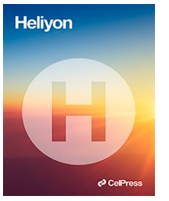
Heliyon 9 (2023) e18127

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



# SP1/RNASEH2A accelerates the development of hepatocellular carcinoma by regulating EMT

Yunhe Hao [a], Rui Zou [a], Jiashou Tao [a], Manfei Jiang [a], Duo Li [a,*]

[a] Department of Hepatobiliary Surgery, Hainan Cancer Hospital, No. 9 West 4th Changbin Street, Xiuying District, Haikou, 570100, Hainan, China

ARTICLE INFO

*Keywords:*
Hepatocellular carcinomas
Proliferation
Invasion
Migration
EMT

ABSTRACT

*Background:* The expression level of Ribonuclease H2, subunit A (RNASEH2A) in hepatocellular carcinoma (HCC) has been reported, but the function of RNASEH2A on HCC cells development and the related molecular mechanisms remain unclear. Herein, we intend to explore the upstream regulator of RNASEH2A and its role in the HCC progression.

*Methods:* GEPIA website was employed to determine the level of RNASEH2A in HCC tissues and get a survival analysis. After reducing RNASEH2A expression by RNA interference, cell counting kit-8, colony formation, Western blot, Transwell and wound healing assays were performed to estimate the malignant properties of HCC cells. The transcriptional factor of RNASEH2A was predicted by UCSC and JASPAR database and confirmed by dual luciferase assay and Ch-IP assay. The expression level of EMT pathway related molecules was determined by western blotting.

*Results:* An increased expression of RNASEH2A was presented in HCC and predicted worse prognosis of HCC patients. Functionally, the results demonstrated that depletion of RNASEH2A suppressed HCC cell proliferation, cell cycle, migration and invasion. Moreover, we illustrated that SP1 targeted to the promoter of RNASEH2A and modulated its expression in HCC cell lines. RNASEH2A knockdown counteracted the function of SP1 overexpression in modulating HCC cell growth, cell cycle, and mobility. Then, our data showed that the SP1/RNASEH2A axis affected the malignant behaviors of HCC cells by regulating EMT process.

*Conclusions:* In summary, these results demonstrated that RNASEH2A promoted HCC cells development through regulating EMT process and was transcriptionally modulated by SP1.

## 1. Introduction

Globally, liver cancer is the most common fatal malignancy [1]. Patients with liver cancer are often diagnosed at advanced stages, resulting in its worse prognosis. Liver cancer is the only deadly cancer whose incidence is increasing year by year [2]. Of all liver cancer cases, greater than 90% are hepatocellular carcinomas (HCC). For patients with advanced HCC, treatment options include: *trans*--arterial chemoembolization, which improves 2-year survival by 23% in patients with advanced HCC compared to conservative treatment; oral sorafenib is the most accepted option in advanced cases. However, less than 1/3 patients benefited from the treatment, and resistance is evident within 6 months of starting the programme [3]. Long-term use of chemotherapeutic agents may also cause other problems, such as toxicity and/or drug ineffectiveness. Therefore, neither ablation nor chemotherapy can significantly improve the prognosis of this devastating disease at present. Further research is necessary to find a better treatment for HCC.

* Corresponding author.
*E-mail address:* 15607605835@163.com (D. Li).

https://doi.org/10.1016/j.heliyon.2023.e18127
Received 5 February 2023; Received in revised form 4 July 2023; Accepted 7 July 2023
Available online 8 July 2023

Ribonuclease H2, subunit A (RNASEH2A), a part of RNASEH2, affects the activity of endoribonuclease [4,5]. Previously, several reports showed that RNASEH2A mutations resulted in hepatitis [6,7]. Additionally, logistic regression analysis presented that RNASEH2A gene expression was closely related to invasive prostate cancer [8]. RNASEH2A, as a highly significant RNA-binding protein, was identified as a targetable putative oncogene in neuroblastoma [9]. Through integrated bioinformatics analysis, a study reported that RNASEH2A was highly expressed in HCC [10]. Moreover, in HCC, hypoxia-induced RNASEH2A limited the activation of cGAS-STING signaling and predicted worse prognosis [11]. Previously, a report revealed that RNASEH2A was an underlying anti-tumor drug target in transformed stem cells [12]. However, how RNASEH2A is implicated in the occurrence and progression of HCC is still unknown.

Specificity protein 1 (SP1), as a transcription factor, expressed throughout the body that drives the activation of oncogenes necessary for tumor progression, metastasis, and survival [13,14]. Analysis from GEPIA website showed that SP1 expression was highly expressed in liver cancer tissues, and high expression SP1 resulted in poor outcome of liver cancer patients. However, the detailed function of SP1 in HCC cells remains to be further studied.

Epithelial-mesenchymal transition (EMT) usually occurs in the initiation of tumor invasion and metastasis [15]. During EMT process, epithelial cells lose polarity and gain invasive abilities to become mesenchymal stem cells [16]. Moreover, mesenchymal markers such as N-cadherin is upregulated. These gene expression changes are regulated by EMT-induced transcription factors such as Snail, Slug, and Twist [17]. Twist and Snail independently regulate and synergistically promote EMT in hepatocellular carcinoma (HCC) [18]. Analysis from GEPIA website demonstrated that RNASEH2A expression was associated to N-cadherin and Snail2. However, the detailed relationship between RNASEH2A and EMT needs to be further explored.

Herein, analysis from GEPIA website demonstrated that RNASEH2A expression was significantly increased in HCC samples, and its overexpression was positively related to the adverse outcomes of HCC patients. Moreover, knockdown of RNASEH2A limited the growth, cell cycle, and mobility of HCC cells, and SP1 (specificity protein 1) was identified as a specific transcription factor of RNASEH2A.

## 2. Materials and methods

### 2.1. Gene expression profiling interactive analysis (GEPIA)

GEPIA website was used to evaluate the mRNA expression of the genes, which from the TCGA and the GTEx projects (http://gepia.cancer-pku.cn/). In our study, GEPIA was used to explore the expression level of RNASEH2A in liver cancer tissues and to get a survival analysis, as well as to evaluate the relationship between RNASEH2A and related genes.

### 2.2. Cell culture

Human normal hepatocyte (L-02) and hepatocarcinoma cell lines (Huh7, HepG2, Hep3B, and BEL-7402) were got from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) including with 10% fetal bovine serum (FBS), 1% antibiotics (penicillin/streptomycin, Gibco, USA). All cells were routinely cultured in a 5% $CO_2$ humidified incubator at 37 °C.

