ORIGINAL ARTICLE

Cancer Science WILEY

Lymphoid-specific helicase promotes the growth and invasion of hepatocellular carcinoma by transcriptional regulation of centromere protein F expression

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Abstract

Lymphoid-specific helicase (LSH) is overexpressed in tumor tissues and its overexpression is associated with poor prognosis in several cancers. However, the role and molecular mechanism of LSH in hepatocellular carcinoma (HCC) remains largely unknown. Herein, we report that LSH was overexpressed in tumor tissues of HCC, and overexpression of LSH was associated with poor prognosis from a public HCC database, and validated by clinical samples from our department. Ectopic LSH expression promoted the growth of HCC cells in vivo and in vitro. Mechanistically, LSH overexpression promoted tumor growth by activating transcription of centromere protein F (CENPF). Clinically, overexpression of LSH and/or CENPF correlated with shorter overall survival and higher cumulative recurrence rates of HCC. In conclusion, LSH promotes tumor growth of HCC through transcriptional regulation of CENPF expression. Therefore, LSH may be a novel predictor for prognosis and a potential therapeutic target for HCC.

KEYWORDS
CENPF, ChIP-seq, HCC, LSH, RNA-seq

1 | INTRODUCTION

Hepatocellular carcinoma is the sixth most common cancer and the third leading cause of cancer-related mortality worldwide.[1] Although some progress has been made in basic and clinical research on HCC, including identification of several diagnostic markers and detection of some genes related to the invasion and metastasis of HCC,[2] the underlying mechanism of HCC remains to be determined.[3,4]

Lymphoid-specific helicase belongs to the SNF2 family of chromatin-remodeling ATPases and plays a critical role in maintaining DNA methylation in development in plants and mammals.[5-8] Recently, LSH has been identified as one of 5-hydroxymethylcytosine (5-hmC) readers in mouse embryonic stem cells, neuronal progenitor cells, and adult mouse brain.[9] Interestingly, LSH maintained genome stability in mammalian somatic cells and also served as a driver in several cancers.[10-15] However, its role in the progression of HCC remains to be determined.

In the present study, we examined the role of LSH in the growth, invasion and metastasis of HCC cells. We also explored the mechanisms of transcription regulation of LSH. We finally established the relationship between LSH expression and HCC prognosis.

2 | MATERIALS AND METHODS

2.1 | Cell lines and cell culture

Human HCC cell lines HCCLM3 (established by the Liver Cancer Institute, Zhongshan Hospital, Fudan University), Huh7, PLC/PRF/5, and Hep3B (purchased from ATCC and raised in Liver Cancer Institute, Zhongshan Hospital, Fudan University) were used in this study. Hep3B cells were cultured in DMEM (HyClone, Logan, UT, USA). HCCLM3, Huh-7, and PLC/PRF/5 cell lines were cultured in DMEM containing 10% FBS (YEASEN, Shanghai, China) supplemented with 100 IU/mL penicillin and 100 µg/mL streptomycin. All cell lines were incubated at 37°C in a humidified atmosphere with 5% CO_2. All cell culture media and FBS were obtained from Gibco (Invitrogen, Carlsbad, CA, USA). Other supplies were obtained from Corning (Corning, NY, USA). This study was approved by the Ethics Committee at Zhongshan Hospital of Fudan University. Full informed consent was obtained from all patients.

2.2 | Quantitative real-time polymerase chain reaction and western blot

Quantitative real-time polymerase chain reaction and WB were carried out as previously described.[16] Primary antibodies against CENPF (20982-1-AP) and LSH (11955-1-AP) were purchased from Proteintech (Chicago, IL, USA). Sequences (5′-3′) of primers used for qPCR are listed below.

Lymphoid-specific helicase, forward primer GAGGCTCC AGCAATGGTTGAA, reverse primer CGCTCTCTCTCTAGTCCAGCA. CENPF, forward primer CTCTCCCGTCAACAGCGTTC, reverse primer GTTGTGCATATTCTTGGCTTGC.

Sequences (5′-3′) of primers used for ChIP-qPCR are listed below. CENPF, forward primer TCTGCTCGGGTTCAAACTGG, reverse primer TGTGAGTCCGTGACCGAGTA.

2.3 | Transfection and clone selection

Lymphoid-specific helicase, LSH-shRNA, and CENPF-shRNA expression lentiviral vectors were purchased from Shanghai GeneChem Co. (Shanghai, China), and the lentiviral vector was transfected using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Transfected HCC cells were selected using puromycin for 7 days prior to assay.

2.4 | Patients and follow up

Fresh tissues were randomly collected from consecutive patients with HCC who underwent curative resection between 2005 and 2009 at the Institute of Liver Cancer, Fudan University. None of the patients received any chemotherapy or radiation treatment prior to the surgery. The study and methods for informed consent were examined and certified by the Ethics Committee of Fudan University.

2.5 | Immunohistochemistry and tissue microarrays

Immunohistochemistry was carried out as described elsewhere.[16] Tissue microarrays (TMA) were constructed as previously described.[17] Primary antibodies against CENPF (20982-1-AP) and LSH (11955-1-AP) were obtained from Proteintech.

2.6 | Cell migration, Matrigel invasion assay, cell viability, and colony formation assay

Cell migration was tested by wound-healing experiment. Matrigel invasion assay was obtained from BD Biosciences (Franklin Lakes, NJ, USA). Cell viability was measured by CCK-8 assay (YEASEN, Shanghai, China). Matrigel invasion assay and CCK-8 assay were carried out according to the manufacturers' protocols. Colony formation assay and wound-healing experiment were done as previously described.[16]

2.7 | Flow cytometry assay

Cell cycle and apoptosis were detected by flow cytometry (FCM; Becton Dickinson, Franklin Lakes, NJ, USA).

2.8 | Tumor xenograft assay

Hepatocellular carcinoma cells were used to establish s.c. xenograft tumor models. Tumor growth was monitored twice weekly, and mice were killed after 4 weeks. Tumors were measured in two dimensions.

