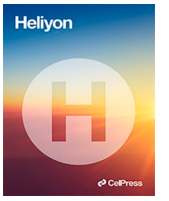
Heliyon 10 (2024) e32409

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

PWP1 is overexpressed in hepatocellular carcinoma and facilitates liver cancer cell proliferation

Rong Huang [a], Fu Xu [a], Luying Su [a], Yan Lu [a], Wei Liu [a], Shuaihu Liu [a], Ling Yang [b], Liya Su [b,*], Wei Song [a,**]

[a] *Department of Biochemistry and Molecular Biology, State Key Laboratory of Common Mechanism Research for Major Diseases, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences, School of Basic Medicine Peking Union Medical College, Beijing, 100005, China*
[b] *Clinical Medical Research Center of the Affiliated Hospital, Inner Mongolia Medical University, Inner Mongolia Key Laboratory of Medical Cell Biology, 1 Tong Dao Street, Hohhot, 010050, Inner Mongolia, China*

ARTICLE INFO

Keywords:
Hepatocellular carcinoma (HCC)
Periodic tryptophan protein 1 (PWP1)
Tumorigenesis
Therapeutic target

ABSTRACT

Identification of novel biomarkers for prediction of disease course and prognosis is needed to reduce morbidity of liver hepatocellular carcinoma (LIHC/HCC) patients. Although dysregulated Periodic tryptophan protein 1 homolog (PWP1/endonuclein) expression has been detected in several tumors, the potential regulatory effect of PWP1 on LIHC remains uncertain. Here we evaluated the expression of PWP1 using multiple online platforms, and demonstrated that PWP1 upregulation was consistently observed in LIHC relative to non-tumor liver tissues and correlated with unfavorable prognosis. Moreover, HCC prognosis was significantly influenced by the methylation status of various CpG sites in the PWP1 gene. Lastly, we provide direct evidence that PWP1 acts as a driver of HCC progression by showing that siRNA-mediated PWP1 silencing significantly suppressed HCC cell proliferation in vitro. These data strongly suggest that PWP1 silencing may be an effective therapeutic strategy to treat LIHC.

1. Introduction

According to GLOBOCAN 2020 statistics, liver cancer represents the third major cause of cancer-related death, accounting for 830,180 deaths (8.3 %) worldwide and surpassed only by lung and colorectal cancer [1]. Hepatocellular carcinoma (HCC) encompasses 75%–85 % of liver cancer cases and is thus the predominant form of primary liver cancer. Although surgical resection is currently the most effective therapeutic intervention [2], both high incidence of recurrence and unsatisfactory clinical outcomes expose the urgency of exploring new methods of treatment [1]. Multilayered processes, including genomic instability, oncogene activation, tumor suppressor inhibition, epigenetic modifications, tumor microenvironment dynamics, as well as anomalous cell signaling, constitute the main determining factors of cancer initiation and progression [3]. Therefore, elucidating the biological mechanisms connected with tumor hyperplasia is needed to identify clinically relevant, sensitive tumor biomarkers for accurate diagnosis and therapy of HCC.

Periodic tryptophan protein 1 homolog (PWP1; endonuclein) is a WD-40 repeat domain-containing protein initially discovered in *Saccharomyces cerevisiae* [4]. Further characterization indicated that PWP1 homologs exist in multiple species, including Drosophila,

* Corresponding author.
** Corresponding author.
E-mail addresses: suliya2307@hotmail.com (L. Su), songwei@ibms.pumc.edu.cn (W. Song).