### 2.3. RNA extraction and qPCR

The whole RNA was generated from cultured cells using a TRIzol Kit, based on the specification of the manufacturers (Invitrogen, USA). Then, 2 μg RNA was used to reverse transcribed into cDNA by using Reverse Transcription Kit (TaKaRa, Dalian, China). qPCR was performed on the ABI 7500 PCR System using SYBR qPCR Mix (TaKaRa). The housekeeping gene GAPDH was used for an internal control, and relative mRNA expression was calculated using $2^{-\Delta\Delta Ct}$ method. The primers were listed as below:

### 2.4. RNASEH2A sense: 5′-AATGGAGGACACGGACTTTG-3′

Antisense: 5′-GACAGGGAGTTCAGGTTGTATT-3.
GAPDH sense: 5′-CCCTTCATTGACCTCAACTACA-3′,
Antisense: 5′-ATGACAAGCTTCCCGTTCTC-3'.

### 2.5. Cell transfection

Small interfering RNA (siRNA) was employed to knockdown the corresponding genes. Briefly, siRNA targeting RNASEH2A (si-RNASEH2A-1, 5′- GCGGGTCAAATACAACCTGAA-3′ or si-RNASEH2A-2, 5′-ACATCCTACTTCCTCAATGAA-3′), SP1 (si-SP1) and siRNA negative control (si-con, 5′-TTCTCCGAACGTGTCACGT-3′) were established by Sangon Biotech (Shanghai, China). For overexpression, SP1 overexpression plasmid was constructed and validated by Sangon Biotech. pcDNA3.1-SP1 was applied to establish HCC cells with SP1 upregulation. The procedures were listed as follows. First, the complete medium was removed, and cells were rinsed and put into 1 mL of Opti-MEM medium. Then, plasmid (1 μg) and lipofectamine 2000 were put in 100 μL Opti-MEM medium.

For transient transfection, cells were implanted in 6-, 24- and 96-well plates. The next day, transfection was performed by using Lipofectamine 2000 (Thermo Fisher Scientific), followed by cultivation at 37 °C for one day.

### 2.6. Measuring cell viability by cell counting Kit-8 (CCK-8) assay

Transfected cells were implanted in 96-well plates with the density of $3 \times 10^3$ cells/well. Subsequently, 10 μL of CCK-8 solution (Beyotime, China) was filled into per well. After cultivation at 37 °C for 1.5 h, the optical density was measured at 450 nm wave length.

### 2.7. Measuring cell proliferation by clone formation assay

HepG2 and Hep3B cells were seeded in 6-well plates (3000 cells/well) and cultivated overnight to make them adherent. Then, cells were treated with si-RNASEH2A/SP1-OE and incubated for 2 weeks. Next, cells were rinsed with PBS, fixed with methanol, and stained with crystal violet. Finally, the colonies were imaged. The assay was performed with 3 replicates.

### 2.8. Western blotting

The same amounts of protein extracts (25 μg) were isolated by SDS-PAGE, and electroblotted to PVDF membranes. The membranes were blotted with the primary antibodies including RNASEH2A (1:1000, ab83943, Abcam), SP1 (1:2000, ab227383, Abcam), CDK1 (1:10000, ab133327, Abcam), CDK2 (1:3000, ab235941, Abcam), N-cadherin (1:1000, ab245117, Abcam), Snail2 (1:1000, ab180714, Abcam), Vimentin (1:1000, ab45939, Abcam) and GAPDH (1:5000, ab8245, Abcam), and then with the corresponding secondary antibody. Enhanced chemilum inescence (ECL) was applied to detect chemiluminescence signals.

### 2.9. Measuring cell invasion by Transwell assay

Cell invasion ability was estimated by using Transwell chamber. First, Matrigel was pre-coated into the upper chamber, cells were cultured without serum for 12 h and were digested with trypsin. Then, mixture was put into the upper chamber, and the complete culture medium was put into the lower chamber. After 24 h, cells were stained, and the number of invaded cells in random 5 fields was measured by microscope.

### 2.10. Measuring cell migration by wound healing assay

Migration of HepG2 and Hep3B cells was analyzed by using 24-well wound healing assay kit basing on supplier's direction. Briefly, cells were implanted and a sterile 20 μL plastic pipette tip was applied to make a wound. Each well was added serum-free DMEM including si-RNASEH2A/SP1-OE and was incubated for 24 h. The wounds were imaged and Image-J software was applied to measure the wound healing areas.

### 2.11. Dual-luciferase reporter assay

Wild type (WT) of RNASEH2A promoter sequences including two SP1 targeting sequences were amplified or mutated (MUT-1 and MUT-2) and then inserted into pGL3-basic vector. Then, the cells were transfected with WT or mutant luciferase reporter vector with control vector or SP1 overexpression plasmid. After 48 h, the cells were tested by using the Dual-luciferase Assay System from Promega.

### 2.12. Chromatin immunoprecipitation (ChIP) assay

To explore whether SP1 could bind to the promoter of RNASEH2A, a ChIP assay was performed based on the ChIP assay kit procedures. In brief, the treated cells were gathered and lysed. Antibody against RNASEH2A and IgG were used for immunoprecipitation. PCR amplification of the precipitated DNA was carried out.

### 2.13. Statistical analysis

All experimental data were processed with SPSS 22.0 and GraphPad Prism 8.0 software, and were expressed as mean ± standard deviation (SD). For two-group comparison, Student's *t*-test was applied. One-way ANOVA followed by Bonferroni's post hoc test was applied for analyzing difference among three or more groups. A $p < 0.05$ was deemed as statistical significant.

## 3. Results

RNASEH2A overexpression was occurred in HCC samples and RNASEH2A knockdown suppressed the growth and mobility in HCC cells.

Analysis from GEPIA website demonstrated that RNASEH2A was over expressed in HCC tissues relative to that in the control tissues (Fig. S1A). Simultaneously, the expression level of RNASEH2A in Huh7, HepG2, Hep3B, and BEL-7402 was significantly increased than that in normal L-02 cells (Fig. 1A–B). HepG2 and Hep3B cells with high RNASEH2A expression were selected for the subsequent functional studies. Survival analysis showed that RNASEH2A overexpression resulted in a shorter survival time in HCC patients (Fig. S1B).

