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High expression of SMPD4 promotes liver cancer and is associated with poor prognosis

Rongyue Wang[1,2], Ting Wang[1], Yanze Su[1], Zhiheng Lin[1], Xiaoping Liu[1], Yuanjun Jiao[1], Jikui Liu[1*] and Erbao Chen[1*]

Abstract

Objectives The expression of sphingomyelin phosphodiesterase 4 (SMPD4), a neutral sphingomyelin enzyme, is intricately associated with tumorigenesis and progression. However, its role in hepatocellular carcinoma (HCC) remains unclear. This study mainly reports the expression, prognostic value and tumor biological function of SMPD4 in HCC.

Methods The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO) and International Cancer Genome Consortium (ICGC) databases were utilized to investigate the expression patterns of SMPD4. Survival Analyses using the Kaplan-Meier method were conducted to assess the predictive value of SMPD4 in HCC. Immunohistochemistry method and real-time quantitative PCR were used to analyze the expression of SMPD4 in our clinical cohort. Immune infiltration analysis was performed to explore the correlation between SMPD4 expression and immune cell infiltration in HCC. Functional enrichment analysis was conducted to depict SMPD4-associated functions and pathways. Using human HCC cell lines, we studied the influence of SMPD4 in cell proliferation, invasion and migration.

Results We found SMPD4 was overexpressed in HCC. The Kaplan–Meier curves demonstrated that higher expression of SMPD4 was associated with worse survival in patients with HCC. Immune infiltration analysis showed that SMPD4 expression exhibited positive correlations with CD4 + T cells, Type 2 T helper cells, and negatively related to neutrophil, eosinophil, nature killer cells, macrophage, activated CD8 T cells. Functional enrichment analysis revealed that SMPD4 expression is associated with cell cycle pathways. Additionally, cell functional studies in HCC cell lines indicated that the knockdown of SMPD4 significantly inhibited cell growth, invasion and migration.

Conclusions These results reveal that high SMPD4 expression is associated with poor prognosis and promotes HCC cell proliferation, invasion and migration. SMPD4 is a promising prognostic biomarker with functional significance for HCC.

Keywords Hepatocellular carcinoma, SMPD4, Prognosis, Immune infiltration, Cell cycle

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Introduction

The Global Cancer Statistics data reveals that liver cancer is a frequently diagnosed digestive tract cancer, corresponding to the sixth in global incidence rate of malignant tumors and third in the mortality rate [1]. Hepatocellular carcinoma (HCC) is the most prevalent liver malignancy, accounting for 75–85% of liver cancer cases and causing approximately 750,000 deaths annually [2]. Although the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer [3] have been widely used to develop diagnostic and treatment strategies, the survival outcome of HCC has not improved significantly because of the elusive molecular mechanisms [4, 5]. It is crucial to comprehensively investigate the molecular mechanisms related to HCC occurrence and development to detect and diagnose HCC early, which may contribute to the development of novel therapeutic strategies for HCC.

SMPD4, a hydrolytic enzyme that hydrolyzes sphingomyelin to ceramides and phosphocholine, has been implicated in sphingomyelin metabolism. SMPD4 is localized on human chromosome 2q21.1; it is a protein consisting of 866 amino acids, with a molecular weight of 97.8 kDa. Its highest expression is reported in the striated muscles and myocardium [6]. In addition, it is expressed in the Golgi apparatus and endoplasmic reticulum [7, 8]. Previous studies [8–11] have found that SMPD4 is also detected on the nuclear membrane, terming it nuclear membrane transmembrane protein-13. Although several studies [12–14] have reported that SMPD4 is related to carcinogenesis and progression, its functions in HCC and the underlying mechanism are largely unknown.

In this study, we investigated the differential expression and potential prognostic value of SMPD4 using TCGA, ICGC and GEO databases. Functional enrichment analysis was performed to investigate SMPD4-associated pathways. Furthermore, the effects of SMPD4 on the proliferation, invasion and migration of HCC cells were assessed. Our results highlight the oncogenic role of SMPD4 in HCC, and determine its potential as a cancer diagnostic and treatment target.

Materials and methods

Sample sources

The HCC specimens were obtained from 20 patients who underwent hepatectomy for HCC in Peking University Shenzhen Hospital from October 2017 to July 2021. Each sample contained its corresponding adjacent tissues. All patients provided informed consent for participation.

Survival analysis

Patients were categorized into high- or low-SMPD4 expression groups according to median SMPD4 mRNA levels. The Kaplan-Meier curves were plotted to assess the overall survival (OS), disease specific survival (DSS), progression free survival (PFS), and recurrence free survival (RFS) in TCGA-HCC patients using the Kaplan-Meier plotter website.

Functional enrichment analysis

The SMPD4-related genes were extracted from GEPIA, and a total of 200 genes were selected with a relevance score > 0.74. Then we applied the DAVID online website to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses based on these 200 genes [15].

Immune infiltration analysis

The infiltration levels of 28 immune cell types between low-SMPD4 and high-SMPD4 group were analyzed, and the enrichment scores were calculated using ssGSEA algorithm. The immune cell gene signatures used in the ssGSEA analysis were listed in Supplementary Table S1. TIMER [16] was used to analyze the correlation of SMPD4 expression with cell cycle genes.

Drug sensitivity analysis

The Cancer Drug Sensitivity Genomics database was used to predict anti-tumor drug responses in patients with HCC.

RNA extraction and quantitative real-time PCR (qRT-PCR)

The TRIzol reagent was used to extract the total RNA from LO2, Hep3B, HepG2, liver cancer tissue and adjacent normal tissues. The Rever Tra Ace qPCR RT kit was adopted for reverse transcription, followed by using the SYBR Green Realtime PCR Master Mix and qRT PCR instrument (Roche Light Cycle 480) for relative quantitative analysis. The expression of genes related to the cell cycle was studied using qRT PCR in si-NC and si-SMPD4 (see Supplementary Table S2 for primer design).

Cell culture and transfection

LO2, Hep3B and HepG2 were purchased from Chinese Academy of Medical Sciences (Beijing, China). We cultivated the cells in DMEM high-glucose medium containing 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (Gibco, USA) at 37 °C and 5% CO_2. Small interfering RNA against SMPD4 was purchased from Guangzhou Ribo-Bio. The Lipofectamine™ 3000 Transfection Reagent (Invitrogen, China) was used for siRNA transfection in cells in line with specific protocols.

Western blot

The protein content was quantified using BCA kit (Beyotime Biotechnology, Shanghai, China). High-temperature denatured protein samples were subjected to SDS-PAGE. The proteins were transferred to a polyvinylidene

fluoride membrane. The membrane was blocked using 5% defatted milk for 2 h, incubated with the anti-SMPD4 (AP5904a, 1:1000, Abcepta, San Diego, USA, RB24190) and anti-beta actin (66009-1-1 g, 1:20000, Proteintech, Wuhan, China, 2D4H5) overnight in a refrigerator under 4 °C. On the following day, the membrane was incubated in the secondary antibody (1:2000) for 2 h under ambient temperature. The protein expression was detected by the BeyoECL Moon.

CCK-8 assay

After suspension in 100 μL of medium, cells (3×10^3/well) were inoculated into 96-well plates. After adhering to the wall, the cells were further cultivated for 24, 48, and 72 h, and the CCK-8 reagent (10 μL) was added to every well, followed by 2 h incubation. The absorbance values were read at 450 nm on a spectrophotometer.