https://doi.org/10.1016/j.heliyon.2024.e32409
Received 6 November 2023; Received in revised form 3 June 2024; Accepted 4 June 2024
Available online 4 June 2024

zebrafish, mice, and humans, and play vital roles in transcriptional gene regulation and tissue growth [5–7]. Research indicated that PWP1 acts as a molecular effector of H3K4 methylation, and deletion of PWP1 in telomeric regions leads to rapid telomere shortening [8]. The multipotent differentiation capacity of mouse embryonic stem cells was also shown to be impaired by PWP1 knockdown, through a mechanism involving upregulated expression of Stat3 secondary to decreased H4K20 methylation in its upstream regulatory region [9]. Importantly, PWP1 is co-expressed with many RNA-processing genes in the mouse genome and is essential for ribosomal RNA (rRNA) biogenesis [10,11]. Research in Drosophila showed that the PWP1 ortholog NLCB regulates nutrient-dependent growth through rRNA transcription mediated by RNA *Pol*I [12]. Furthermore, NLCB co-localizes with RNA Pol II in Drosophila polytene chromosomes, and its deficiency impairs germline stem cell maintenance in male flies, leading to sterility [13].

The impact of PWP1 on the biology of solid tumors has been revealed by previous studies. Overexpression of PWP1 was detected in human pancreatic adenocarcinoma, in association with cell cycle regulation. Interestingly, and suggestive of PWP1 diagnostic and therapeutic potential, upregulated PWP1 expression has been correlated with unfavorable survival in head and neck squamous cell carcinoma, as well as in lung cancer [12,14]. In turn, molecular assays indicated that PWP1 enhances lung cancer cell malignancy through its interaction with DVL2 and Merlin to upregulate and downregulate, respectively, intracellular signaling mediated by the Wnt and Hippo pathways [14]. While these findings support a prominent role of PWP1 in different cancer types, the potential function of PWP1 in HCC remains largely undetermined.

Therefore, with the aid of The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) datasets, the present research intended to illustrate the transcriptional and translational expression profiles of PWP1 in HCC and other tumor types. Moreover, the association between PWP1 expression levels and genomic alterations, DNA methylation status, functional partner genes, cellular processes, and clinical outcomes was also analyzed in HCC datasets. Lastly, proliferation and tumorigenesis assays were conducted in PWP1-silenced human HCC cells in vitro to validate the pro-oncogenic activity of PWP1. Based on the multiple associations with potential clinical relevance detected for dysregulated PWP1 expression in HCC, we propose that PWP1 may represent a robust therapeutic target for HCC treatment.

2. Materials and methods

2.1. Online data mining

PWP1 gene expression was systematically explored in normal tissues and tumor samples in the TIMER2.0 portal (https://cistrome.shinyapps.io/timer/) [15]. The “Diff exp” module was used to visualize gene expression profiles.

HCCDB (lifeome.net/database/hccdb/), an integrated visual database that provides expression profiles of over 3000 HCC samples [16], was addressed to estimate mRNA levels of PWP1 in LIHC and control paratumoral tissues.

We next browsed UALCAN (http://ualcan.path.uab.edu/index.html) to evaluate associations between PWP1 mRNA expression and survival [17]. PWP1 methylation level in primary tumor and adjacent normal tissues was also analyzed in this platform. Besides, we analyzed PWP1 protein levels in primary liver tumors and control normal samples within the “CPTAC” module. Significance was assessed as $P < 0.05$.

The Kaplan-Meier Plotter (https://kmplot.com/analysis/) portal [18,19] was interrogated to evaluate the prognostic features of PWP1 in LIHC by assessing the correlation between PWP1 mRNA levels and clinical outcomes. The optimal threshold was defined by the KM plotter algorithm and $P < 0.05$ was considered significant.

GEPIA2 (http://gepia.cancer-pku.cn/), a bioinformatics atlas for large-scale expression profiling and interactive analysis [20], was used to predict the clinical outcomes of high and low PWP1 expression in LIHC. The median mRNA expression level was chosen for dividing patients into high- and low-PWP1 subgroups. The relationship between PWP1 and its 10 closest interacting proteins was estimated in the “Correlation Analysis” module, while the “Expression DIY” section was used to visualize the expression of PWP1 in liver tumor and control samples.