2.9 | Chromatin immunoprecipitation

Chromatin immunoprecipitation was carried out as follows. Briefly, DNA was cross-linked using 1% formalin, the cells were lysed in SDS buffer, and DNA was fragmented by sonication. ChIP for LSH was done using an anti-Flag antibody (SAB4301135; Sigma Chemical Co., St Louis, MO, USA).
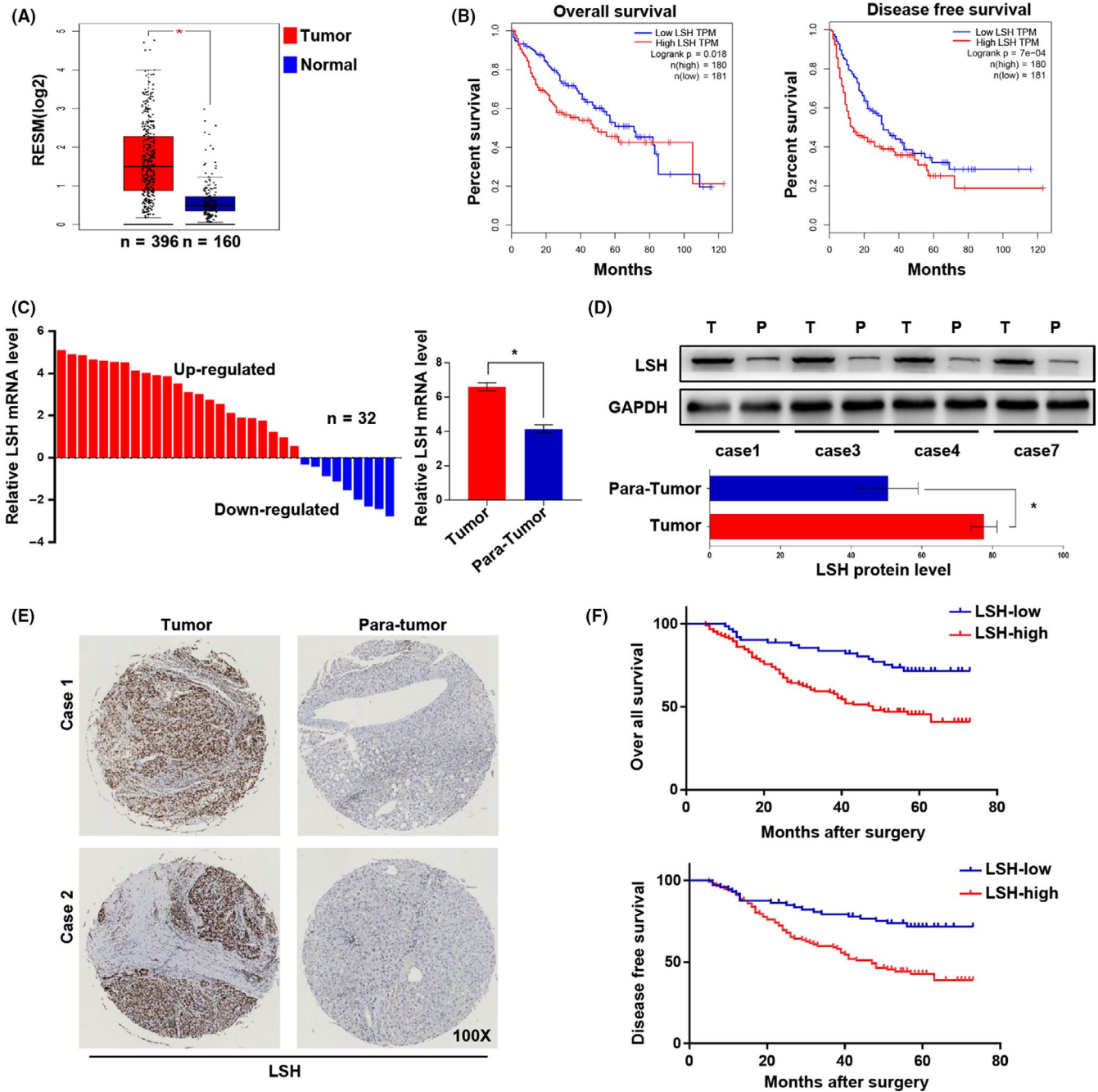
FIGURE 1 Lymphoid-specific helicase (LSH) is overexpressed in tumor tissues and its expression correlates with overall survival of hepatocellular carcinoma (HCC) patients. A, Results from public database GEPIA indicated LSH expression in HCC is higher than that in para-tumor tissues. B, Online analysis of overall survival (OS) and disease-free survival (DFS) shows that higher LSH expression indicates a poorer prognosis. P-values are shown in the figure. C and D, mRNA and protein expressions of LSH were tested by qPCR and western blot. E, HCC tissue microarrays were tested by immunohistochemistry (IHC) using LSH antibody. F, IHC scores of LSH were analyzed for OS and DFS. *P < 0.05

2.10 | High-throughput sequencing

The resulting DNA library was sequenced on Illumina Hiseq2500 (San Diego, CA, USA). The results obtained were analyzed using Hisat2, StringTie and Ballgown tools to obtain deferentially expressed genes. The UCSC Genome Browser (University of California, Santa Cruz) was used for data visualization.

