdown decreased the expression levels of CyclinD1, CyclinD3, CDK2 and CDK4 in Huh7 cells relative to the sh-NC group (Fig. 3C, $p < 0.001$).

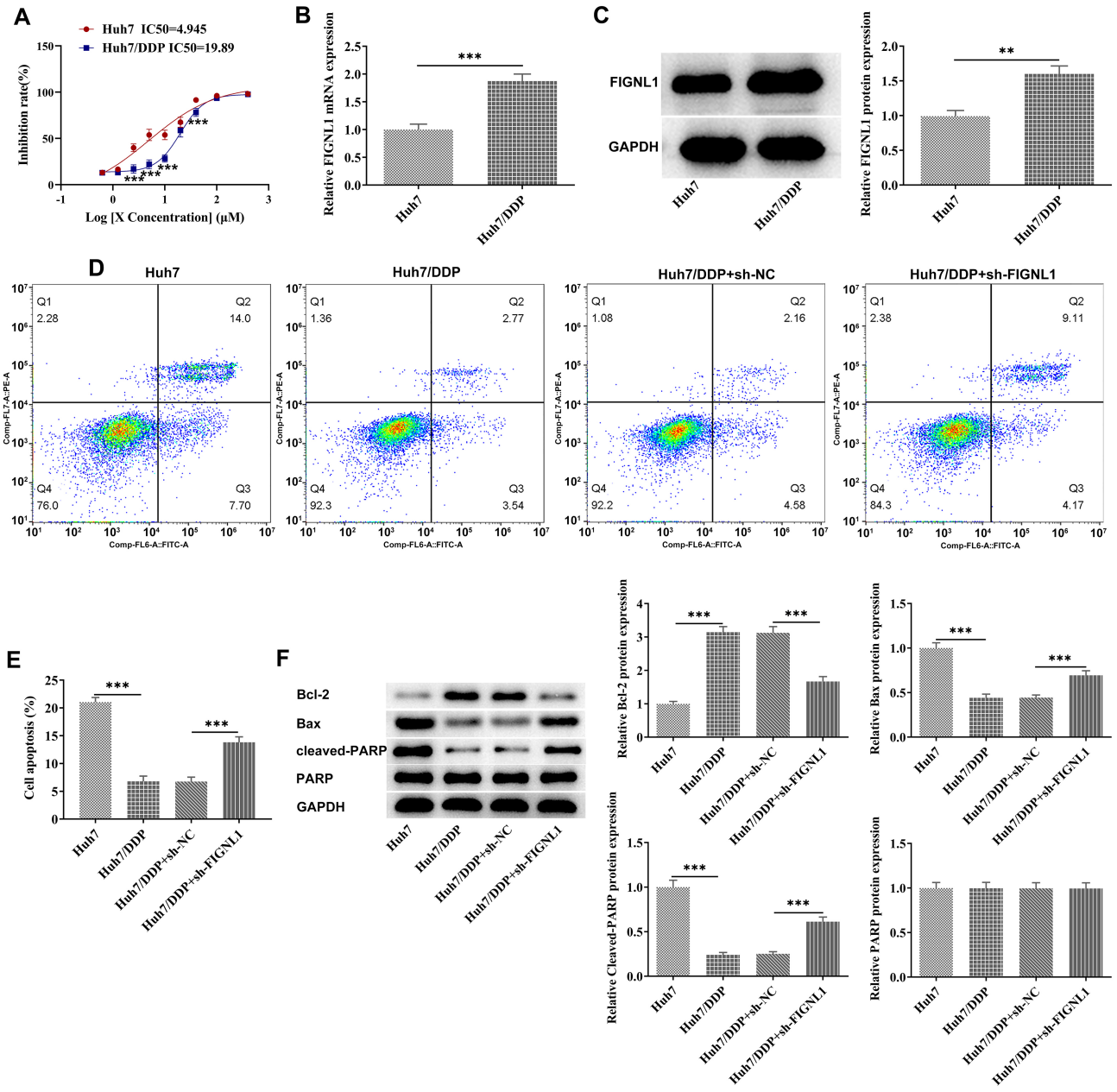


**Fig. 4. Knockdown of FIGNL1 restrains cisplatin resistance in Huh7 cells.** (A) The half maximal inhibitory concentration (IC50) was estimated employing CCK-8 assay. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). ***$p < 0.001$ vs. Huh7 group. (B) *FIGNL1* mRNA level in Huh7 and Huh7/DDP cells with FIGNL1 silence was appraised utilizing qRT-PCR. (C) FIGNL1 protein expression in Huh7 and Huh7/DDP cells with FIGNL1 silence was appraised utilizing western blot. (D,E) Cell apoptotic level was appraised employing flow cytometry. (F) The expressions of proteins associated with apoptosis were appraised employing western blot. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). **$p < 0.01$, ***$p < 0.001$. PARP, poly (ADP-ribose) polymerase; Bcl-2, B cell lymphoma protein-2; Bax, Bcl-2 associated X; DDP, cisplatin; FITC, fluorescein isothiocyanate.

### *Knockdown of FIGNL1 Restrains Cisplatin Resistance in Huh7 Cells*

Cisplatin resistance is a common trait in cancer treatment [28]. To observe the effects of FIGNL1 silencing on cisplatin resistance in HCC, DDP-resistant Huh7/DDP cell line was constructed by increasing the concentrations of cisplatin. CCK-8 assay showed that the half maximal inhibitory concentration (IC50) of DDP for Huh7 cells was 4.945 μM and the IC50 of DDP for Huh7/DDP cells was 19.89 μM (Fig. 4A). As Fig. 4B,C depicted, FIGNL1 expression was markedly increased in Huh7/DDP cells compared with that in untreated Huh7 cells ($p < 0.01$, $p <$

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BIOLOGICAL REGULATORS
and Homeostatic Agents