The cBioPortal (https://www.cbioportal.org/) platform was accessed to obtain comprehensive tumor genomics information. PWP1 gene alterations in LIHC were summarized by the “Cancer Types Summary” module [21]. The methylation status of CpG sites in the PWP1 gene was visualized in the MethSurv portal (https://biit.cs.ut.ee/methsurv/) [22]. In addition, we estimated hazard ratios for overall survival (OS) for the PWP1 CpG methylation variants detected.

A protein-protein interaction (PPI) network for PWP1 was constructed by mining the STRING database (https://cn.string-db.org/) [23]. With a confidence score > 0.9, 10 interacting genes were finally procured for correlation analysis with PWP1. Univariate Cox regression analyses of PWP1 and partner genes were conducted using the ‘survival’ package in R.

2.2. Correlation analysis of PWP1 expression and PWP1 DNA alterations

Copy number alterations (CNA) and methylation status of the PWP1 gene were analyzed in TCGA-LIHC cohorts using R software. Correlations between PWP1 CNA and mRNA expression were assessed and visualized by box plots. Correlation between PWP1 mRNA expression and DNA methylation status was calculated by linear regression. $P < 0.05$ was considered significant.

2.3. Functional enrichment analyses

RNA-Seq and clinical data for patients with LIHC were retrieved from TCGA repositories (https://www.cancer.gov/) [24]. Differentially expressed genes (DEGs) in high and low PWP1 tumors were identified by the “edgeR” package in R using |log2-fold

change (FC)| ≥1.0 and adjusted p-value (padj) < 0.05 as cut-off values. The identified DEGs were subjected to Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using the R package "clusterProfiler", with padj < 0.05 as threshold [25,26]. Gene Set Enrichment Analysis (GSEA) for PWP1-high and PWP1-low groups was conducted by GSEA v4.0.3 software [27]. FDR < 0.05 and normalized enrichment score (NES) ≥ 1.5 were the cutoffs for significance.

2.4. Cell culture and PWP1 gene silencing

Human liver cancer cell lines (Huh7, HepG2, PLC and SMMC7721) were obtained from the National Infrastructure of Cell Line Resource (Beijing, China), while cancer cell (HLE) line and normal liver (LO2) cell were obtained from ATCC (ATCC, Manassas, USA). All the cells were grown in DMEM (Gibco, Waltham, MA, USA) supplemented with 10 % FBS (Ausbian, Australia) and 1× penicillin/streptomycin. Incubation was done at 37 °C in a humidified 5 % CO_2 incubator. After thawing, the cells were used for no more than 15 passages. RNAi oligo sequences targeting PWP1 (synthesized at RiboBio Co., Ltd., China) are shown in the supplementary file (Table S1). PLC and SMMC7721 cells were transfected with pooled siRNAs (siPWP1 1: 2: 3 = 1: 1: 1) using an RNAiMAX kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and used for proliferation and colony formation assays 24 h later. Expression analysis of PWP1 via real-time quantitative PCR (RT-qPCR) and western blotting was performed in cells transfected for 48 h.

2.5. Real-time quantitative PCR

Cellular RNA was extracted with TRIzol reagent (Invitrogen). Two μg of RNA per sample was used for cDNA synthesis based on the TranScript First-Strand cDNA Synthesis SuperMix (Transgen Biotech, Beijing, China). RT-qPCR was carried out in triplicate using a PowerUp™ SYBR™ Green Master Mix (Applied Biosystems; Thermo Fisher Scientific) on a CFX Connect Real-Time PCR System (BioRad, Hercules, CA, USA). Target gene levels were determined by the $2^{-\Delta\Delta CT}$ method with human β-actin as internal control. Primers used in this assay are shown in the supplementary file (Table S1).