2.11 | Statistical analysis

Statistical analysis was carried out using SPSS software (version 19.0; SPSS, Inc.). Values are expressed as mean and standard deviation (SD). Student's t test and one-way ANOVA were used for comparisons between groups. Categorical data were analyzed by chi-squared or Fisher's exact tests. Correlation analysis was carried

TABLE 1 Correlation between LSH expression and clinicopathological characteristics in 208 HCC patients

| Variable | No. of patients | LSH expression | | P-value |
		LSH high	LSH low	
Gender				
Female	30	11	19	0.064
Male	178	57	121	
Age (years)				
<52	110	39	71	0.135
≥52	98	39	59	
Cirrhosis				
Yes	186	72	114	**<0.001**
No	22	6	16	
HBsAg				
Positive	36	14	22	0.028
Negative	172	64	108	
HCV				
Positive	6	1	5	0.190
Negative	202	77	125	
AFP (ng/mL)				
<20	77	30	47	0.755
≥20	131	48	83	
Tumor size (cm)				
<5	116	50	66	**<0.001**
≥5	92	28	64	
No. tumors				
Single	174	68	106	**<0.001**
Multiple	34	10	24	
Tumor encapsulation				
Complete	102	43	59	0.023
None	106	35	71	
Tumor differentiation				
I + II	151	56	95	**<0.001**
III + IV	57	22	35	
Tumor thrombus				
Positive	133	66	67	**<0.001**
Negative	75	12	63	
TNM stage				
I	147	58	89	**<0.001**
II + III	61	20	41	

Bold values are statistically significant ($P < 0.05$).
AFP, alpha fetoprotein; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LSH, lymphoid-specific helicase.

out to assess the relationship between LSH and CENPF expression. Cumulative recurrence and survival rates were analyzed using Kaplan-Meier's method and log-rank test. Cox's proportional hazards regression model was used to analyze independent prognostic factors. $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Lymphoid-specific helicase is overexpressed in tumor tissues and its expression correlates with overall survival of HCC patients

To explore the expression and potential role of LSH in HCC, we first used the publicly available HCC database (GEPIA, http://gepia. cancer-pku.cn) to analyze LSH mRNA expression between tumor specimens and normal tissues.[18] As shown in Figure 1A, LSH mRNA expression was significantly elevated in HCC tissues compared to para-tumor liver tissues. Importantly, LSH mRNA expression was negatively associated with overall survival ($P = 0.018$, Figure 1B) and relapse-free survival ($P < 0.001$, Figure 1B), suggesting that LSH expression may be an indicator of the prognosis of HCC patients.

Lymphoid-specific helicase expression was significantly higher in tumor tissues than in para-tumor tissues (Figure 1C,D). To validate the relationship between LSH expression and the prognosis of HCC patients, 208 HCC tissues and corresponding para-tumor liver tissues were subjected to IHC staining for LSH. Positive staining was located in the nucleus of tumor cells (Figure 1E). We further analyzed the correlation between LSH expression and clinical features, as shown in Table 1. Furthermore, Kaplan-Meier analysis showed that higher level of LSH expression was associated with shorter overall survival (OS) ($P < 0.001$; Figure 1F) and disease-free survival (DFS) ($P < 0.001$; Figure 1F). Moreover, univariate and multivariate analyses showed that LSH expression was an independent prognostic factor of OS for patients with HCC ($P = 0.001$; Table 2). Taken together, these data indicate that upregulation of LSH contributes to recurrence and is associated with a poorer prognosis in HCC.

3.2 | Knockdown of LSH expression inhibits cell growth and invasion of HCC cells in vitro and in vivo

To further explore the function of LSH in HCC, we analyzed its expression in four different metastatic potential HCC cell lines to select the most appropriate cell models for loss-of-function and gain-of-function assays (Figure 2A). Results showed that high metastatic potential HCC cell lines LCCLM3 and Huh-7 tended to express a high level of LSH, whereas low metastatic potential HCC cell lines PLC/PRF/5 and Hep3B had low LSH expression (Figure 2A). Then, we successfully constructed HCCLM3 cells with stable knocked-down LSH (HCCLM3-shLSH) and PLC/PRF/5 cells with upregulated LSH expression (PLC/PRF/5-LSH), confirmed by WB and qPCR (Figure 2B).

CCK-8 assay showed that cell proliferation was significantly decreased in LSH knockdown cells (Figure 2C). The capacity for colony formation of HCC cells was clearly reduced after LSH was knocked down, whereas the capacity for colony formation was enhanced when LSH was overexpressed (Figure 2D). Flow cytometry analyses showed that the proportion of cells in G0/G1 in HCCLM3 cells was higher than that of HCCLM3-shLSH and vice versa (Figure 2E). Similarly, downregulated expression of LSH reduced the increased rate of apoptosis (Figure 2F). Invasion capacity was also inhibited in LSH knockdown

TABLE 2 Univariate and multivariate analyses of factors associated with overall survival

Factor	Univariate *P*-value	Multivariate analysis		
		HR	95% CI	*P*-value
Gender (female vs male)	0.173			NA
Age (years) (≥52 vs <52)	0.381			NA
Liver cirrhosis (yes vs no)	0.843			NA
HBsAg (positive vs negative)	0.167			NA
HCV (positive vs negative)	0.981			NA
Serum AFP, ng/mL (≥20 vs <20)	0.371			NA
Tumor encapsulation (yes vs no)	0.534			NA
Tumor differentiation (III/IV vs I/II)	0.193			NA
Tumor number (multiple vs single)	0.074			NA
Tumor thrombus (positive vs negative)	**0.003**	1.729	1.138-2.191	**0.001**
Tumor size (diameter, cm) (≥5 vs <5)	**0.001**	1.942	1.276-2.334	**0.003**
TNM stage (I/II vs III/IV)	0.021			NA
LSH expression (high vs low)	**<0.001**	2.115	1.562-3.156	**0.001**
CENPF expression (high vs low)	**<0.001**	2.225	1.361-3.638	**0.001**

Multivariate analysis, Cox proportional hazards regression model. Variables were adopted for their prognostic significance by univariate analysis with forward stepwise selection (forward, likelihood ratio). Variables were adopted for their prognostic significance by univariate analysis (*P* < 0.01). Bold indicates *P* < 0.05.
AFP, alpha fetoprotein; CENPF, centromere protein F; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HR, hazard ratio; LSH, lymphoid-specific helicase; NA, not applicable.

cells and enhanced in cells overexpressing LSH (Figure 2G). Wound healing assay showed that the migratory ability of HCC cells was significantly inhibited after LSH expression was decreased (Figure 2H,I).

In an in vivo experiment, 5×10^6 HCCLM3-shLSH, PLC/PRF/5-LSH cells and controls were s.c. implanted into nude mice, respectively. Mice were killed on the 35th day after inoculation. The results showed that xenografts from HCCLM3 cells and PLC/PRF/5-LSH cells were significantly bigger than those from HCCLM3-shLSH and PLC/PRF/5 cells, respectively (Figure 2J,K). Together, these findings indicate that LSH effectively promotes the growth and invasion of HCC cells.