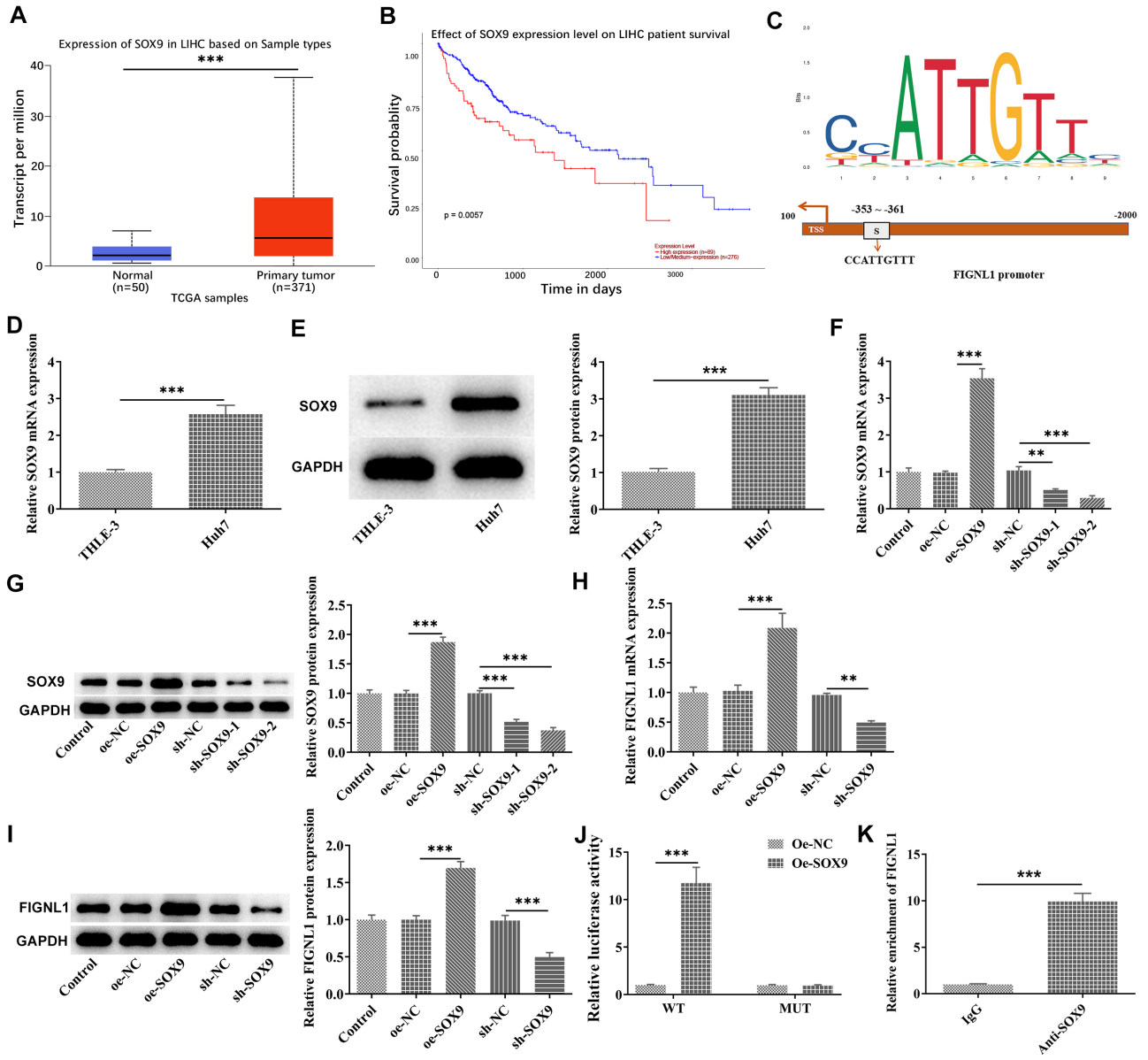


**Fig. 5. Sex determining region Y (SRY)-related High Mobility Group (HMG) box-containing gene 9 (SOX9) is distinctly elevated in HCC tissues and cells and regulates FIGNL1 expression.** (A) GEPIA database demonstrated the upregulation of SOX9 in HCC patients. (B) GEPIA database displayed the relationship between SOX9 expression and the survival of HCC patients. (C) The binding site of SOX9 and FIGNL1 promoter. (D) *SOX9* mRNA level in Huh7 cells was appraised employing qRT-PCR. (E) SOX9 protein expression in Huh7 cells was appraised employing western blot. (F) *SOX9* mRNA level in Huh7 cells with SOX9-specific pcDNA overexpression (oe) vector (oe-SOX9) or sh-SOX9 was appraised employing qRT-PCR. (G) SOX9 protein expression in Huh7 cells with oe-SOX9 or sh-SOX9 was appraised employing western blot. (H) *FIGNL1* mRNA level in Huh7 cells with oe-SOX9 or sh-SOX9 was appraised employing qRT-PCR. (I) FIGNL1 protein expression in Huh7 cells with oe-SOX9 or sh-SOX9 was appraised employing western blot. (J) Luciferase reporter assay detected the luciferase activity of FIGNL1 promoter. (K) Chromatin immunoprecipitation (ChIP) assay verified the binding of SOX9 and FIGNL1 promoter. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). $^{**}p < 0.01$, $^{***}p < 0.001$. LIHC, liver hepatocellular carcinoma; WT, wild-type; MUT, mutated.

0.001). Then, Huh7 and Huh7/DDP cells were treated with 5 μM DDP, and cell apoptosis was evaluated by flow cytometry and western blot. As shown in Fig. 4D–F, the apoptosis rate of Huh7/DDP cells was obviously decreased compared with Huh7 cells, which was then partially increased by FIGNL1 silencing ($p < 0.001$), accompanied by diminished Bcl-2 expression and elevated Bax and cleaved PARP expressions in Huh7/DDP + sh-FIGNL1 group ($p < 0.001$).