2.6. Western blotting

Cells were lysed and quantified by a bicinchoninic acid assay kit (Thermo Scientific Pierce, USA). Proteins (20 μg per lane) were subsequently resolved on SDS-PAGE, transferred onto PVDF membranes (Millipore, Boston, USA), blocked with 5 % skimmed milk at 37 °C for 1–2 h, and then reacted with primary antibodies, including anti-CCND1 (1:1,000, Cell Signaling Technology, MA, USA), anti-CDK4 (1:1,000, Cell Signaling Technology, MA, USA), anti-PWP1 (1:1,000, ABclonal, Wuhan, China), anti-β-Tubulin (1:2,000, Proteintech, Chicago, IL, USA) and anti-GAPDH (1:3,000, Proteintech, Chicago, IL, USA) overnight at 4 °C. Thereafter, HRP-conjugated anti-mouse and anti-rabbit IgG secondary antibodies (1:10,000, ZSGB-BIO, Beijing, China) were incubated for 1 h at 37 °C. Protein bands were detected with an Enhanced Chemiluminescence (ECL) detection system (Millipore, Boston, USA).

2.7. Cell proliferation and colony formation assays

Transfected cells were seeded at a density of 1500 cells/well into 96-well plates (Corning-Costar, NY, USA). On days 1, 2, 3, 4, and 5, the medium was removed and 100 μl of CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added to each well. The cells were incubated at 37 °C for 1 h and absorbance next determined at 450 nm with a microplate reader (Thermo Scientific). To conduct colony formation experiments, transfected cells (500 cells/well) were evenly seeded into 12-well plates and then cultured for 14 days. Cells were then immobilized with methanol, stained with crystal violet, and cell colony numbers counted under transmitted light microscopy. Each experiment was done in triplicate.

2.8. Cell cycle analysis

The distribution of the cell cycle phases was analyzed by flow cytometry. Cells were seeded onto 6-well plates and transfected with pooled siRNAs, 48 h later, the cells were collected using 0.25 % EDTA/trypsin and washed twice in 1 × PBS. Then, 70 % ethanol was used to fix the cells at −20 °C for 12 h. Next, the cells were resuspended in 500 μL work solutions prepared as Rnase A: propidium iodide = 1: 9 in volume (KeyGEN, Jiangsu, China), and incubated at room temperature from light for 60 min. The Cytoflex was used to read the fluorescence, and the percentage of cells in each phase of the cell cycle (subG1, G1, S and G2) was calculated using the FlowJo software (version 10.4.2, BD, USA).

2.9. Statistical analyses

Results of the in vitro experiments are shown as the mean and SD. All statistical analyses were conducted on GraphPad Prism 7.0 software. An unpaired *t*-test was applied to assess differences between means from two categories. P < 0.05 (two-tailed) indicated significance.