Given the importance of RNASEH2A in HCC, we conducted several biological assays to detect the function of RNASEH2A in HCC cells. To explore whether RNASEH2A served as a carcinogenic factor in HCC progression, gene knockdown experiments were carried out. In brief, si-con, si-RNASEH2A-1 or si-RNASEH2A-2 was transfected into HepG2 and Hep3B cells, and the interfering efficiency of si-RNASEH2A was examined by qPCR and western blotting assays (Fig. 1C–D). Analysis from GEPIA website showed that RNASEH2A expression was positively connected to the proliferative marker genes Ki67 and PCNA (Figs. S1C–D). Data from CCK-8 assay showed that si-RNASEH2A-1 or si-RNASEH2A-2 transfection resulted in obviously decreasing proliferative ability in HepG2 and Hep3B cells (Fig. 1E–F). Colony formation assay demonstrated that transfection with si-RNASEH2A-1 or si-RNASEH2A-2 significantly decreased

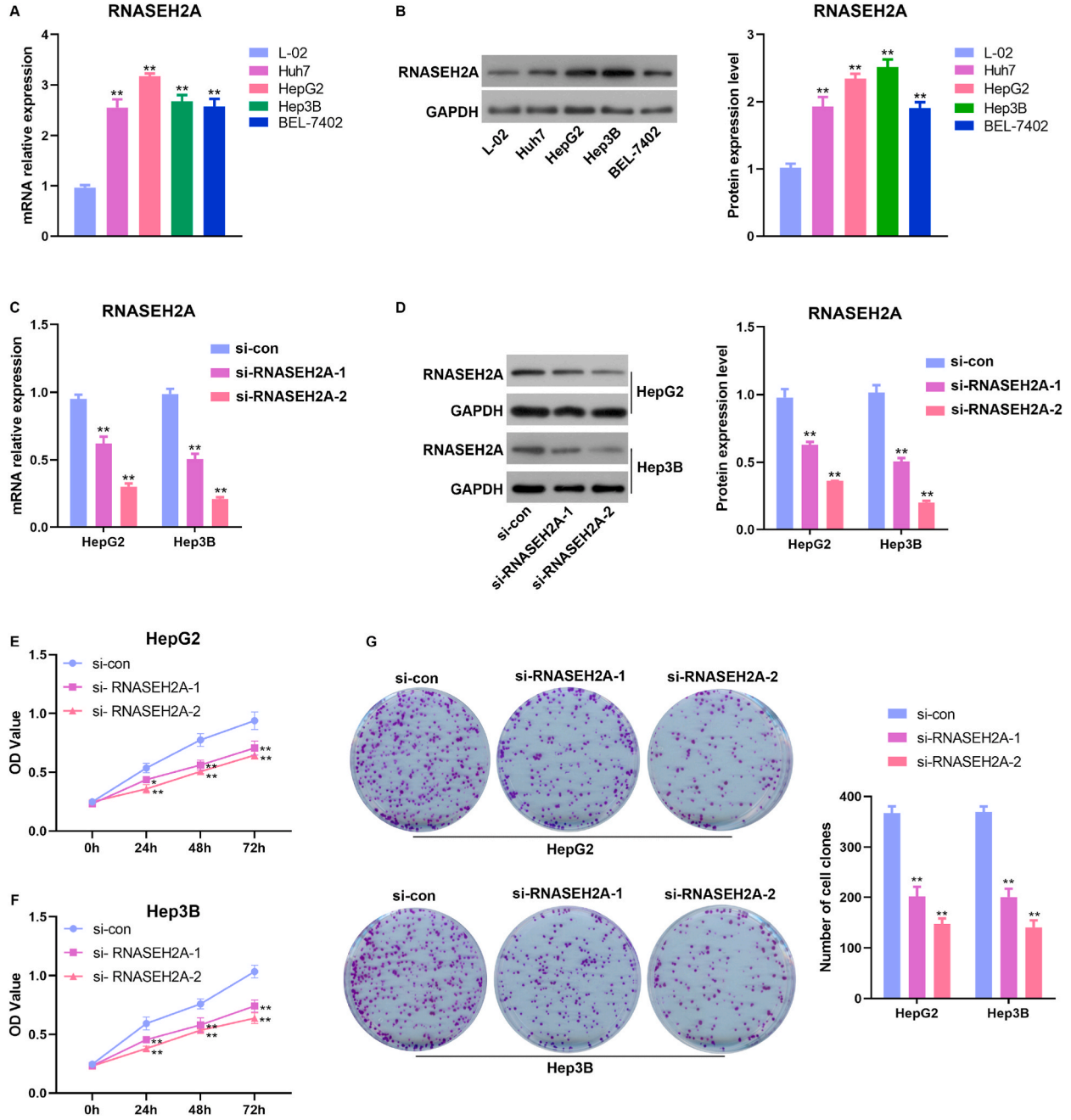



**Fig. 1.** RNASEH2A was highly expressed in HCC cell lines and promoted the proliferation of HCC cells. A-B. The mRNA and protein levels of RNASEH2A were significantly increased in HCC cell lines, **p < 0.01 vs. L-02. C-D. In HepG2 and Hep3B cells, transfection with si-RNASEH2A-1 or si-RNASEH2A-2 significantly decreased the mRNA and protein levels of RNASEH2A. E-F. Transfection with si-RNASEH2A-1 or si-RNASEH2A-2 obviously reduced the viability in HepG2 and Hep3B cells. G. In HepG2 and Hep3B cells, treatment with si-RNASEH2A-1 or si-RNASEH2A-2 obviously decreased the number of cell clones. *p < 0.05, **p < 0.01 vs. si-con.