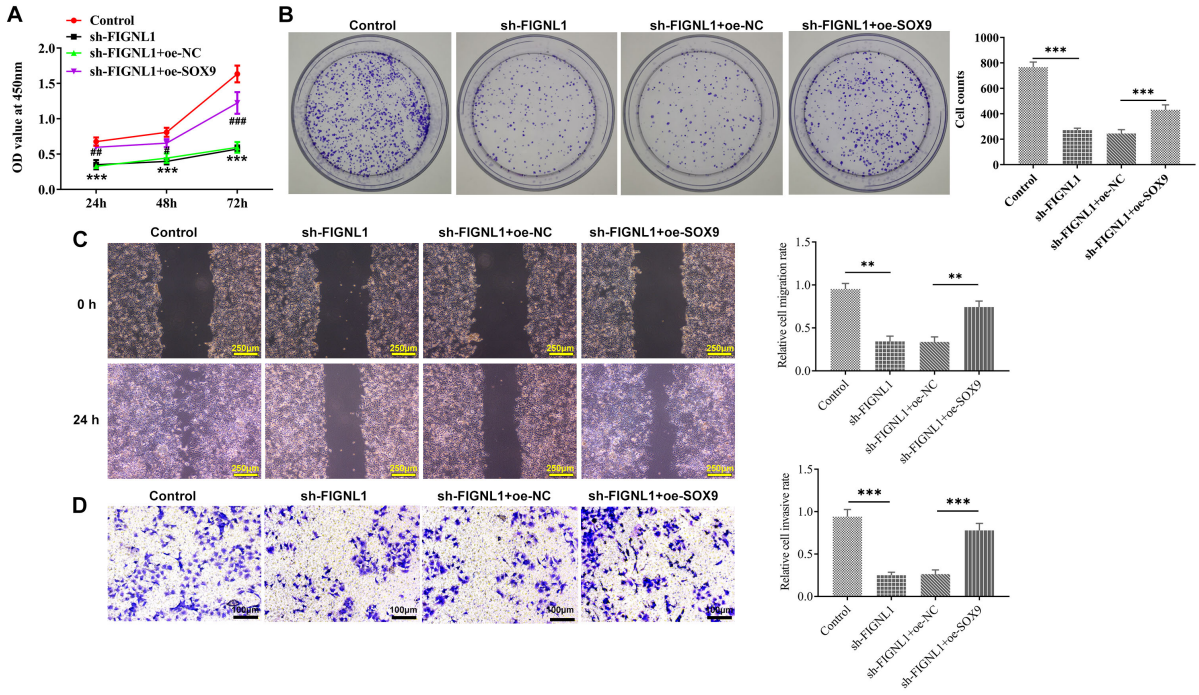


**Fig. 6. SOX9 overexpression facilitated the proliferative, migratory and invasive capabilities of Huh7 cells.** (A) Cell proliferative capability was evaluated employing CCK-8 assay. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). ***$p < 0.001$ vs. control group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. sh-FIGNL1 + empty vector (oe-NC) group. (B) Cell colony-forming ability was appraised employing colony formation assay. (C) Cell migratory capability was appraised employing wound healing assay. (D) Cell invasive capability was appraised employing transwell assay. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). **$p < 0.01$, ***$p < 0.001$. OD, optical density.

### *SOX9 is Distinctly Elevated in HCC Tissues and Cells and Regulates FIGNL1 Expression*

SOX9 is supposed to be an emerging driver of cancer progression and drug resistance [29]. Considering this, the expression of SOX9 was also assessed. As shown in Fig. 5A,B, SOX9 expression was remarkably increased in HCC tissues relative to the normal tissues and SOX9 elevation had close association with the poor prognosis of patients suffering from HCC ($p < 0.001$). The binding site of SOX9 and FIGNL1 promoter was predicted by JASPAR database (Fig. 5C). As Fig. 5D,E illustrated, a significant increase in SOX9 expression was observed in Huh7 cells relative to the THLE-3 cells ($p < 0.001$). To explore the biological role that SOX9 played in Huh7 cells, SOX9 was overexpressed and silenced in Huh7 cells. It was noted that SOX9 had lower expression in sh-SOX9-2 group, thus, sh-SOX9-2 was chosen for follow-up studies (Fig. 5F,G). As presented in Fig. 5H,I, SOX9 overexpression