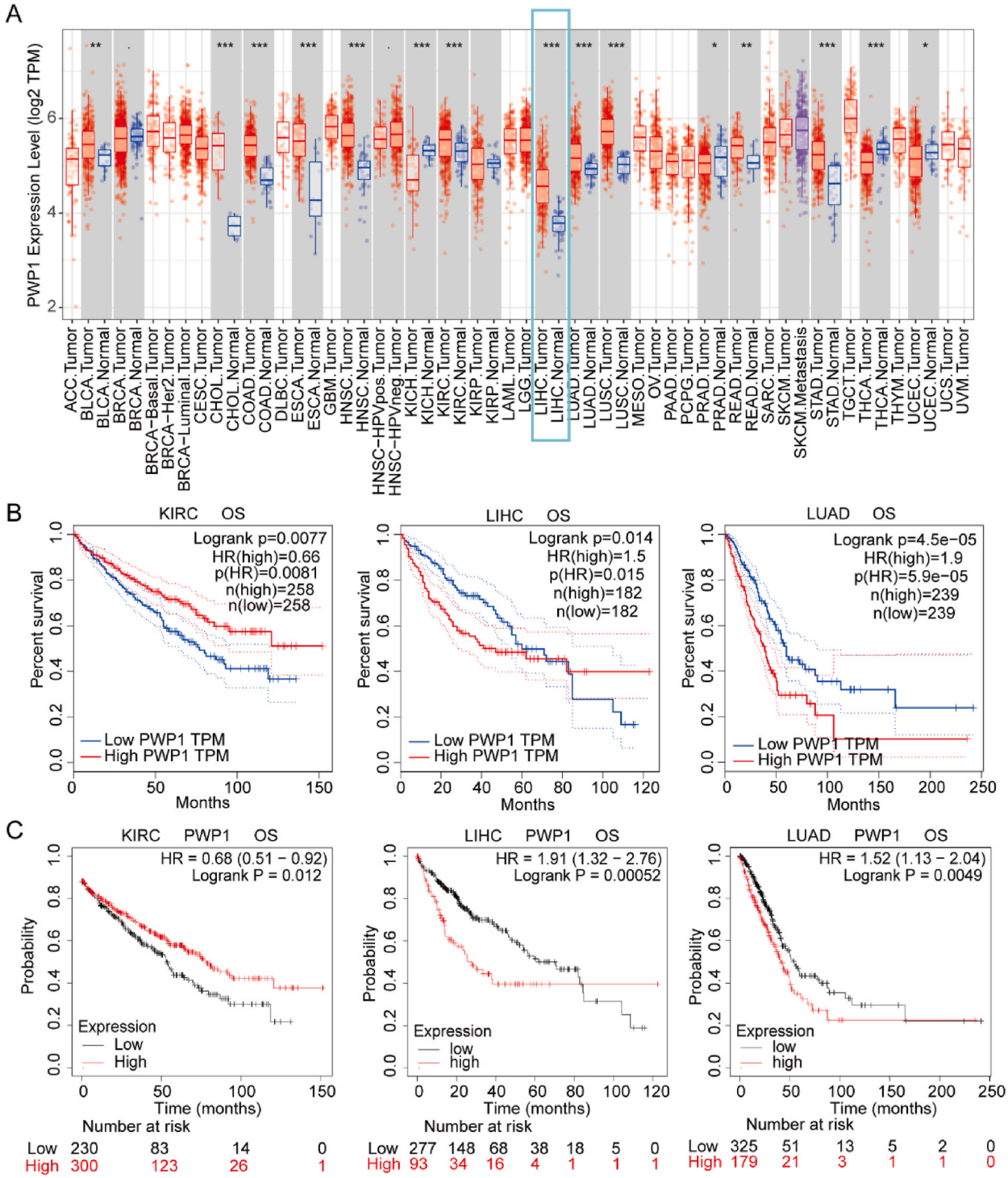
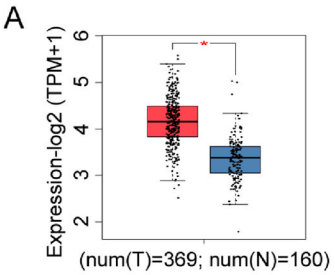
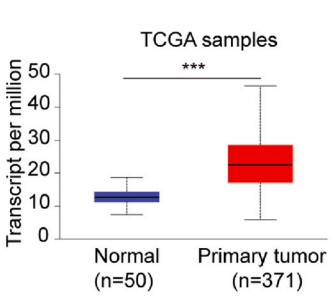
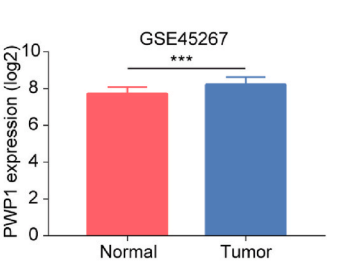
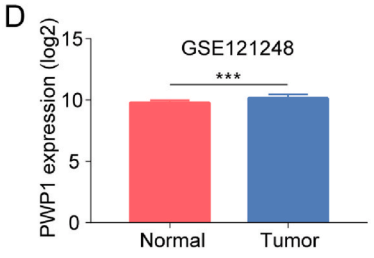