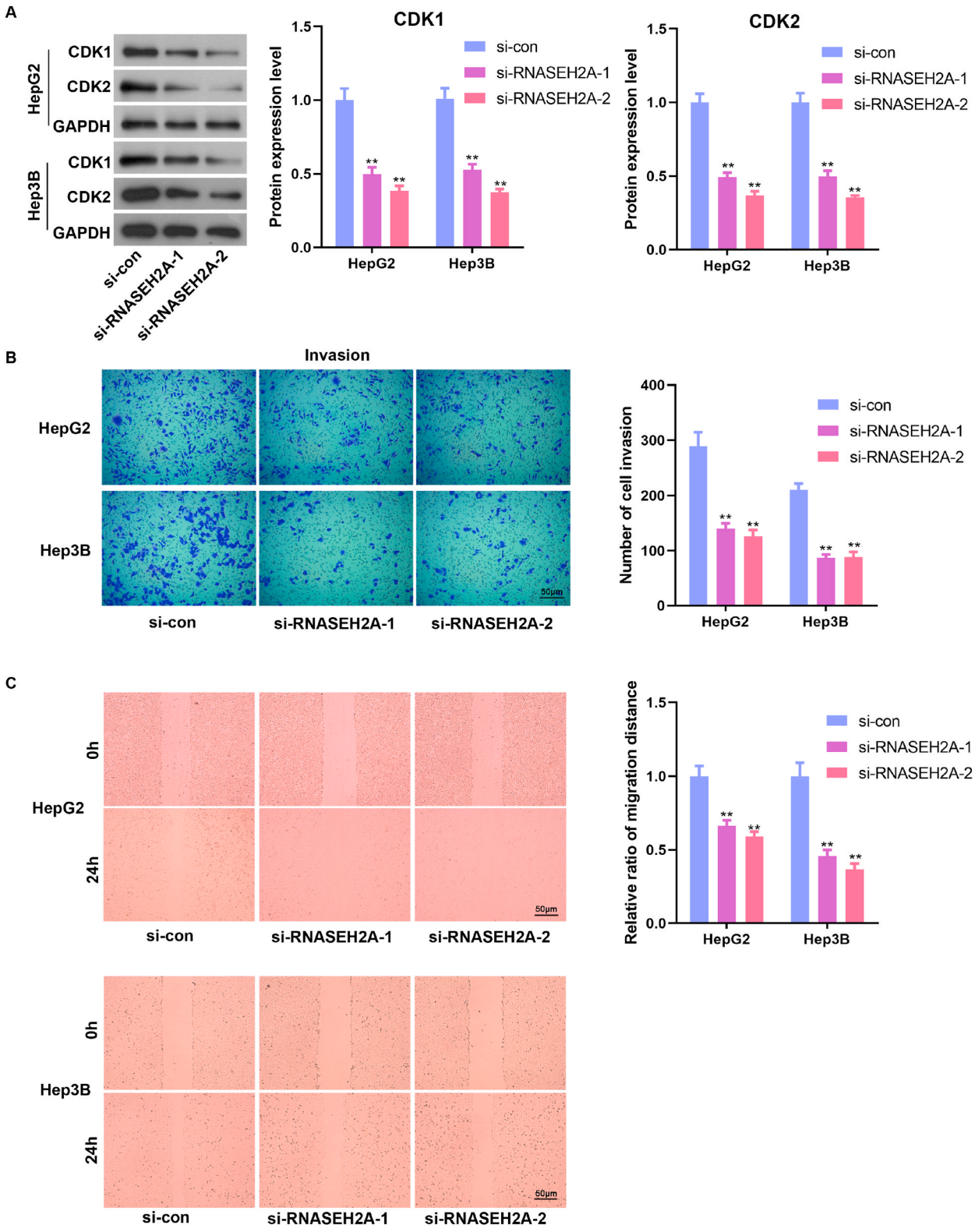



**Fig. 2.** Depletion of RNASEH2A significantly blocked the cell cycle, invasion and migration in HepG2 and Hep3B cells. A. In HepG2 and Hep3B cells, si-RNASEH2A-1 or si-RNASEH2A-2 stimulation obviously decreased the protein levels of CDK1 and CDK2. B. In HepG2 and Hep3B cells, si-RNASEH2A-1 or si-RNASEH2A-2 stimulation obviously reduced the number of cell invaded. C. In HepG2 and Hep3B cells, si-RNASEH2A-1 or si-RNASEH2A-2 stimulation obviously reduced the cell migration distance. **$p < 0.01$ vs. si-con. Scale bar = 50 μm.

the number of cell clones (Fig. 1G). Moreover, analysis from GEPIA website showed that RNASEH2A expression was positively connected to the cell cycle marker genes CDK1 and CDK2 (Figs. S1E–F). And data from the western blotting assay suggested that the protein patterns of cell cycle-related genes CDK1 and CDK2 in transfected HepG2 and Hep3B cells were obviously suppressed after si-RNASEH2A-1 or si-RNASEH2A-2 transfection (Fig. 2A). Besides, Transwell assay showed that transfection with si-RNASEH2A-1 or si-RNASEH2A-2 obviously diminished the invasion ability of HCC cells (Fig. 2B). Data from wound healing assay showed that si-RNASEH2A-1 or si-RNASEH2A-2 treatment obviously reduced the migratory distance of HepG2 and Hep3B cells (Fig. 2C). Collectively, the findings insinuated that RNASEH2A depletion suppressed the malignant properties of HCC cells.

Transcription factor SP1 directly bound to the promoter of RNASEH2A and modulated the expression of RNASEH2A in HCC cells.

To explore the regulatory mechanism of RNASEH2A in HCC, bioinformatics analysis was performed (UCSC, https://genome.ucsc.edu/), and the two binding sites between SP1 and RNASEH2A were presented (Fig. 3A). The results presented that SP1-OE treatment increased the luciferase activity, while transfection either MUT-1 or MUT-2 reduced the luciferase activity (Fig. 3B). Subsequently, data from Ch-IP assay showed that SP1 depletion diminished RNASEH2A (Fig. 3C). Furthermore, we also discovered that SP1 depletion remarkably inhibited the protein level of RNASEH2A in HepG2 or Hep3B cells, whereas overexpression of SP1 increased RNASEH2A expression (Fig. 3D). Besides that, analysis from GEPIA website indicated that SP1 was upregulated in HCC samples, and SP1 overexpression led to an unfavorable prognosis in HCC patients (Figs. S1G–H). Collectively, these results illustrated that SP1 bound to promoter region of RNASEH2A gene and regulated RNASEH2A expression.

RNASEH2A knockdown counteracted the effect of SP1 overexpression in regulated HCC cell proliferation, invasion, migration and cell cycle by regulating EMT process.