EdU cell proliferation assay

The cells were inoculated in 96-well plates before incubating for 2 h using 5-ethynyl-2'-deoxyuridine (EdU, 10 μM). After immersion within 4% paraformaldehyde for fixation, the cells were subjected to 10 min permeabilization using 0.5% Triton X-100. Hoechst 33,342 was used to stain the nucleus for 10 min. The light microscope (Olympus BX53) was used for image capturing.

Wound healing assay

A 200 μL pipette tip was used to create a wound in the confluent monolayer. After washing with phosphate-buffered saline, we captured the images. After incubation at 37 °C and 5% CO_2, we captured the images again at 24 h and 48 h.

Transwell experiments

Matrigel-coated or uncoated (BD, USA) Transwell chambers were used for migration and invasion assays. We introduced 1×10^5 cells into the upper chambers, and a medium containing 10% FBS (600 μL) was added to the lower chamber. At 48 h post-incubation, 4% paraformaldehyde was added to fix cells migrating onto the lower chamber surface for 30 min, followed by another 15 min staining using 0.1% crystal violet. Afterward, the light microscope (BX53, Olympus) was used to quantify three random fields for each well. The number of migrating and invading cells was counted.

Statistical analysis

Continuous variables were compared using the Student's t-test if they were normally distributed or using the Mann–Whitney U test if they were nonnormally distributed. Comparing categorical variables, chi-square test and Fisher's exact test was used. Kaplan-Meier curves were used to assess the prognostic value of SMPD4 expression. Spearman's test was used for correlation analysis of immune cell infiltration. Every experiment was conducted thrice independently, and the Student's *t*-test was used to analyze the differences between the two groups. GraphPad Prism 7.0 and R software (3.6.3) were used for statistical analysis. A two-sided p-value < 0.05 was considered significant.

Results

SMPD4 expression in HCC

The pan-caner data analysis showed that SMPD4 is upregulated in numerous cancers, such as lung adenocarcinoma (LUAD), colon adenocarcinoma (COAD) and HCC (Fig. 1A). Notably, we found that SMPD4 was significantly overexpressed in HCC tissues (Fig. 1B). In the paired samples of the TCGA data (Fig. 1C), SMPD4 showed a significantly higher expression compared to normal tissues ($p < 0.05$), which was validated in ICGC-LIHC database and GSE22058 database (Fig. 1D, E). The diagnostic potential of SMPD4 for HCC and non-tumor tissues was evaluated using the receiver operating characteristics (ROC) curve analysis (Fig. 1F). The area under curve of SMPD4 was calculated as 0.952 (95% confidence interval [CI]: 0.932–0.971, $P < 0.0001$), suggesting the good diagnostic performance of SMPD4. Then we investigate the correlation among clinical characteristics and SMDP4 expression levels in HCC. Patients were categorized into high- and low-expression groups according to median SMPD4 mRNA levels, and their corresponding clinical characteristics were summarized in Table 1. The correlation between SMPD4 levels and clinical characteristics was also analyzed. Our results indicated a significant association between SMPD4 expression and sex ($P = 0.009$), T stage ($P = 0.04$), N stage ($P = 0.049$), TNM stage ($P = 0.019$), and differentiation grade ($P = 0.001$). Higher SMPD4 expression was related to a higher T stage, a higher N stage, an advanced TNM stage and worse differentiation grade.

Prognostic significance of SMPD4

The Kaplan-Meier curves indicated that patients with higher SMPD4 expression had significantly worse outcome. We found that the OS (Fig. 1G, HR, 1.49 [95% CI, 1.05–2.11]; $P = 0.025$), DSS (Fig. 1H, HR, 1.88, [95% CI, 1.19–2.97], $P = 0.006$), RFS (Fig. 1I, HR, 1.92, [95% CI, 1.42–2.6], $P < 0.001$), and PFS (Fig. 2J, HR, 1.87, [95% CI,1.34, 2.62], $P < 0.001$) were significantly shorter in the patients with high expression of SMPD4, indicating that mRNA of SMPD4 could be potential prognostic biomarkers for HCC patients.