3.3 | mRNA sequencing and ChIP-seq show target genes of LSH

To further investigate the molecular mechanism of the role of LSH in HCC cells, we used mRNA-seq and ChIP-seq to analyze mRNA expression profiles of alteration of LSH expression and DNA-protein interactions with LSH, respectively. First, gene expression profiles of HCCLM3 cells stably transfected with two different shRNA sequences were analyzed by mRNA-seq, and the differentially expressed genes (>2-fold) were identified (shown in heat map, Figure 3A). We found that 4342 differentially expressed genes overlapped in these two cells. Then, we used to ChIP for LSH and sequence to identify the target genes of LSH in the above HCCLM3 cells transfected with two different shRNA sequences. We identified 1238 differentially expressed genes (>2-fold) and they intersected with the above 4342 overlapped differential genes. Results showed 146 overlapping genes (Figure 3B). KEGG and gene ontology (GO) analyses for these 146 genes were carried out. The results showed that cell biological pathways, such as cell cycle, division, and response to drugs and hormones were increased (Figure 3B). Gene set enrichment analysis (GSEA) was carried out and significant pathways were identified for both up- and downregulated gene sets. As LSH is located mainly in the nucleus (Figure 1E), pathways related to chromosome, nuclear division and cell cycle were analyzed.
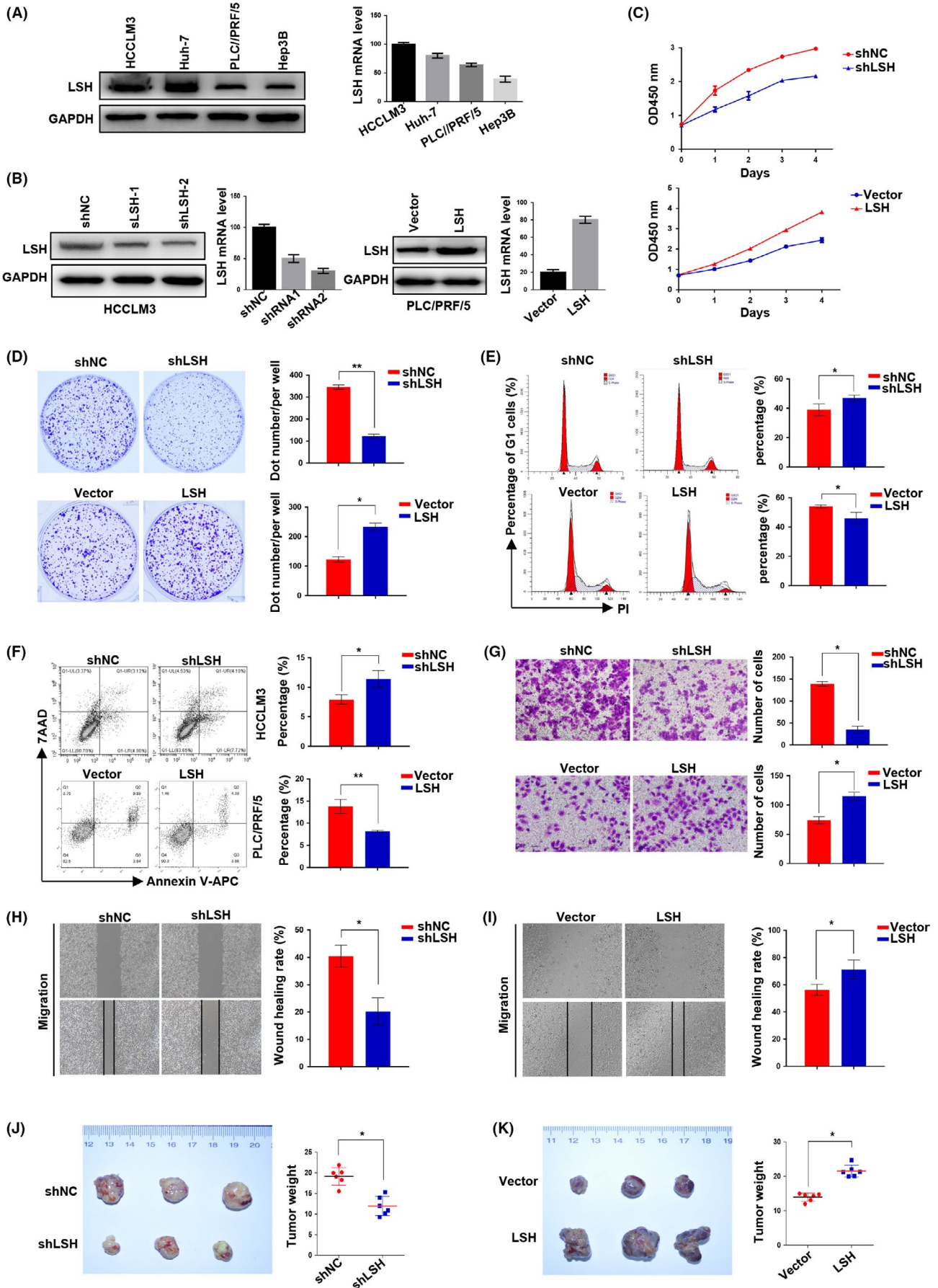
FIGURE 2 Knockdown of lymphoid-specific helicase (LSH) expression inhibits cell growth and invasion of hepatocellular carcinoma (HCC) cells in vitro and in vivo. A, Western blot (WB) and qPCR experiments for testing LSH in HCC cell lines. B, Knockdown and overexpression of LSH confirmed by WB and qPCR. C, CCK-8 assays show decreased OD450 after LSH knockdown and elevated OD450 after overexpression. D, Colony formation assays for the HCC cell lines used above. E and F, Cell cycle and apoptosis rates tested by flow cytometry. G, Transwell assay carried out in these cell lines. H and I, Results of wound-healing experiments. J and K, Subcutaneous tumors in nude mice after death. *$P < 0.05$, **$P < 0.01$

Results showed knockdown of LSH obviously disrupted these pathways (Figure 3C). Of note, one of the genes most frequently included in these gene sets was CENPF (Figure 3D).