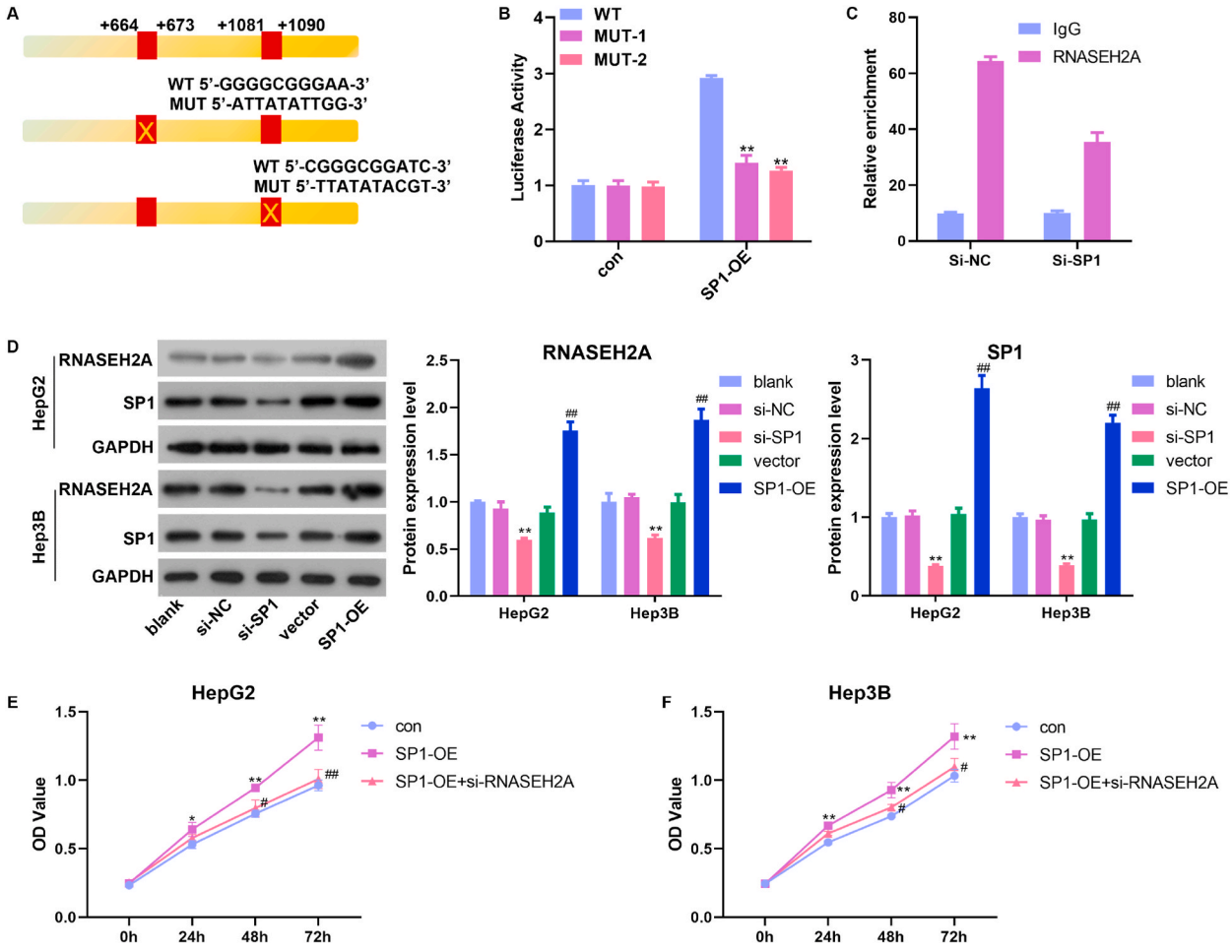



**Fig. 3.** SP1 was conformed as a transcriptional factor of RNASEH2A and modulate RNASEH2A expression. A. The targeting sequences between SP1 and RNASEH2A were presented. B. Overexpression of SP1 promoted luciferase activity, while mutated MUT-1 or MUT-2 resulted in diminished luciferase activity, **p < 0.01 vs. WT group. C. After si-con or si-SP1 treatment, Ch-IP assay was carried out, and RNASEH2A was detected by qPCR. D. The interference efficiency of si-SP1 and overexpression efficiency of SP1-OE were presented, knocked down of SP1 reduced RNASEH2A expression, while overexpression of SP1 increased RNASEH2A expression, **p < 0.01 vs. si-con, ##p < 0.01 vs. vector. E-F. The promoting effects of SP1-OE on the OD values of HepG2 and Hep3B cells were reduced after co-treatment of SP1-OE and si-RNASEH2A. *p < 0.05, **p < 0.01 vs. con, #p < 0.05, ##p < 0.01 vs. SP1-OE.