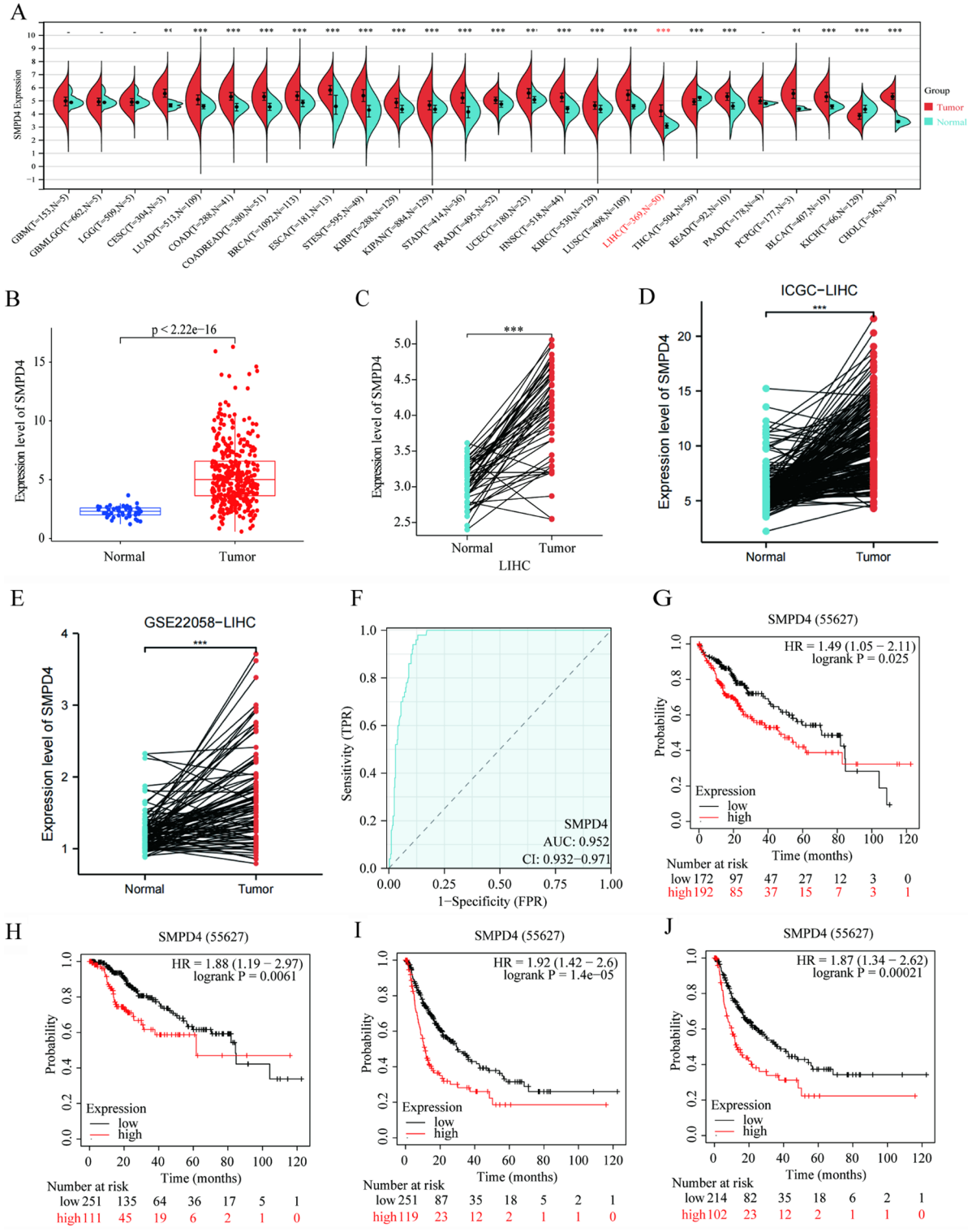

Fig. 1 Expression pattern and prognostic value of SMPD4 in HCC. (**A**) SMPD4 expression in pan-cancer from TCGA database. SMPD4 expression in (**B**) unpaired and (**C**) paired samples from the TCGA-LIHC database. **D** SMPD4 expression in paired samples from ICGC-LIHC database. **E** SMPD4 expression in paired samples from GSE 22,058 database. **F** ROC curve analysis of the diagnostic potential of SMPD4 in HCC. Kaplan-Meier analysis of (**G**) Overall survival, (**H**) Disease-specific survival, (**I**) Relapse-free survival, (**J**) Progression-free survival

Table 1 Clinical characteristics of patients with HCC

	Level	Overall	High expression of SMPD4	Low expression of SMPD4	*P* value
Number		342	170	172	
Age70 (%)	<=70	271 (79.24)	139 (81.76)	132 (76.74)	0.3119
	> 70	71 (20.76)	31 (18.24)	40 (23.26)	
Sex (%)	FEMALE	109 (31.87)	66 (38.82)	43 (25.00)	0.0086
	MALE	233 (68.13)	104 (61.18)	129 (75.00)	
T (%)	T1	171 (50.00)	72 (42.35)	99 (57.56)	0.0352
	T2	85 (24.85)	46 (27.06)	39 (22.67)	
	T3	76 (22.22)	46 (27.06)	30(17.44)	
	T4	10 (2.92)	6 (3.53)	4(2.33)	
N (%)	N0	251 (73.39)	133 (78.24)	118 (68.60)	0.0489
	N1	4 (1.17)	3 (1.76)	1 (0.58)	
	NX	87 (25.44)	34(20.00)	53 (30.81)	
M(%)	M0	261 (76.32)	136 (80.00)	125 (72.67)	0.2662
	M1	4 (1.17)	2 (1.18)	2 (1.16)	
	MX	77 (22.51)	32 (18.82)	45 (26.16)	
Stage (%)	Stage I	169 (49.42)	71 (41.76)	98 (56.98)	0.0186
	Stage II	83 (24.27)	44 (25.88)	39 (22.67)	
	Stage III	85 (24.85)	53 (31.18)	32 (18.60)	
	Stage IV	5(1.46)	2 (1.18)	3 (1.74)	
Grade (%)	G1	45 (13.16)	17 (10.00)	28 (16.28)	0.001
	G2	168 (49.12)	71 (41.76)	97 (56.40)	
	G3	117 (34.21)	74 (43.53)	43 (25.00)	
	G4	12 (3.51)	8 (4.71)	4 (2.33)	
Status (%)	Alive	229 (66.96)	115 (67.65)	114 (66.28)	0.8776
	Dead	113 (33.04)	55 (32.35)	58 (33.72)	

SMPD4 expression correlates with immune infiltration levels

The correlation between SMPD4 expression and immune cell infiltration levels were investigated using ssGSEA method. SMPD4 expression exhibited positive correlations with CD4 + T cells, Type 2 T helper cells, and negatively related to neutrophil, eosinophil, nature killer cells, macrophage, activated CD8 T cells (Fig. 2A), suggesting that SMPD4 may play a crucial role in the tumor immune microenvironment of HCC. To investigate the potential association between the SMPD4 expression and immunotherapy efficacy, IMvigor210 cohort, a widely used cohort of patients receiving immunotherapy was selected. The results showed that patients with lower SMPD4 expression had better survival, but the difference was not statistically significant (Fig. 2B, $P = 0.12$).

SMPD4-associated functions and pathways in HCC

We performed enrichment analysis based on SMPD4-correlated genes. KEGG pathway analysis demonstrated that the SMPD4 overexpression was associated with spliceosomes, cell cycle, and ubiquitin-mediated proteolysis (Fig. 3A). The oncogene role of SMPD4 was remarkable correlated with these molecular pathways. Subsequently, we performed the GO analysis. The biological process results (Fig. 3B) showed that genes interacting with SMPD4 were primarily associated with mRNA splicing, via spliceosome, and RNA splicing. For cellular components (Fig. 3C), the SMPD4-interacting genomes were mostly associated with nucleoplasm, nucleus and nuclear speck. For molecular function (Fig. 3D), the SMPD4-interacting genomes were largely enriched in RNA binding, protein binding, and chromatin binding. Furthermore, we focused on the cell cycle and examined the correlations between SMPD4 and cell cycle-related genes, suggesting that CDK4, CDK7, E2F1, and E2F4 were highly correlated with SMPD4 expression (Fig. 3E-F).

Drug sensitivity test

We next selected nine FDA-approved chemotherapeutics to evaluate the sensitivities of patients in the low-SMPD4 and high-SMPD4 expression groups to these drugs. We found that patients with low expression of SMPD4 exhibited significantly lower IC50 value for these nine drugs. The results showed that SMPD4 expression was related to drug sensitivity (Fig. 4A-H).

Biological significance of SMPD4 in HCC cells

we detected the mRNA level of SMPD4 in 20 HCC tissues and 20 paired normal liver tissues by qRT-PCR. The results showed that the expression of SMPD4 were higher in HCC tissues than normal liver tissues ($P < 0.05$, Fig. 5A). The qRT-PCR results showed that SMPD4 mRNA levels were overexpressed in Hep3B and HepG2 cells compared to normal liver cells (LO) ($P < 0.05$, Fig. 5B), which was consistent with the difference analysis based on TCGA database.

We next investigated the effect of SMPD4 on HCC. The level of SMPD4 was reduced in Hep3B and HepG2 cell lines by silencing SMPD4 using siRNA (Fig. 5C-D). We found that inhibition of SMPD4 remarkably decreased the proliferative abilities in si-SMPD4 cells in CCK-8 assays (Fig. 6A-B). Furthermore, we performed EdU assays and found that the ratios of EdU-positive cells within si-SMPD4 cells were reduced compared with si-NC cells (Fig. 6C-F). The wound healing assay demonstrated that inhibition of SMPD4 remarkably decreased the migration abilities in si-SMPD4 cells (Fig. 7A-D). The transwell assay showed that inhibition of SMPD4 remarkably decreased the migration and invasion abilities in si-SMPD4 cells (Fig. 8A, B). Taken together, SMPD4 enhances HCC cell proliferation, migration and invasion in vitro.