elevated FIGNL1 expression and SOX9 silencing inhibited FIGNL1 expression. In addition, the luciferase activity of the wild-type FIGNL1 promoter was significantly increased after overexpressing SOX9 (Fig. 5J). It was also illuminated that FIGNL1 was abundant in anti-SOX9 group, by contrast with the IgG group (Fig. 5K, $p < 0.001$).

### *Overexpression of SOX9 Reverses the Effects of FIGNL1 Silencing on Huh7 and Huh7/DDP Cells*

To explore the mechanism of FIGNL1 associated with SOX9 in Huh7 cells, the cellular experiments were conducted. As presented in Fig. 6A, SOX9 overexpression significantly enhanced the decreased proliferation ability of Huh7 cells mediated by FIGNL1 silencing ($p < 0.05$, $p < 0.01$, $p < 0.001$). Consistently, the decreased number of colonies in Huh7 cells due to FIGNL1 deficiency was increased after the transfection with oe-SOX9 (Fig. 6B, $p < 0.01$, $p < 0.001$). Additionally, the decreased migration and invasion of FIGNL1-silenced Huh7 cells were partially increased after overexpressing SOX9 (Fig. 6C,D, $p < 0.01$, $p < 0.001$). Moreover, SOX9 overexpression reduced the cell population in G1 phase whereas elevated the fraction of S-phase cells in FIGNL1-silenced Huh7 cells (Fig. 7A,B). Western blot results revealed that SOX9 overexpression counteracted the suppressive impacts of FIGNL1 silencing on the expression of CyclinD1, CyclinD3, CDK2 and CDK4 in Huh7 cells (Fig. 7C, $p < 0.05$, $p < 0.01$, $p < 0.001$). Furthermore, an obvious decrease in cell apoptosis rate in FIGNL1-silenced Huh7/DDP cells was observed after overexpressing SOX9 when compared with the Huh7/DDP + sh-FIGNL1 + oe-NC group (Fig. 8A,B, $p < 0.05$, $p < 0.01$, $p < 0.001$). Meanwhile, the upregulation