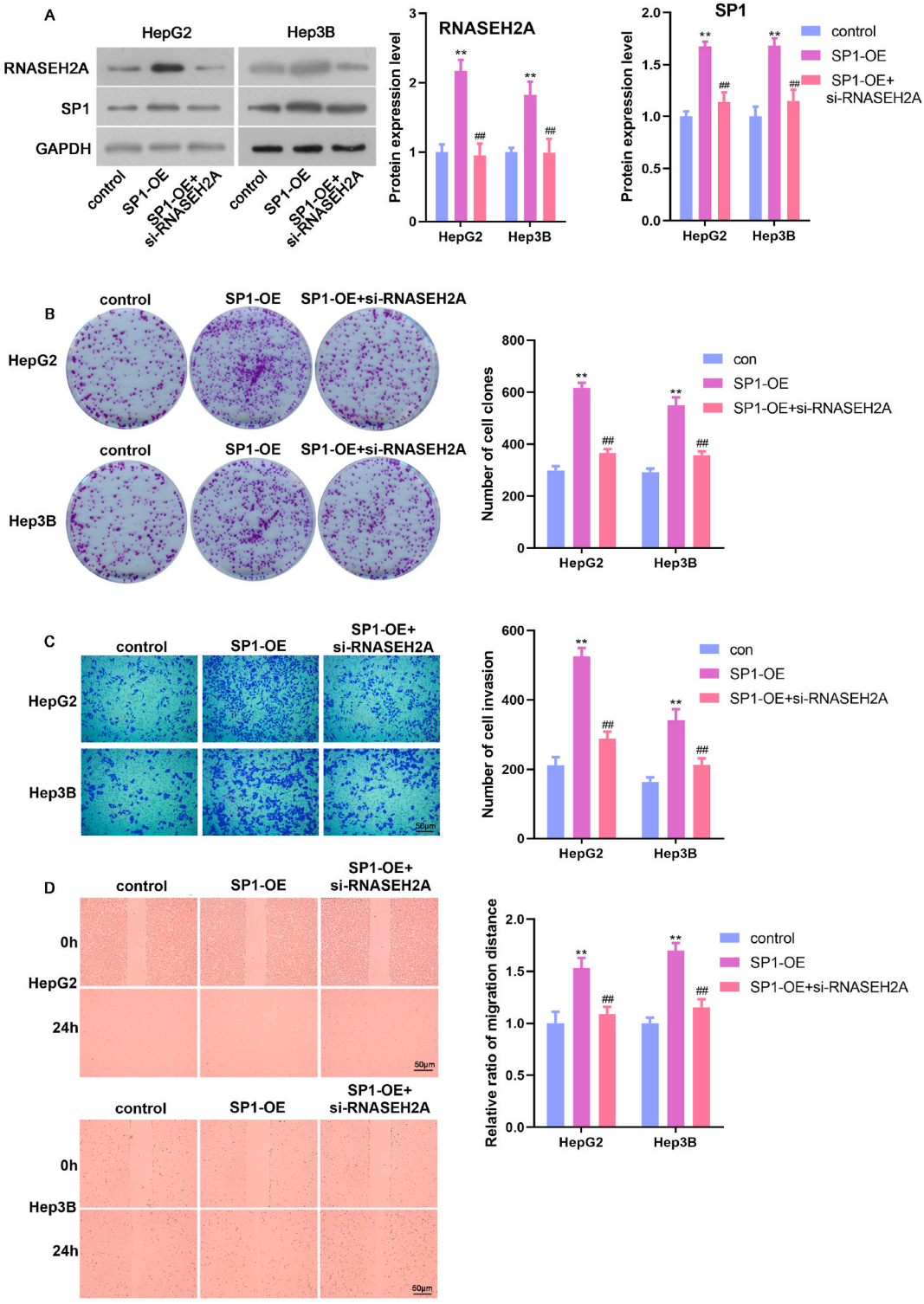



**Fig. 4.** Depletion of RNASEH2A antagonized the effect of SP1-OE in modulating HCC cell proliferation, invasion and migration. A. The protein levels of RNASEH2A and SP1 were measured in HepG2 and Hep3B cells. B. The promoting effects of SP1-OE on the colony number of HCC cells were diminished after co-treatment of SP1-OE and si-RNASEH2A. C. Upregulation of SP1 elevated the invasion number of HCC cells, while the function of SP1-OE was suppressed by si-RNASEH2A treatment. D. Overexpression of SP1 increased the migrated distance of HepG2 and Hep3B cells, while the effect of SP1-OE was diminished by si-RNASEH2A treatment. **p < 0.01 vs. con, ##p < 0.01 vs. SP1-OE. Scale bar = 50 μm.

Subsequently, HepG2 or Hep3B cells were transfected with SP1 overexpression plasmid, or SP1 overexpression plasmid & si-RNASEH2A. Analysis from CCK-8 demonstrated that SP1 overexpression obviously promoted the proliferation ability in HepG2 and Hep3B cells (Fig. 3E–F). However, the facilitation of SP1-OE on HCC cells growth was suppressed by si-RNASEH2A transfection. We measured the protein levels of RNASEH2A and SP1 in HepG2 and Hep3B cells after SP1-OE or SP1-OE + si-RNASEH2A treatment

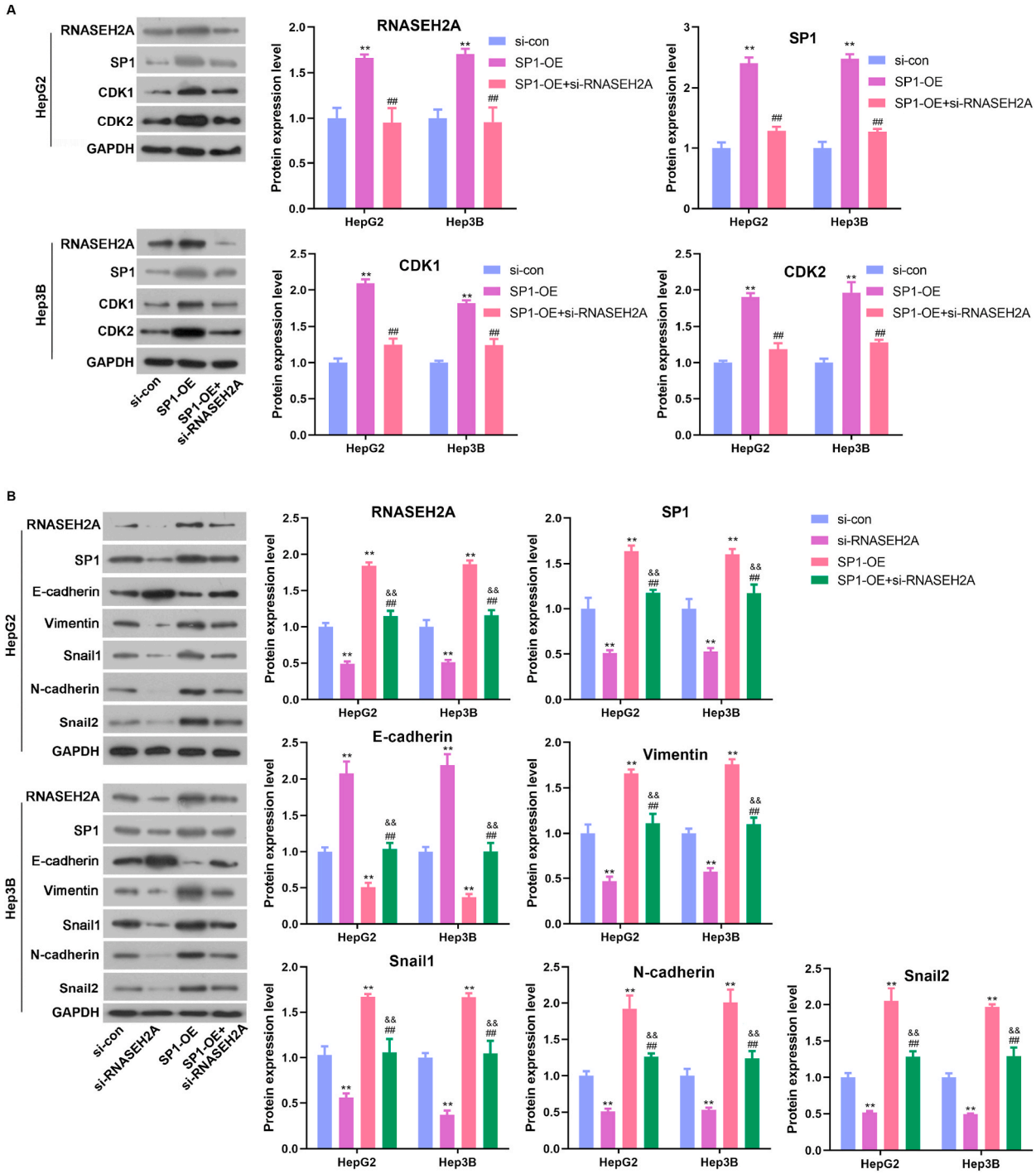



**Fig. 5.** The key genes related to cell cycle and EMT process were regulated by SP1/RNASEH2A. A. Overexpression of SP1 strengthened the level of CDK1 and CDK2, whereas the addition of si-RNASEH2A diminished the effect of SP1-OE. B. Upregulation of SP1 increased the level of N-cadherin, Snail1, Snail2, Vimentin, and decreased E-cadherin, whereas the administration of si-RNASEH2A inhibited the effect of SP1-OE on N-cadherin, Snail1, Snail2, Vimentin and E-cadherin expression. **p < 0.01 vs. si-con, ##p < 0.01 vs. si-RNASEH2A, &&p < 0.01 vs. SP1-OE.