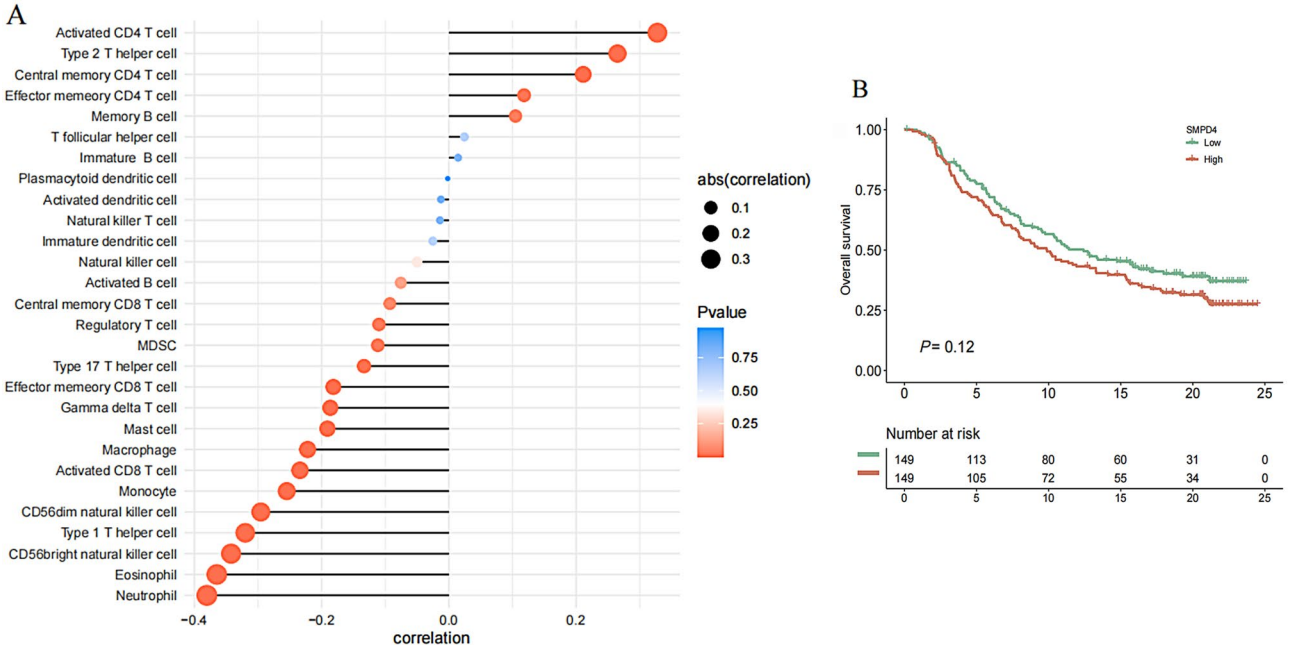

Fig. 2 Immune infiltration analysis of SMPD4. **A** The lollipop plot represented the correlation between 28 immune cells and SMPD4. **B** Survival curves of high-SMPD4 expression and low-SMPD4 expression group in IMvigor210 cohort

Correlation between SMPD4 and cell cycle genes

The qRT-PCR assay revealed that the expression of CDK7, CDK4, and E2F1 was reduced, while the expression of E2F4 was upregulated following SMPD4 knockdown in Hep3B cells (Fig. 9A-D). In HepG2 cells, the expression of CDK7, CDK4, and E2F1 decreased following SMPD4 knockdown, while E2F4 was overexpressed (Fig. 9E-H).

Discussion

Genes of the SMPD family have significant regulatory value in malignant tumors. SMPD4, as a sphingomyelinase of the SMPD family, has been implicated in tumor metastasis and recurrence. Increasing studies have demonstrated the significant role of SMPD4 in tumors. Sherry et al. screened SMPD4 and other eight expression-based genetic biomarkers to distinguish patients with early-stage from those with advanced renal clear cell carcinoma [13]. Hu et al. filtered five lipid metabolism genes and reported upregulated SMPD4 expression in liver cancer samples compared to adjacent samples by qRT-PCR [14]. Our results showed that the expression of SMPD4 is increased in HCC tissues, and acts as an oncogene, leading to worse survival outcomes. The KEGG analysis showed that similar genes were largely associated with cell cycle, spliceosome, and ubiquitin-mediated proteolysis. In order to verify its function, we observed that knockout of SMPD4 significantly inhibited the proliferation, invasion, and migration of HCC cells. We hypothesized that SMPD4 promotes the occurrence and growth of HCC cells by regulating cell cycle. We next verified the expression of cell cycle-related genes after knocking down SMPD4. The results showed that CDK7, CDK4 and E2F1 were downregulated, while E2F4 was upregulated. These findings further confirmed that SMPD4 was involved in the HCC cell cycle and contribute to the invasion and metastasis of HCC cells.

The cell cycle is the basis of all other cell phenotypes, and its accurate regulation is crucial for cell survival and proliferation [17, 18]. CDK4 forms a complex with cyclin D that phosphorylates the retinoblastoma protein, releasing the E2F1 transcription factor, promoting G1 to S phase, and promoting DNA replication [19, 20]. Inhibitors targeting CDK4 can inhibit the growth of breast cancer tumor cells [20–22]. CDK7 is activated by binding to cyclin H and participates in G1 and G2 progression [23]. The inhibition of CDK7 in pancreatic cancer reduces gene transcription, inhibits cell cycle progression, and induces cell apoptosis [24]. E2F1 expression is upregulated in HCC and promotes the development of HCC by targeting MYBL2 or by regulating SIRT5 [25, 26]. E2F4 is a major repressor of cell cycle progression and prevents malignant cell proliferation. Upon binding to the DNA, E2F4 induces cell transition from G0 to G1 and S phases [27, 28].

Increasing studies have showed that the immune microenvironment plays an important role in tumorigenesis and cancer progression. In addition to tumor cells, the tumor microenvironment (TME) consists of immune and non-immune stromal components [29].