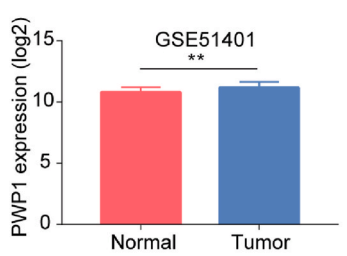
Fig. 1. PWP1 expression levels and prognostic value in different human cancers. **A** PWP1 transcriptional expression in different cancer types and corresponding normal tissues. **B, C** Analysis of OS for PWP1-high and PWP1-low KIRC, LIHC, and LUAD patients via GEPIA (**B**) and Kaplan-Meier Plotter (**C**) datasets, respectively.

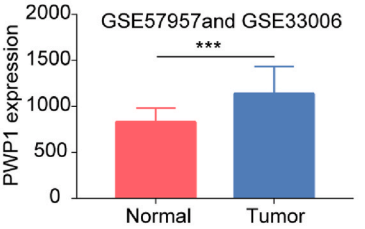






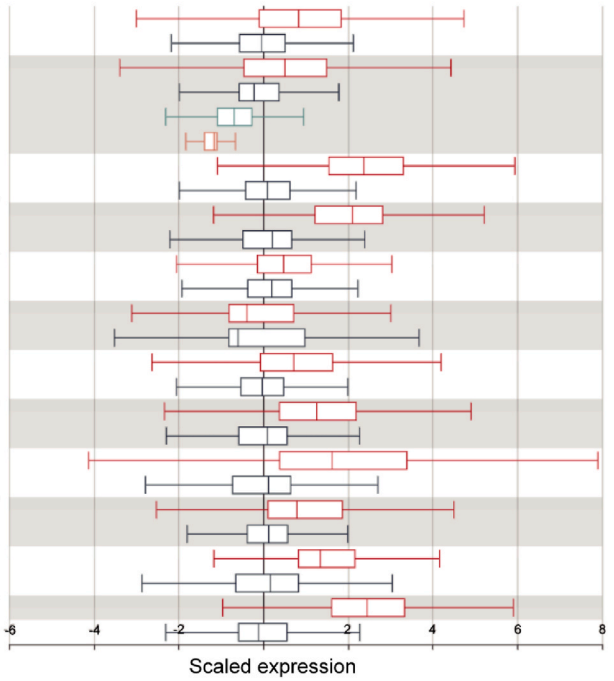


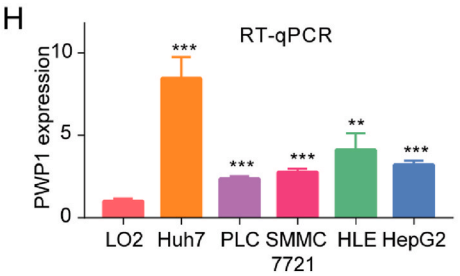


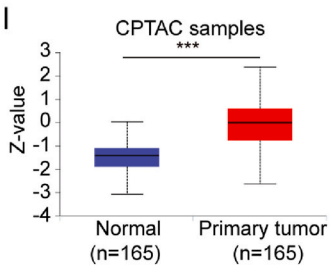


G

Dataset	Source	P-value	Nums	Type
HCCDB1	GSE22058	***	100 97	HCC Adjacent
HCCDB3	GSE25097	***	268 243 40 6	HCC Adjacent Cirrhotic Heathy
HCCDB4	GSE36376	***	240 193	HCC Adjacent
HCCDB6	GSE14520 (GPL3721 Subset)	***	225 220	HCC Adjacent
HCCDB7	GSE10143	0.3549	80 82	HCC Adjacent
HCCDB11	GSE46444	0.9228	88 48	HCC Adjacent
HCCDB12	GSE54236	***	81 80	HCC Adjacent
HCCDB13	GSE63898	***	228 168	HCC Adjacent
HCCDB15	TCGA-LIHC	***	351 49	HCC Adjacent
HCCDB16	GSE64041	***	60 60	HCC Adjacent
HCCDB17	GSE76427	***	115 52	HCC Adjacent
HCCDB18	ICGC-LIRI-JP	***	212 177	HCC Adjacent