(Fig. 4A). Knockdown of RNASEH2A reversed the promotion of cell colony caused by SP1 overexpression (Fig. 4B). Moreover, SP1 upregulation increased the number of HepG2 and Hep3B cells that invaded and migrated, while knockdown of RNASEH2A together with SP1 overexpression counteracted the enhancement of cell invasion and migration (Fig. 4C–D). Additionally, SP1 upregulation remarkably elevated the protein levels of CDK1 and CDK2, whilst, the promoting effect of SP1 overexpression on CDK1 and CDK2 expression was suppressed by si-RNASEH2A transfection (Fig. 5A).

Considering the influence of RNASEH2A on HCC cell invasion and migration, GEPIA website was applied to explore the connection between RNASEH2A and EMT-related genes. Analysis from GEPIA website demonstrated that RNASEH2A level was positively connected to N-cadherin, Snail2 and Vimentin (Figs. S1I–K). Data from western blotting assay demonstrated that transfection with si-RNASEH2A significantly reduced N-cadherin, Snail1, Snail2 and Vimentin levels, as well as elevated E-cadherin. Whereas SP1 upregulation obviously elevated the protein levels of N-cadherin, Snail1, Snail2 and Vimentin, as well as reduced E-cadherin. However, RNASEH2A knockdown together with SP1 overexpression counteracted the effect of si-RNASEH2A or SP1-OE on N-cadherin, Snail1, Snail2, Vimentin and E-cadherin expression (Fig. 5B).

## 4. Discussion

In our study, bioinformatics and biological assays were combined to detect the malignant properties of RNASEH2A in HCC cell lines. First, RNASEH2A expression was obviously elevated both in HCC tissues and cell lines. Then, RNASEH2A knockdown led to decreased growth ability of HCC cells. Moreover, cell invasion and migration abilities were obviously reduced after RNASEH2A knockdown. SP1 was confirmed as a specific transcription factor and bound to the promoter region of RNASEH2A. Moreover, SP1 upregulation contributed to the growth, cell cycle, and mobility of HCC cells.

RNASEH2A has been predicted to be an important target gene in a variety of cancers by bioinformatics analysis, including HCC. However, the specific role and regulatory mechanism of RNASEH2A in HCC are still unclear. Analysis from GEPIA website suggested that RNASEH2A expression was positively correlated with PCNA and Ki67 expression. Therefore, we detected the relationship between RNASEH2A and cell growth in HCC cells. Our data exhibited that depletion of RNASEH2A reduced the viability and proliferation of HCC cells. Then, analysis from GEPIA website suggested that RNASEH2A expression was positively correlated with CDK1 and CDK2 expression, and western blotting experiment was employed to identify the hypothesis, which exhibited that the protein levels of CDK1 and CDK2 were obviously diminished after RNASEH2A knockdown. Moreover, analysis from GEPIA website demonstrated that RNASEH2A was positively connected to EMT markers (N-cadherin, Snail2 and Vimentin), and our results exhibited that knockdown of RNASEH2A suppressed the movement of HCC cells.

SP1, a well-known member of the transcription factor family, is involved in numerous important physiological processes and plays significant roles in cell proliferation, apoptosis and carcinogenesis [19]. SP1 is often overexpressed in some tumors, whereas the suppression or knockdown of SP1 can reduce tumor formation, growth and metastasis [20,21]. In our study, analysis from GEPIA website exhibited that SP1 level was significantly increased in HCC samples, and its overexpression resulted in worse prognosis of HCC patients. SP1 was confirmed as a specific transcription factor and bound to the promoter region of RNASEH2A. Another study reported that SP1 was considered to be a primary modulator of genes implicated in the development of pancreatic cancer [22]. Besides, suppression of SP1 by siRNA can limit the proliferative ability of colon cancer stem cells, thereby contributing to apoptosis of cultured and xenografts cells [23]. Depletion of SP1 inhibited cell proliferation, clonogenicity and the expression of stem cell markers in nasopharyngeal carcinoma [24]. Increasing studies revealed that transcription factors are important factors in nearly all kinds of tumor, whereas SP1 downregulation suppressed the expression of genes implicated in driving the cancer development [25]. In this study, overexpression of SP1 increased the malignant properties of HCC cells. Besides, knockdown of SP1 also suppressed RNASEH2A expression, whereas RNASEH2A expression was significantly increased after SP1 overexpression. Furthermore, RNASEH2A knockdown counteracted the function of SP1 overexpression in modulating HCC cell viability, growth, invasion and migration.

It is well known that EMT is implicated in the migration and invasion of tumor cells and performs a critical function in tumor metastasis, including HCC [26,27]. A marked augmentation of N-cadherin, Snail2 and Vimentin expression is significant indicators of EMT process [28]. In this study, analysis from GEPIA website demonstrated that RNASEH2A expression was positively connected to N-cadherin, Snail2 and Vimentin. Moreover, RNASEH2A knockdown resulted in decreased protein levels of N-cadherin, Snail1, Snail2, Vimentin and increased E-cadherin, whereas the phenomena were reversed after SP1 overexpression. The above information insinuated that function of SP1/RNASEH2A in HCC cells was achieved by regulating EMT process.

Several shortcomings should be listed out. First, the effects of SP1/RNASEH2A were merely verified in vitro. Second, the downstream target of SP1/RNASEH2A has not been reported. Third, other pathways that may be implicated in the function of SP1/RNASEH2A on malignant properties of HCC cells have not been revealed.

Taken together, the whole data insinuated that RNASEH2A promoted the growth and mobility of HCC cells via regulating EMT process and was transcriptionally modulated by SP1. These results also afford new perspectives to consider SP1 and RNASEH2A in targeted therapeutic treatments to suppress HCC cells growth, invasion and migration.