3.4 | Centromere protein F is overexpressed in HCC tissues and is positively correlated with LSH protein overexpression

Given the important role of CENPF in the cell cycle, mitosis and regulation of PLK1 activity at G2/M transition, we further explored the interaction of LSH with CENPF. We first analyzed the relationship between LSH and CENPF expression in The Cancer Genome Atlas (TCGA) database. We observed that *LSH* and *Cenpf* mRNA were consistently upregulated in HCC tissues, compared to para-tumor liver tissues (Figure 4A,B,

Pearson 0.69, Spearman 0.8 by cBioPortal). Moreover, survival analysis showed that higher *Cenpf* expression in tumor tissues was associated with poorer prognosis in HCC patients (Figure 4C,D, by GEPIA).

We also investigated the expression of CENPF in 208 HCC samples and analyzed the correlation between CENPF and LSH. Results showed that CENPF expression in tumor tissues was significantly higher than that in para-tumor tissues at the level of mRNA and protein (Figure 4E). Importantly, we found that increased LSH protein level is associated with increased CENPF protein level (Figure 4F). Furthermore, survival analysis also showed that high CENPF expression in tumor tissues was associated with short overall survival (OS) ($P < 0.001$) and DFS ($P < 0.001$; Figure 4G). Similarly, univariate and multivariate analyses were carried out and showed that CENPF expression was an independent prognostic factor of OS for patients with HCC ($P = 0.001$; Table 3).
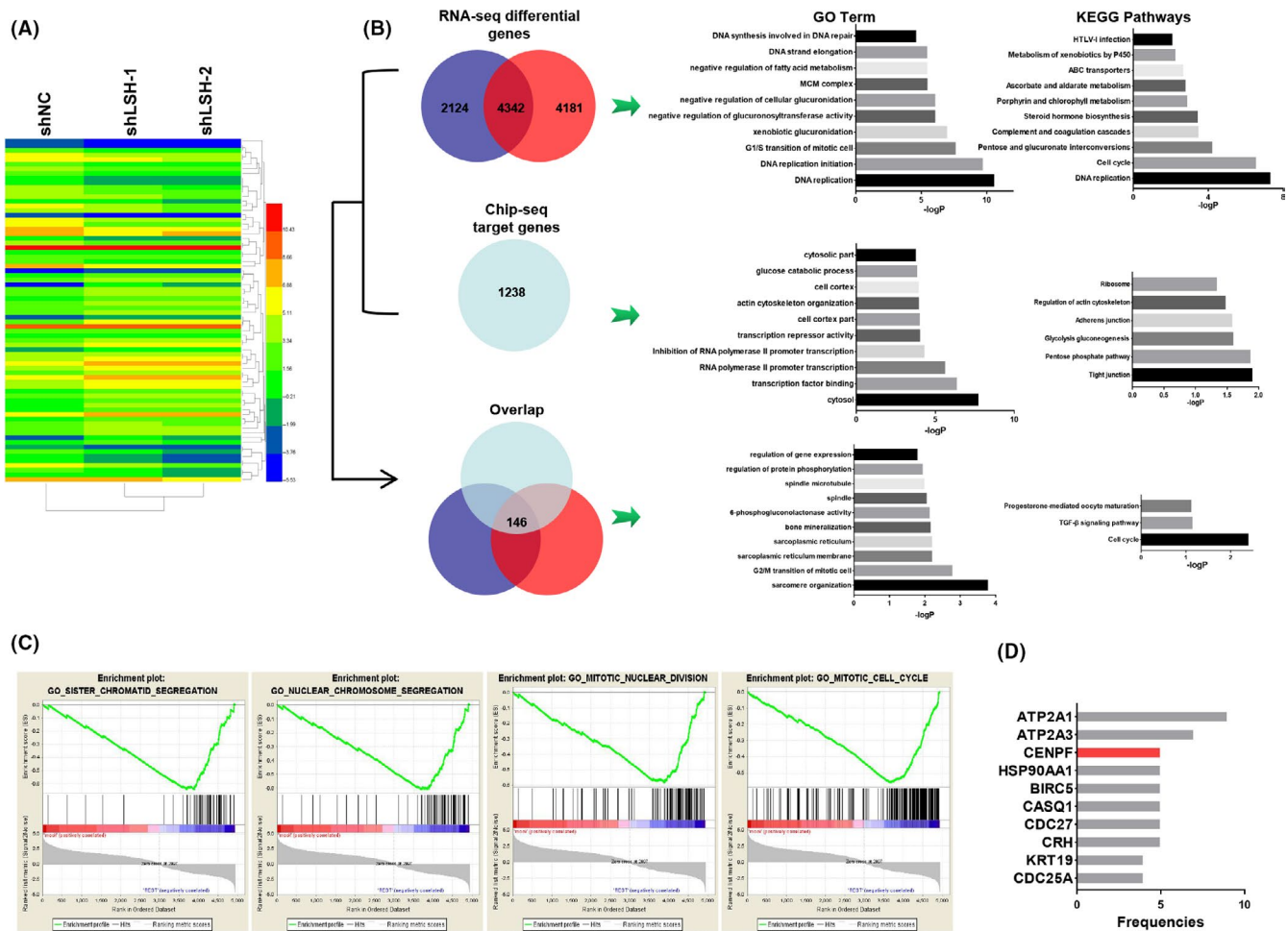