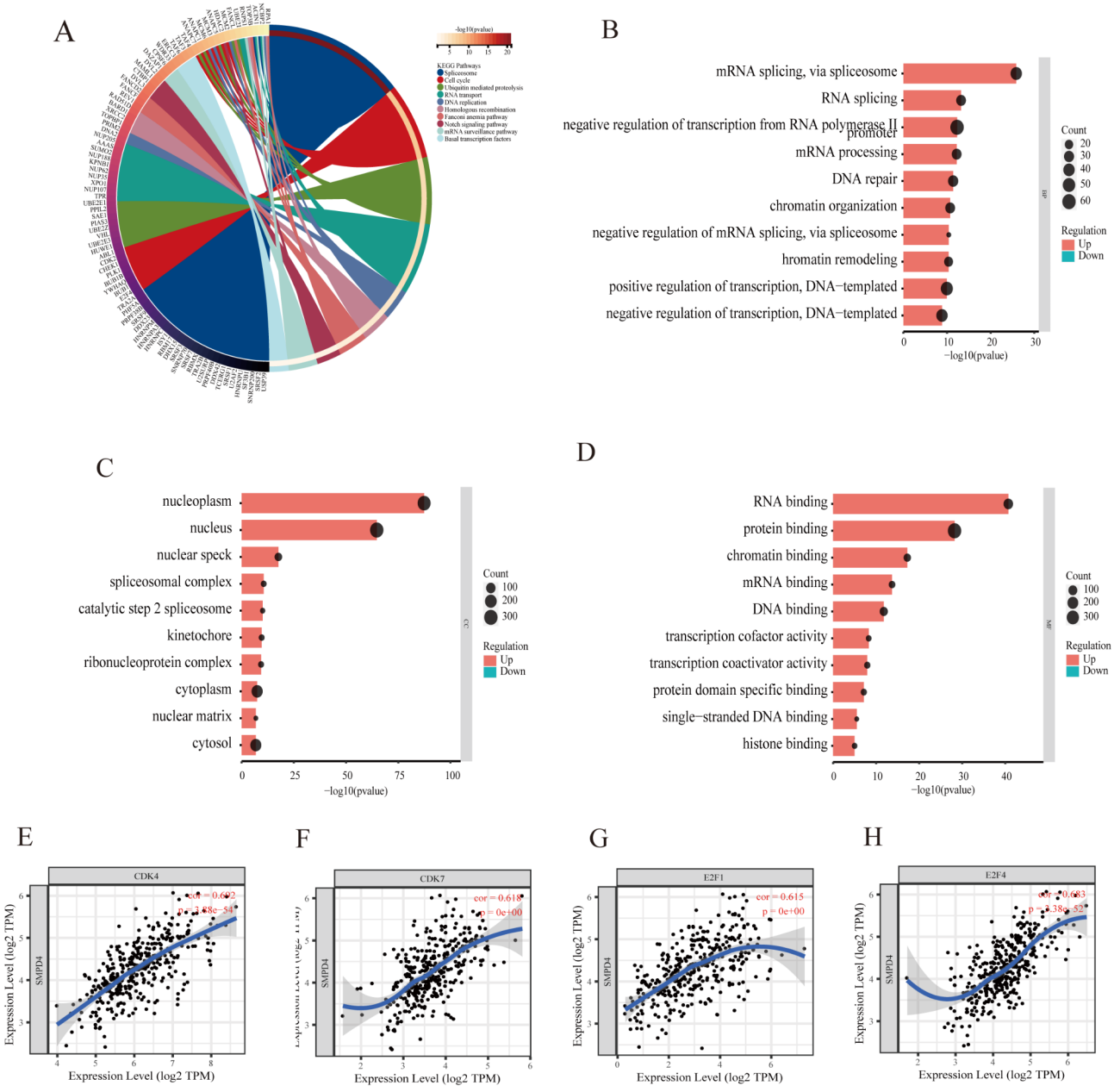

Fig. 3 GO and KEGG pathways enrichment analysis. **A** Chord plot represented the KEGG pathway analysis of DEGs between high-SMPD4 expression and low-SMPD4 expression group. **B-D** Bar plots demonstrated GO pathway analysis of DEGs between high-SMPD4 expression and low-SMPD4 expression group. **E**, **F** Correlations of SMPD4 expression with cell cycle-related genes, including CDK7, E2F1, and E2F4

HCC commonly experience immune system impairment, resulting in the accumulation of immune suppressive cells in the TME, such as Th2 cells and regulatory T cells [30]. Immune infiltration analysis revealed that SMPD4 expression was positively related to type 2 T helper cells, indicating that SMPD4 might participate in the tumor immune escape by mediating Th2 cell infiltration. Our results also showed that SMPD4 expression was positively related CD4 + T cell, and negatively related to neutrophil, eosinophil, nature killer cells, macrophage, activated CD8 T cells. These results suggests that SMPD4 might be involved in promoting tumorigenesis but also in influencing immune cell recruitment, polarization, or activation. This intricate role could potentially affect tumor progression and immune evasion, and further studies are needed to elucidate whether SMPD4 is influencing immune cell dynamics in a way that enhances tumor growth or alters immune responses.

There are limited studies exploring the relationship between SMPD4 and drug sensitivity. Chemotherapy

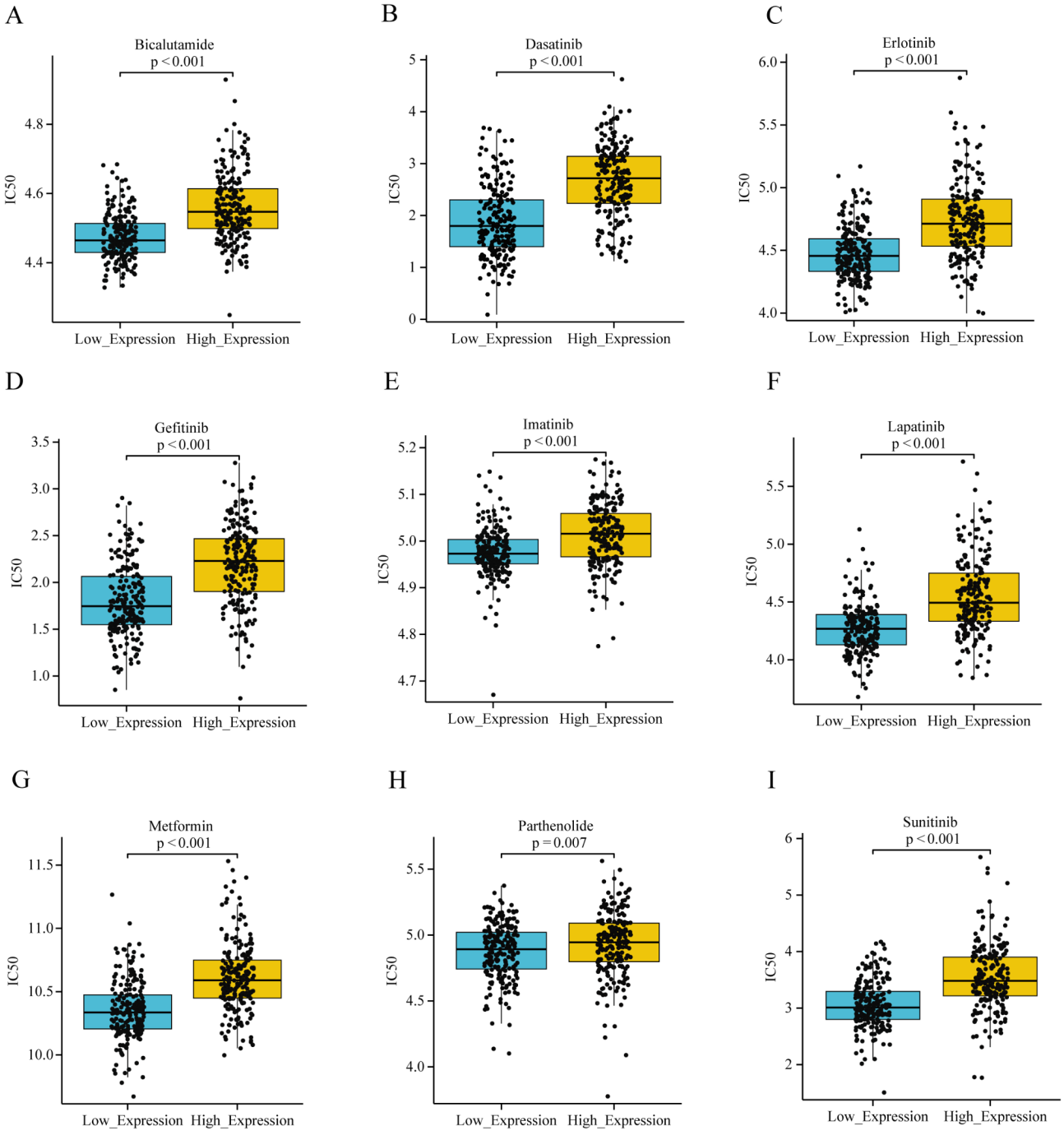

Fig. 4 Drug sensitivity analysis. **A-H** The boxplots represented the estimated IC50 for Bicalutamide, Dasatinib, Erlotinib, Gefitinib, Imatinib, Lapatinib, Metformin, Parthenolide and Sunitinib in the high- and low-SMPD4 expression groups

and targeted therapy are important treatments for HCC, yet clinical outcomes remain unsatisfactory due to the drug resistance [31, 32]. We analyzed the correlation between the expression of SMPD4 and drug sensitivities. The results showed that patients with low expression of SMPD4 exhibited significantly higher sensitivity to these drugs. SMPD4 expression may affect the efficacy of chemotherapy drugs and targeted drugs in HCC patients, which imply the significance of SMPD4 for HCC treatments.