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BIOLOGICAL REGULATORS
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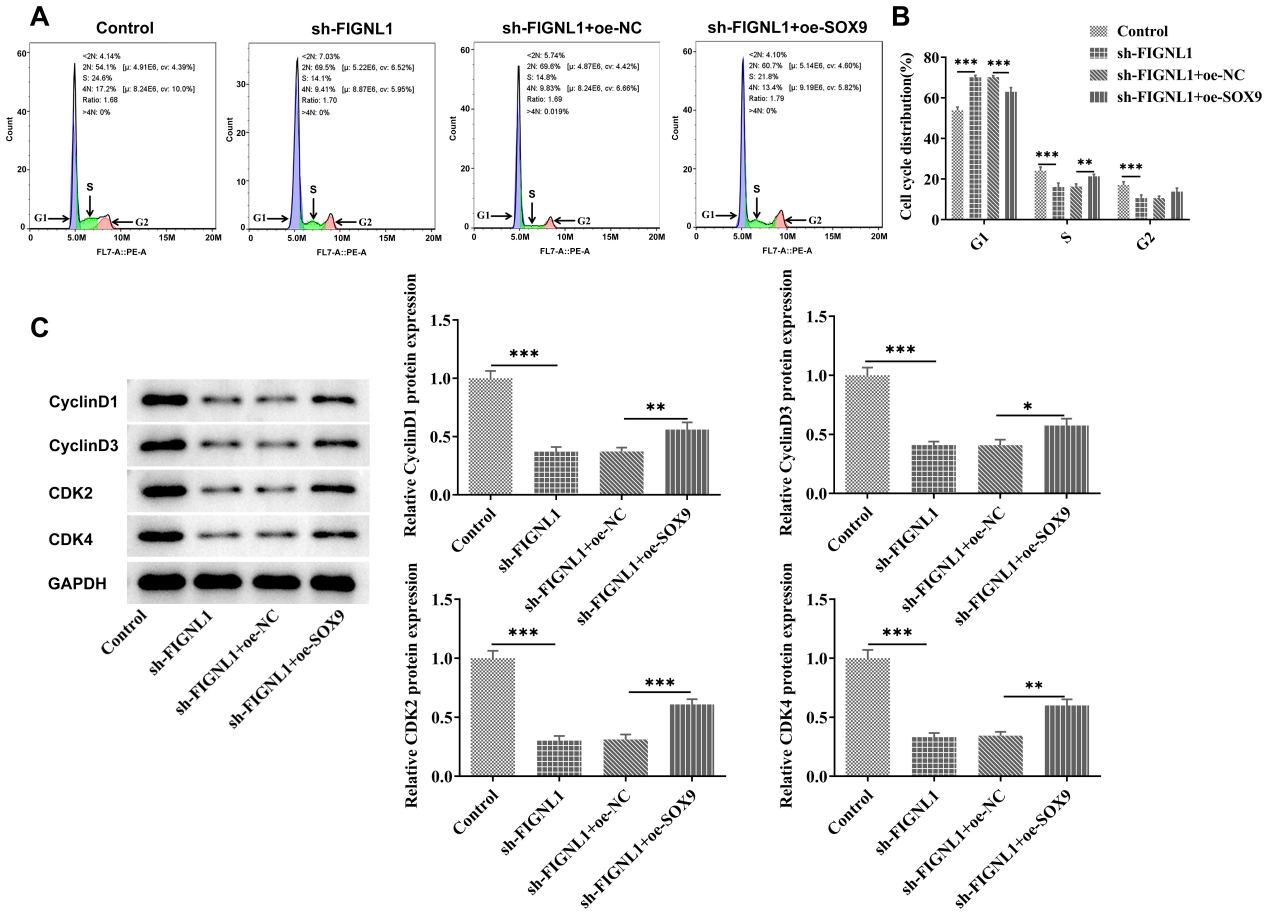


**Fig. 7. SOX9 overexpression inhibits the cell cycle progression of Huh7 cells.** (A,B) Cell cycle was appraised employing flow cytometry. (C) The expression levels of proteins associated with cell cycle were appraised employing western blot. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

of SOX9 led to increased Bcl-2 expression level and reduced Bax and cleaved PARP expression levels in FIGNL1-silenced Huh7/DDP cells (Fig. 8C).