## Funding

Hainan Provincial major science and technology project, ZDYF2022SHFZ118.

## Authors' contributions

Yunhe Hao: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Rui Zou: Conceived and designed the experiments; Wrote the paper.

Jiashou Tao: Analyzed and interpreted the data; Wrote the paper.

Manfei Jiang: Performed the experiments; Wrote the paper.

Duo Li: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

## Data availability statement

Data will be made available on request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18127.

**Supplementary figure**: Fig. S1. Analysis from GEPIA website showed the expression of RNASEH2A and SP1 in HCC samples and the corresponding survival curve, and showed the correlation between RNASEH2A and related genes. A. RNASEH2A was highly expressed in HCC samples, *$p < 0.05$. B. High expression of RNASEH2A resulted in unfavorable prognosis in HCC patients, $p = 0.038$. C–F. RNASEH2A expression was positively related to Ki67, PCNA, CDK1 and CDK2. G. SP1 was highly expressed in HCC samples. H. High expression of SP1 resulted in unfavorable prognosis in HCC patients, $p = 0.03$. I–K. RNASEH2A expression was positively related to N-cadherin, Snail2 and Vimentin.

## References


[1] D. Anwanwan, et al., Challenges in liver cancer and possible treatment approaches, Biochim. Biophys. Acta Rev. Canc 1873 (1) (2020), 188314.
[2] R.L. Siegel, K.D. Miller, Cancer statistics, 2019 69 (1) (2019) 7–34.
[3] H.B. El-Serag, et al., Diagnosis and treatment of hepatocellular carcinoma, Gastroenterology 134 (6) (2008) 1752–1763.
[4] S. Feng, Z. Cao, Is the role of human RNase H2 restricted to its enzyme activity? Prog. Biophys. Mol. Biol. 121 (1) (2016) 66–73.
[5] M.A. Reijns, et al., The structure of the human RNase H2 complex defines key interaction interfaces relevant to enzyme function and human disease, J. Biol. Chem. 286 (12) (2011) 10530–10539.
[6] Y.J. Crow, Aicardi-Goutières syndrome, Handb. Clin. Neurol. 113 (2013) 1629–1635.
[7] Y.J. Crow, N. Manel, Aicardi-Goutières syndrome and the type I interferonopathies, Nat. Rev. Immunol. 15 (7) (2015) 429–440.
[8] K.A. Williams, et al., A systems genetics approach identifies CXCL14, ITGAX, and LPCAT2 as novel aggressive prostate cancer susceptibility genes, PLoS Genet. 10 (11) (2014), e1004809.
[9] J.L. Bell, S. Hagemann, J.K. Holien, Identification of RNA-Binding Proteins as Targetable Putative Oncogenes in Neuroblastoma 21 (14) (2020).
[10] S. Hua, et al., Identification of hub genes in hepatocellular carcinoma using integrated bioinformatic analysis, Aging (Albany NY) 12 (6) (2020) 5439–5468.
[11] F. Zhao, et al., Hypoxia-induced RNASEH2A limits activation of cGAS-STING signaling in HCC and predicts poor prognosis, Tumori 108 (1) (2022) 63–76.
[12] J.M. Flanagan, et al., Genomics screen in transformed stem cells reveals RNASEH2A, PPAP2C, and ADARB1 as putative anticancer drug targets, Mol. Cancer Ther. 8 (1) (2009) 249–260.
[13] M.R. Briggs, et al., Purification and biochemical characterization of the promoter-specific transcription factor, Sp1, Science 234 (4772) (1986) 47–52.
[14] R. Bajpai, G.P. Nagaraju, Specificity protein 1: its role in colorectal cancer progression and metastasis, Crit. Rev. Oncol. Hematol. 113 (2017) 1–7.
[15] Y. Dong, et al., Higher matrix stiffness as an independent initiator triggers epithelial-mesenchymal transition and facilitates HCC metastasis, J. Hematol. Oncol. 12 (1) (2019) 112.
[16] J.P. Thiery, et al., Epithelial-mesenchymal transitions in development and disease, Cell 139 (5) (2009) 871–890.
[17] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Invest. 119 (6) (2009) 1420–1428.
[18] M.H. Yang, et al., Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma, Hepatology 50 (5) (2009) 1464–1474.
[19] C. Vizcaíno, S. Mansilla, J. Portugal, Sp1 transcription factor: a long-standing target in cancer chemotherapy, Pharmacol. Ther. 152 (2015) 111–124.
[20] U.T. Sankpal, et al., Expression of specificity protein transcription factors in pancreatic cancer and their association in prognosis and therapy, Curr. Med. Chem. 19 (22) (2012) 3779–3786.
[21] S. Previdi, et al., Inhibition of Sp1-dependent transcription and antitumor activity of the new aureolic acid analogues mithramycin SDK and SK in human ovarian cancer xenografts, Gynecol. Oncol. 118 (2) (2010) 182–188.
[22] B.S. Wang, et al., Identification of genes and candidate agents associated with pancreatic cancer, Tumour Biol. 35 (1) (2014) 81–88.
[23] Y. Zhao, et al., Inhibition of the transcription factor Sp1 suppresses colon cancer stem cell growth and induces apoptosis in vitro and in nude mouse xenografts, Oncol. Rep. 30 (4) (2013) 1782–1792.
[24] J.P. Zhang, et al., Down-regulation of Sp1 suppresses cell proliferation, clonogenicity and the expressions of stem cell markers in nasopharyngeal carcinoma, J. Transl. Med. 12 (2014) 222.
[25] S. Safe, et al., Transcription factor Sp1, also known as specificity protein 1 as a therapeutic target, Expert Opin. Ther. Targets 18 (7) (2014) 759–769.

[26] Z. Zha, J. Li, MicroRNA-125a-5p regulates liver cancer cell growth, migration and invasion and EMT by targeting HAX1, Int. J. Mol. Med. 46 (5) (2020) 1849–1861.
[27] K.R. Jakobsen, et al., The role of epithelial to mesenchymal transition in resistance to epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer, Transl. Lung Cancer Res. 5 (2) (2016) 172–182.
[28] V.P. Ramamurthy, et al., The retinamide VNLG-152 inhibits f-AR/AR-V7 and MNK-eIF4E signaling pathways to suppress EMT and castration-resistant prostate cancer xenograft growth, FEBS J. 285 (6) (2018) 1051–1063.