In conclusion, SMPD4 is upregulated in various cancers. Its expression is associated with the immune infiltration and survival outcome. In addition, knocking down SMPD4 can affect the HCC cell proliferation, invasion and migration via regulating the transformation of the cell cycle. Further investigation of SMPD4's involvement

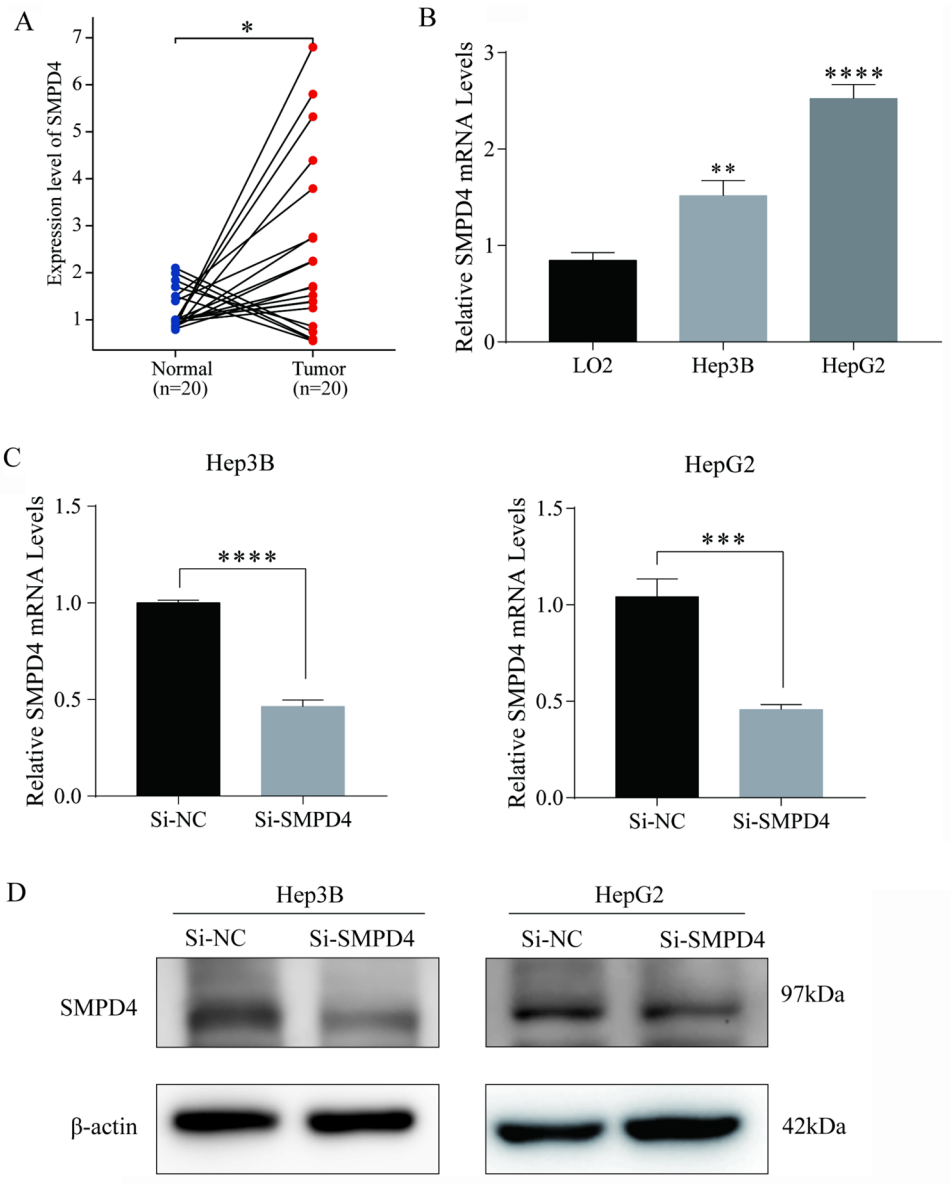

Fig. 5 SMPD4 expressions in HCC detected by qRT-PCR and Western blot. **A** qRT-PCR analysis of SMPD4 mRNA in 20 HCC tissues and 20 paired normal liver tissues. **B** Expression of SMPD4 in HCC cell lines was detected by qRT-PCR. **C** The efficiency of transfection in Hep3B and HepG2 were confirmed by qRT-PCR. **D** The efficiency of transfection in Hep3B and HepG2 were confirmed by Western blot

in cell cycle may contribute to the development of novel therapeutic strategies for HCC.

Limitations

Our study had certain limitations. In vivo animal experiments are needed to verify the function of SMPD4 in HCC. The underlying mechanism of how SMPD4 regulates HCC cell behavior remains to be further explored.