FIGURE 3 mRNA sequencing (mRNA-seq) and ChIP-seq shows target genes of lymphoid-specific helicase (LSH). A, Heat map of RNA-seq after LSH knockdown. B, Gene ontology (GO) and KEQQ analysis of differential genes of RNA-seq, ChIP-seq and their overlap. C, Gene set enrichment analysis (GSEA) analysis of differential genes of RNA-seq. D, Frequencies of included genes in GO and KEGG gene sets
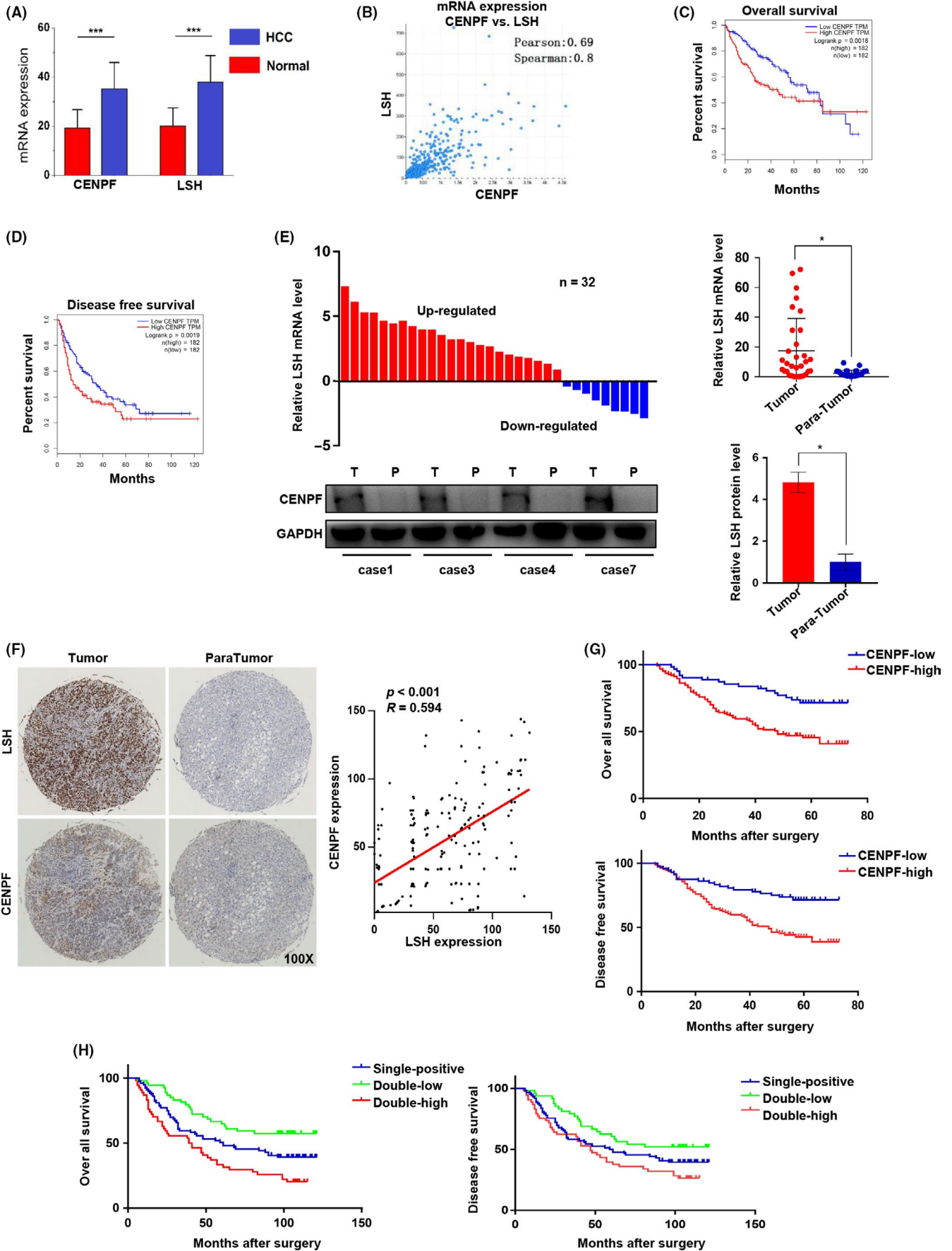
(A) mRNA expression — CENPF, LSH; HCC, Normal; ***

(B) mRNA expression CENPF vs. LSH; Pearson: 0.69; Spearman: 0.8

(C) Overall survival; Low CENPF TPM, High CENPF TPM; Logrank p = 0.0018; n(high) = 182; n(low) = 182

(D) Disease free survival; Low CENPF TPM, High CENPF TPM; Logrank p = 0.0019; n(high) = 182; n(low) = 182

(E) Relative LSH mRNA level; n = 32; Up-regulated; Down-regulated; CENPF, GAPDH; T, P; case1, case3, case4, case7; Relative LSH mRNA level — Tumor, Para-Tumor *; Relative LSH protein level — Tumor, Para-Tumor *

(F) Tumor, ParaTumor; LSH, CENPF; 100X; p < 0.001; R = 0.594; CENPF expression vs. LSH expression

(G) Over all survival; CENPF-low, CENPF-high; Months after surgery; Disease free survival; CENPF-low, CENPF-high; Months after surgery

(H) Over all survival; Single-positive, Double-low, Double-high; Months after surgery; Disease free survival; Single-positive, Double-low, Double-high; Months after surgery

FIGURE 4 Centromere protein F (CENPF) is overexpressed in hepatocellular carcinoma (HCC) tissues and is positively correlated with lymphoid-specific helicase (LSH) protein overexpression. A, CENPF and LSH mRNA expression. B, Correlation of mRNA level between CENPF and LSH. C and D, Online analysis for overall survival (OS) and disease-free survival (DFS) shows that higher CENPF expression indicates a poorer prognosis. E, mRNA and protein levels tested by qPCR and western blot (WB). F, Immunohistochemistry (IHC) test of CENPF protein expression in clinical samples, and the correlation between LSH and CENPF expression. G, IHC scores of CENPF were analyzed for OS and DFS. H, OS and DFS analysis integrated CENPF and LSH IHC scores. *P < 0.05. ***P < 0.001

TABLE 3 Correlation between CENPF and clinicopathological characteristics in 208 HCC patients