## Discussion

Despite the fact that great advances have been made in medical, locoregional as well as surgical therapies, HCC is still a main contributor to cancer death worldwide [30]. In clinical trials, targeted therapies have been shown to have great potential for HCC treatment, but there are still some problems such as low objective response rate (ORR) as well as adaptive or acquired resistance due the existence of HCC heterogeneity attributed to its morphological diversity, signal transduction network and discrepancies of microenvironment [31,32]. Therefore, researches on HCC classification and response to therapy are of great significance. In this study, FIGNL1 expression was elevated in HCC tissues and cells and had association with the poor prognosis of patients suffering from HCC. FIGNL1 silencing was uncovered to repress the proliferative, migratory and invasive capabilities of Huh7 cells, induce cell cycle arrest, facilitate cell apoptosis, as well as suppress cisplatin resistance. In addition, SOX9 was evidenced to regulate the transcriptional activity of FIGNL1 and SOX9 overexpression reversed the impacts of FIGNL1 silencing on HCC cells.

A study has elaborated that FIGNL1 expression is greatly increased in non-small cell lung cancer (NSCLC), especially in cisplatin-resistant cell lines [33]. Li and coworkers [34] have put forward that FIGNL1 overexpression can promote cell proliferation, reduce G1 phase arrest, suppress cell apoptosis, and increase migration ability. Additionally, Ma *et al*. [35] have showed that relative to normal tissues, FIGNL1 expression is upregulated in small cell lung cancer (SCLC) patients, and FIGNL1 overexpression enhances the resistance of NCI-H446 cells to cisplatin and etoposide. According to Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE101728) (GSE101728), FIGNL1 expression was elevated in HCC tissues relative to paracancerous tissues. Nevertheless, the special role of FIGNL1 in HCC is still obscure. Results from UALCAN showed that FIGNL1 was conspicuously upregulated in HCC and had close association with poor prognosis. Moreover, our results also re-