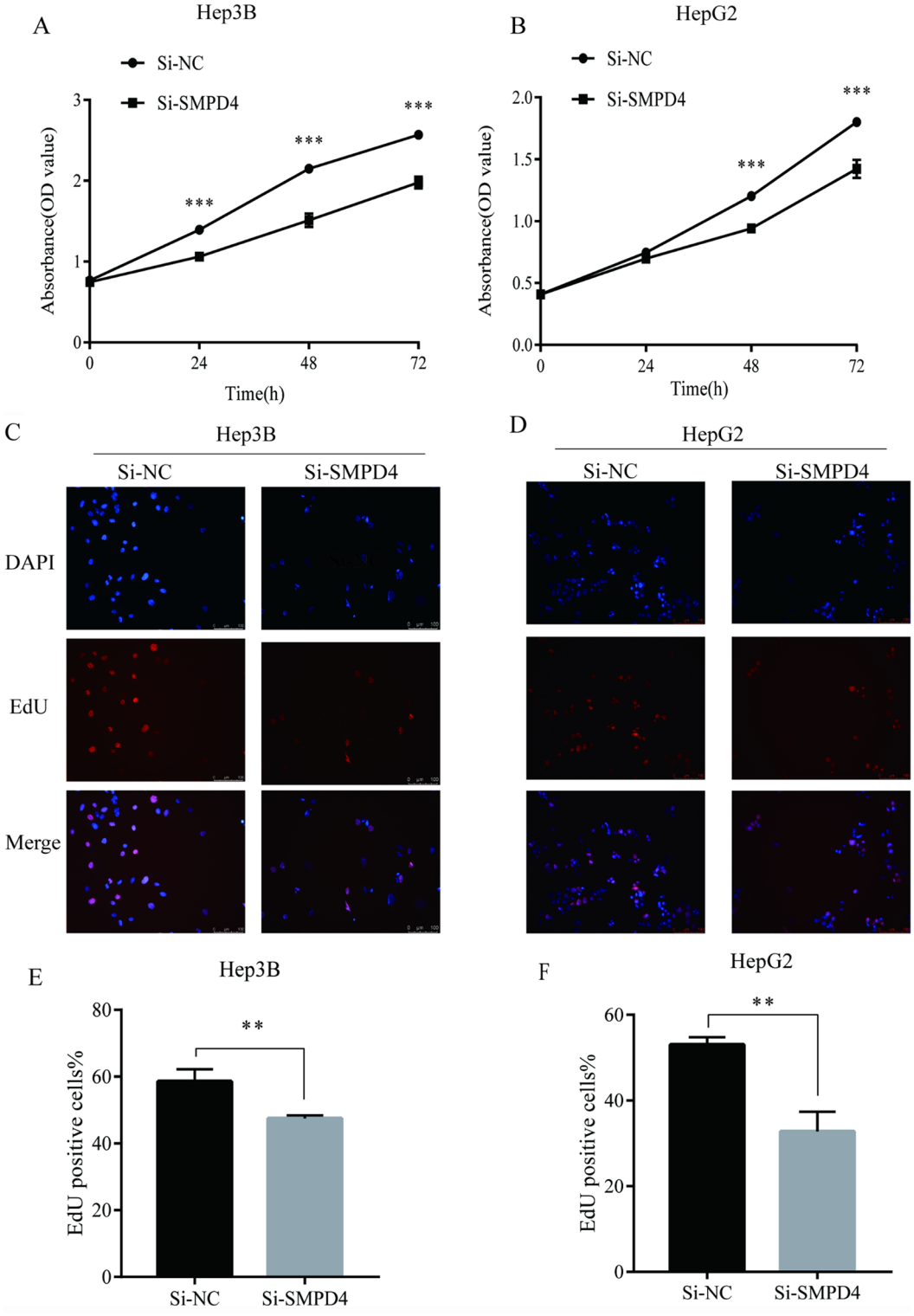

Fig. 6 CCK-8 and EdU experiment to detect the effect of SMPD4 on cell proliferation ability. **A**, **B** CCK-8 experimental results of Hep3B and HepG2. **C**, **E** EdU experimental results of Hep3B. **D**, **F** EdU experimental results of HepG2

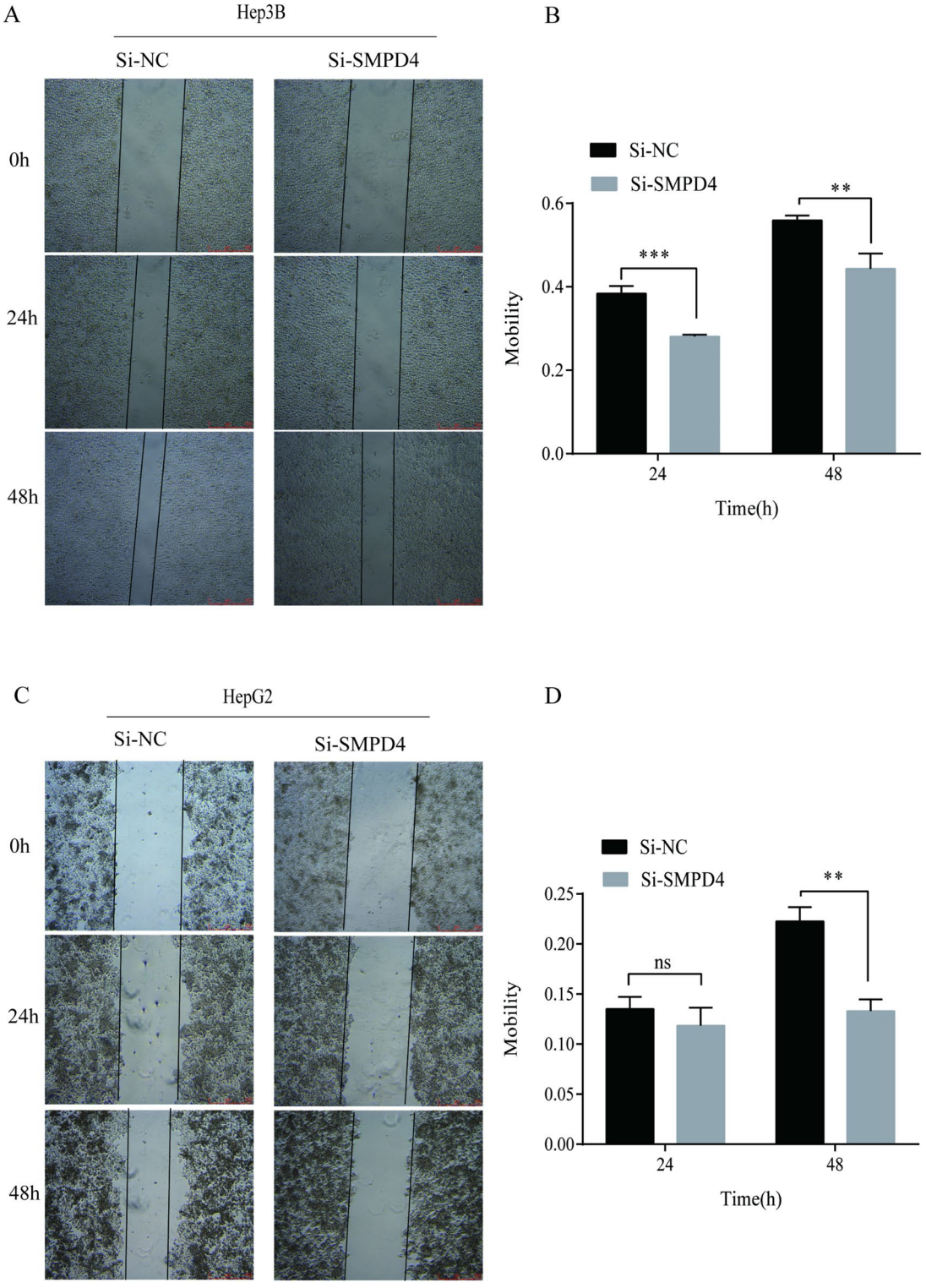

Fig. 7 Wound healing assay detection of the effect of SMPD4 on the migration ability of HCC cells. **A**, **B** Wound healing experimental results of Hep3B. **C**, **D** Wound healing experimental results of HepG2