Variable	No. of patients	CENPF expression		P-value
		CENPF high	CENPF low	
Gender				
Female	30	7	23	0.214
Male	178	66	112	
Age (years)				
<52	98	35	63	0.885
≥52	110	38	72	
Hepatic cirrhosis				
Yes	186	66	120	0.817
No	22	7	15	
HBsAg				
Positive	172	114	58	0.443
Negative	36	21	15	
HCV				
Positive	6	3	3	0.667
Negative	202	132	70	
AFP (ng/mL)				
<20	73	44	29	0.654
≥20	135	86	49	
Tumor size (cm)				
<5	116	73	43	0.560
≥5	92	62	30	
No. tumors				
Single	174	110	64	0.327
Multiple	34	25	9	
Tumor encapsulation				
Complete	102	41	61	0.198
None	106	33	73	
Tumor differentiation				
I + II	151	102	49	0.198
III + IV	57	33	24	
Tumor thrombus				
Positive	64	40	24	0.640
Negative	144	95	49	
TNM stage				
I	146	95	51	**0.017**
II + III	62	40	22	

Bold values are statistically significant (P < 0.05).
AFP, alpha fetoprotein; CENPF, centromere protein F; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.
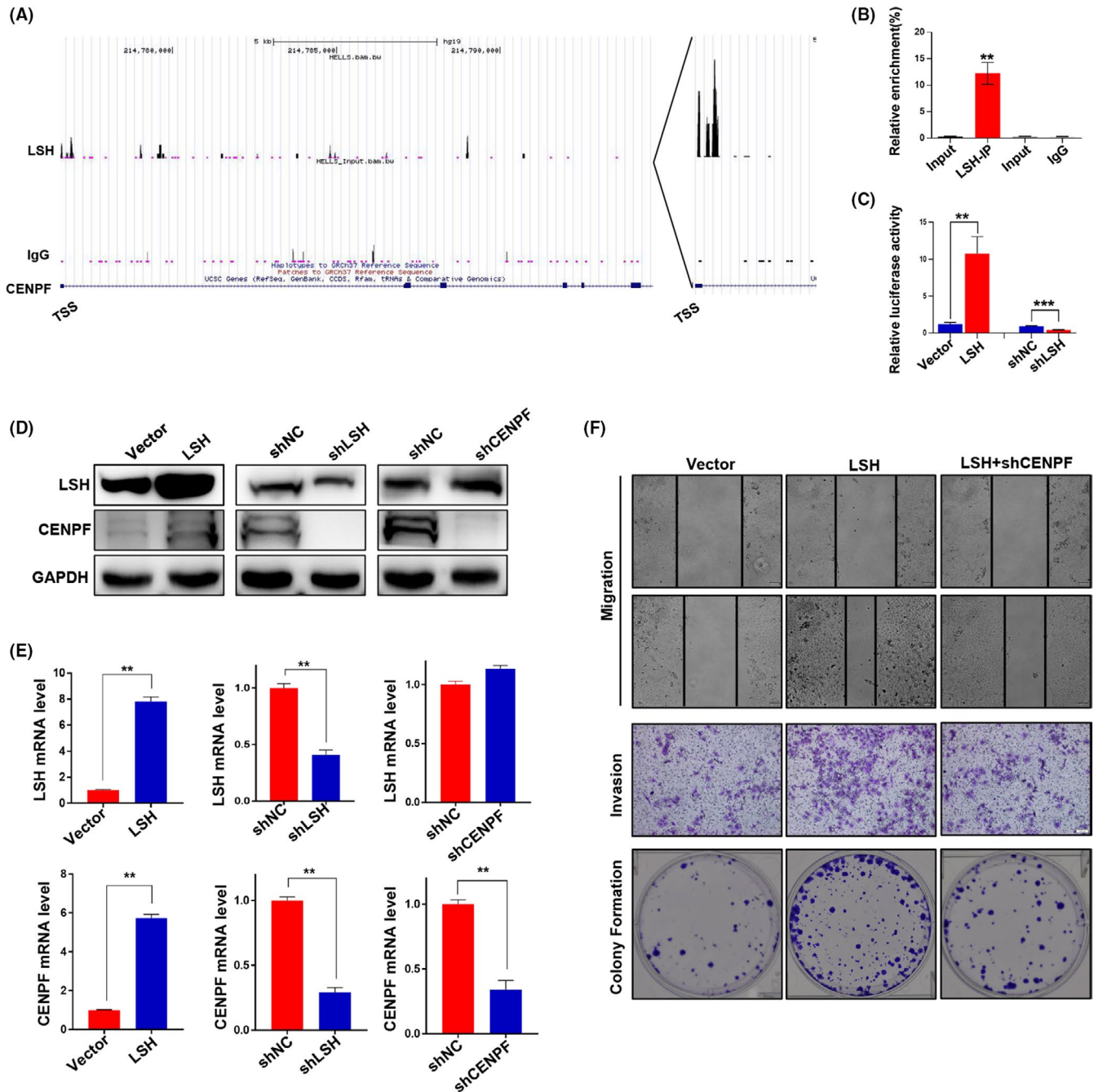
(A)

(B)

(C)

(D)

(E)

(F)

FIGURE 5 Lymphoid-specific helicase (LSH) binds to the transcription start site of *cenpf* and promotes the growth of hepatocellular carcinoma in a centromere protein F (CENPF)-dependent way. A, ChIP-seq visualized by UCSC at the *cenpf* gene body and transcription start site (TSS). B, ChIP-qPCR verified for LSH binding. C, Luciferase activity in the indicated cells. D, Western blot results for LSH and CENPF correlations. E, qPCR results for LSH and CENPF correlations. F, Influence on phenotypes of combined CENPF and LSH. **$P < 0.01$, ***$P < 0.001$

Considering the positive relationship between LSH and CENPF expression, we divided the cohorts into three subgroups based on the expression of LSH and CENPF (both high, both low, single high). We then carried out Kaplan-Meier analysis and log-rank test and found that HCC patients with high expression of both CENPF and LSH ("both high") showed the worst OS ($P < 0.001$) and DFS ($P < 0.001$), whereas the subgroup with low expression of both CENPF and LSH ("both low") had the best prognosis (Figure 4H). These clinical data indicate that LSH probably plays a substantial role in HCC in a CENPF-dependent manner.