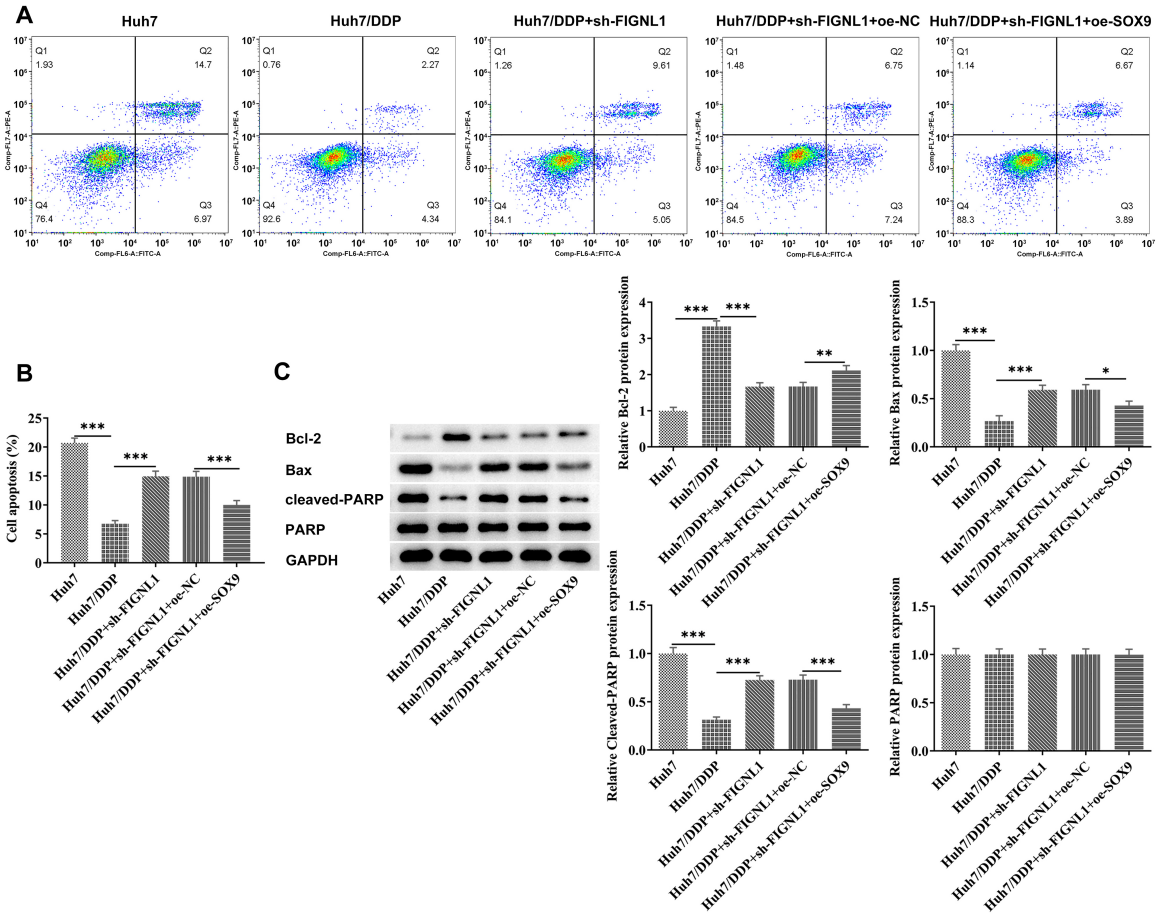


**Fig. 8. SOX9 overexpression suppresses Huh7/DDP cell apoptotic level.** (A,B) Cell apoptotic level was appraised employing flow cytometry. (C) The expressions of proteins associated with apoptosis were appraised employing western bolt. Data from three independent replicates (n = 3) were presented as mean ± standard deviation (SD). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

vealed that FIGNL1 expression was ascending in HCC cell lines. When FIGNL1 was silenced, the proliferative and migratory capabilities of Huh7 cells were inhibited, the cell cycle arrest was promoted and cisplatin resistance was repressed.

SOX9, which belongs to a family of twenty SRY-related HMG box-containing proteins, is a pivotal transcription factor in chondrocytes and a lineage essential for skeletogenesis [36]. Emerging evidence have suggested that SOX9 plays an oncogenic role in different types of human cancers [37,38]. By contrast with the adjacent normal tissues, SOX9 expression was ascending in HCC tumor tissues and the inhibition of SOX9 can restrain the proliferative, migratory and invasive capabilities of hepatoma cells and facilitate cell apoptosis [39]. What's more, SOX9 expression level is increased in cisplatin-resistant cervical cancer cells, and the regulatory axis of lncRNA ANXA2P2/miR-361-3p/SOX9 promotes cell growth and cisplatin resistance in cervical cancer [40]. Wang *et al.* [41] have claimed that SOX9 knockdown facilitates cell apoptosis in breast cancer, and represses the cell cycle in G0/G1 phase. Herein, JASPAR database predicted the binding of SOX9 and FIGNL1 promoter, suggesting that SOX9 might transcriptionally activate FIGNL1 expression by binding to FIGNL1 promoter region, which was subsequently attested applying luciferase reporter assay and ChIP assay. SOX9 expression was markedly up-regulated in HCC patients and SOX9 elevation had close association with the poor prognosis of patients suffering from HCC. Rescue experiments implicated that SOX9 overexpression partially counteracted the impacts of FIGNL1 silencing on cell proliferative, migratory, invasive abilities, cell cycle and cisplatin resistance in HCC.

## Conclusion

All in all, the study revealed the inhibitory role that FIGNL1 knockdown played in the malignant phenotypes of HCC. Mechanically, FIGNL1 could be transcriptionally activated by SOX9 to influence the advancement of HCC. These findings might be informative for the investigation of targeted therapies for HCC.

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## Availability of Data and Materials

Enquiries about data availability should be directed to the corresponding author.

## Author Contributions

WZ and NL contributed to the concept and designed the research study. WZ, PC, WLZ and FYL performed the research. WZ, PC and NL contributed to the analysis and interpretation of the data. WZ drafted the manuscript and NL revised it. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

## Acknowledgment

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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