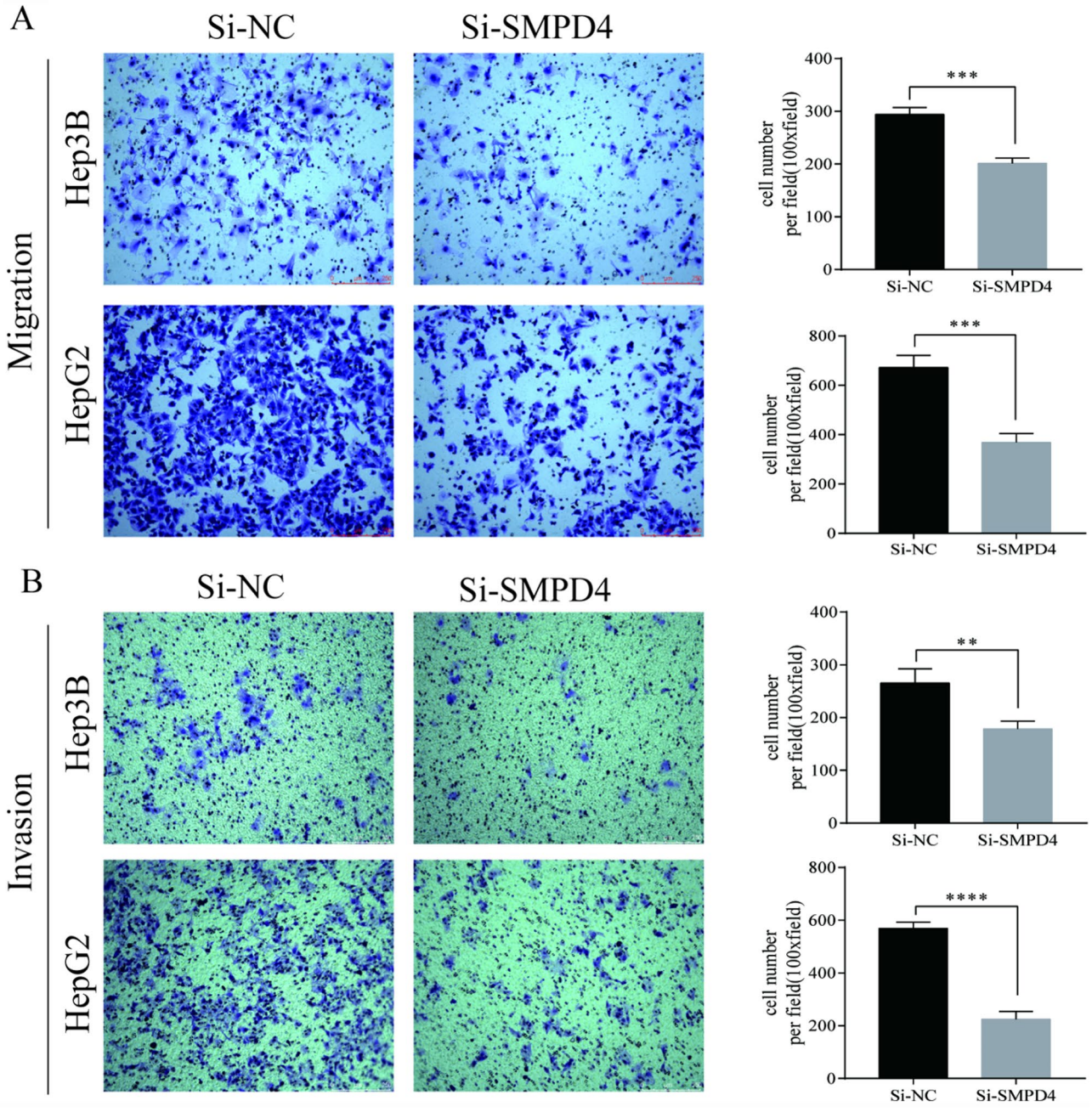

Fig. 8 Transwell experiment detecting the impact of SMPD4 on the invasion ability of HCC cells. **A** Migration experimental results of Hep3B and HepG2. **B** Invasion experimental results of Hep3B and HepG2

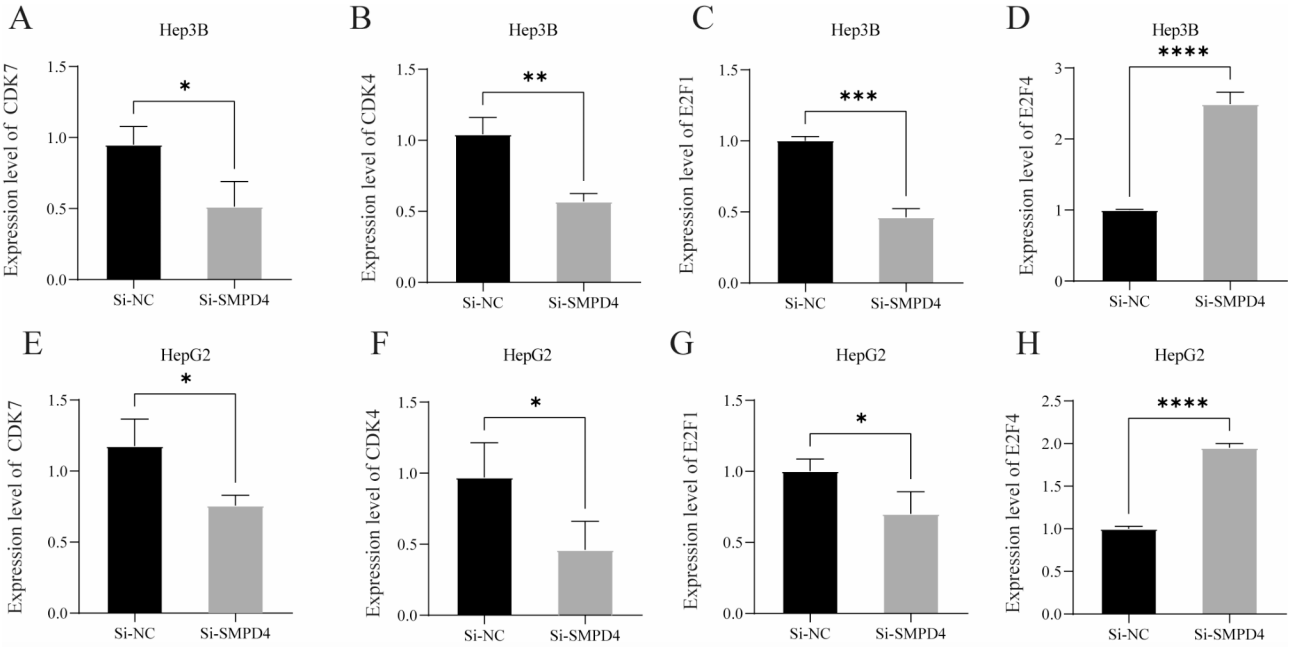

Fig. 9 qRT-PCR detection of cell cycle genes. **A-D** Expression of SMPD4 and CDK7, CDK4, E2F1 and E2F4 in Hep3B. **E-H** Expression of SMPD4 and CDK7, CDK4, E2F1 and E2F4 in Hep G2

Abbreviations

SMPD4	Sphingomyelin phosphodiesterase 4
HCC	Hepatocellular carcinoma
CCK-8	Cell counting kit 8
DFS	Disease free survival
FBS	Fetal bovine serum
GEPIA	Gene Expression Profiling Interactive Analysis
GEO	Gene Expression Omnibus
GO	Gene Ontology
ICGC	International Cancer Genome Consortium
KEGG	Kyoto encyclopedia of genes and genome
ssGSEA	Single sample Gene Set Enrichment Analysis
OS	Overall survival
PFS	Progression free survival
RFS	Relapse free survival
ROC	Receiver operating characteristic
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TCGA	The Cancer Genome Atlas
TIMER	Tumor Immune Estimation Resource
qRT-PCR	Quantitative reverse transcription PCR

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-025-07212-4.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Author contributions

RW and TW designed the study. TW, YS and ZL obtained the data from database. EC and RW performed analysis of the data. RW wrote the manuscript. RW performed experimental analysis. XL, YJ, EC and JL revised the manuscript. All authors contributed to the article and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

The study was approved by the Peking University Shenzhen Hospital ethics committee (2019-052). All methods were carried out in accordance with relevant guidelines and regulations (e.g. Declaration of Helsinki). All patients studied signed informed consent for participation. The patients/participants provided their written informed consent to participate in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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