3.5 | Lymphoid-specific helicase binds to the transcription start site of *Cenpf* and promotes growth of HCC in a CENPF-dependent way

To further address the interaction of LSH with CENPF, we thoroughly analyzed the above ChIP-seq data. We observed that a specific peak of LSH overlapped at the transcription start site (TSS) of the *cenpf* gene (Figure 5A), which is located at chr1: 214 776 582-214 776 968, and verified these results by qPCR using
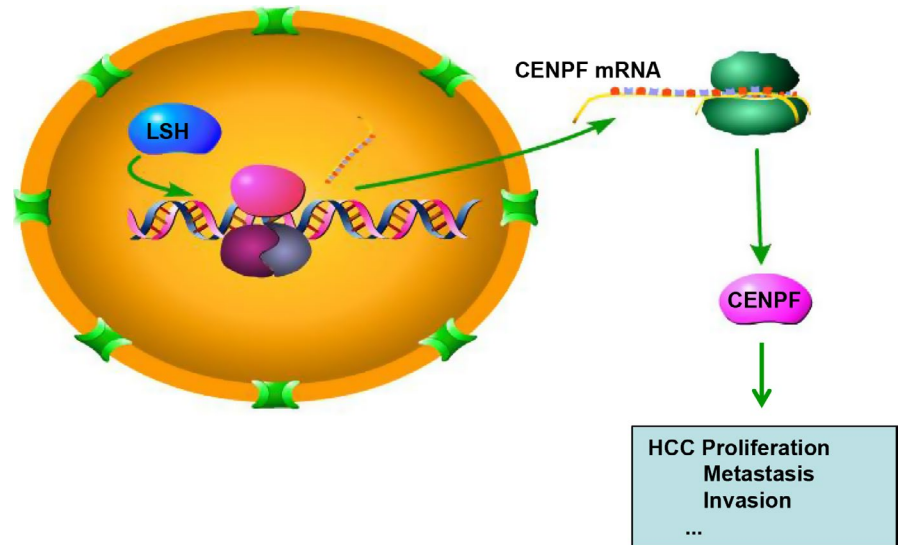
specific primers targeting the TSS region (Figure 5B). Interaction of LSH with CENPF was further validated in HCC cells. Luciferase activity and CENPF expression were dramatically reduced after LSH expression was inhibited (Figure 5C-E). Similarly, luciferase activity and expression of CENPF were obviously increased after LSH was overexpressed (Figure 5C-E). However, LSH expression was not significantly changed when CENPF was knocked down (Figure 5D,E). These results showed that CENPF was one of the downstream targets of LSH. Functional analysis also showed that cell proliferation and migration influenced by LSH overexpression could be partially inhibited by CENPF knockdown (Figure 5F).

4 | DISCUSSION

Lymphoid-specific helicase plays a critical role in the development of mammals through maintaining DNA methylation and remodeling chromatin.[6,7] Recent studies also showed upregulated expression of LSH in several malignant tumors, such as prostate cancer, melanoma, head and neck cancer; and LSH is probably involved in the tumor progression.[12] Moreover, LSH has been reported to be linked to glioma biology as a downstream target of LRP6-GSK3β-E2F1 signaling;[19] however, the detailed mechanism of LSH and its downstream targets in cancers still need to be thoroughly addressed. A recent study has reported that by altering nucleosome occupancy at the nucleosome-free region (NFR) and enhancer, LSH epigenetically suppresses multiple tumor suppressor genes including E-cadherin, FBP1, IGFBP3, XAF1 and CREB3L3 to promote HCC progression.[20] In the present study, we first showed upregulated expression of LSH in HCC samples from a public database, and established the linkage of high expression of LSH with poor prognosis of HCC patients. Second, we validated these relationships of LSH and prognosis of patients in a larger cohort of HCC patients. Third, we used transfection or interference technology to modify the expression of LSH in HCC cells and found that upregulation of LSH expression in HCC cells promoted growth,

migration and invasion in vitro. Last, but not least, the in vivo experiment showed that enforced expression of LSH hastened tumor growth. These data provide sufficient evidence to support the notion that LSH plays a substantial role in the growth and progression of HCC, which is consistent with previous reports of HCC.

Although both we and a previous report found that LSH promotes HCC progression, we have formulated a new explanation for the mechanism.[20] In addition to its role in DNA methylation, LSH is also considered a nucleoprotein. Our important finding from the present study is that LSH plays a key role in tumor growth through regulation of downstream target CENPF. In the present study, we used a combination of mRNA-seq with ChIP-seq and confirmed the interaction of LSH and CENPF. qPCR analysis then showed that LSH combined with the *cenf* TSS area. Importantly, modification of LSH expression in HCC cells could correspondingly alter the expression of CENPF. In turn, alteration of CENPF expression did not influence LSH expression, indicating that CENPF is a downstream target of LSH (summarized in Figure 6). CENPF protein is a component of the nuclear matrix during the G2 phase of interphase and is required for kinetochore function and chromosome segregation in mitosis. Previous studies have shown that CENPF is upregulated in breast cancer,[21] nasopharyngeal cancer,[22] hepatocellular carcinoma,[23] esophageal squamous cell carcinoma,[24] gastrointestinal stromal tumors[25] and, in some cases it is associated with aggressive tumor phenotype and poor survival.[21,22,24] However, the mechanism of CENPF expression control remains unclear. In the present study, inhibition of LSH in HCC cells significantly decreased the proportion of cells in G0/G1. More importantly, increased expression of CENPF could rescue the growth, migration and invasion of HCC cells. Clinically, HCC patients expressing high CENPF and LSH showed the poorest prognosis. These data not only broaden our understanding of the mechanism of the role of LSH in tumor progression, but also provide convincing evidence to support the notion that LSH may be a novel therapeutic target for HCC patients.

In conclusion, LSH promotes tumor progression through transcription regulation of *cenpf*, and may be an effective therapeutic target for a subgroup of HCC patients with high expression of LSH.

The datasets supporting the conclusions of this article are included within the article and its supplementary material. The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

DISCLOSURE

Authors declare no conflicts of interest for this article.

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