(caption on next page)

Fig. 2. PWP1 is overexpressed in HCC. **A, B** Comparison of PWP1 transcriptional expression in LIHC and normal tissues according to GEPIA and UALCAN portals. **C–F** PWP1 mRNA expression in the GSE45267, GSE121248, GSE51401, GSE57957, and GSE33006 GEO datasets. **G** Analysis of PWP1 transcriptional expression in LIHC and normal liver tissues in HCCDB cohorts. **H** RT-qPCR detection of PWP1 expression in normal hepatocytes and HCC cell lines. **I** Analysis of PWP1 protein expression in LIHC and non-tumorous liver tissues in the CPTAC database. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

3. Results

3.1. PWP1 expression is upregulated in LIHC

We used the GEPIA platform to analyze PWP1 expression profiles in different human cancers and their corresponding normal tissues in TCGA. Tumor tissues displayed higher expression of PWP1 than normal tissues in most cancer types except for KICH (kidney chromophobe), PRAD (prostate adenocarcinoma) and THCA (thyroid carcinoma). Compared with other paired samples, particularly low PWP1 mRNA levels were detected in LIHC and normal liver tissues (Fig. 1A). Next, we investigated the prognostic value of PWP1 in those cancers exhibiting differential PWP1 mRNA expression levels. Interestingly, both GEPIA and KM plotter analyses revealed that high PWP1 expression was correlated with prolonged survival in KIRC, but predicted instead poor survival in LIHC and LUAD (Fig. 1B and C).

Consistent with the above pan-cancer analysis results, further examination of TCGA datasets confirmed that PWP1 was overexpressed in LIHC (Fig. 2A and B). Moreover, this finding was validated in the Gene Expression Omnibus (GEO) datasets GSE45267, GSE121248, GSE51401, GSE57957, and GSE33006 ($P < 0.05$; Fig. 2C–F) as well as in 12 HCC cohorts in the HCCDB database (Fig. 2G). Consistent with these data, our RT-qPCR assays showed that PWP1 expression is upregulated in human HCC (Huh7, PLC, SMMC7721, HLE and HepG2) cells compared to normal hepatocytes (LO2) (Fig. 2H). Moreover, analysis of proteomic data from the CPTAC database indicated that PWP1 protein levels were higher in LIHC relative to normal liver tissues (Fig. 2I). Overall, our results indicated that PWP1 displays significantly increased expression in LIHC versus control liver samples at both transcriptional and translational levels.

3.2. Genetic and epigenetic profiles of PWP1 in HCC

To investigate the potential mechanisms that mediate PWP1 dysregulation in LIHC, we identified PWP1 gene copy number alterations (CNA) and methylation patterns in TCGA-LIHC data retrieved in cBioPortal. The overall PWP1 alteration frequency in HCC patients was approximately 0.5 % (Fig. 3A). Based on transcriptome and classic CNA data, analysis of HCC samples ($n = 364$) indicated that PWP1 was homozygously deleted (Homdel) in 44 patients, heterozygously deleted (Hetloss) in 271 patients, showed normal diploid expression in 48 patients, and exhibited gene copy number gain in one patient. PWP1 diploidy was significantly associated with increased PWP1 transcription in comparison with the homdel and hetloss phenotypes, with the latter exhibiting in turn higher PWP1 transcription relative to homdel patients ($P < 0.05$) (Fig. 3B).

UALCAN analysis showed that PWP1 DNA methylation levels were overall decreased in LIHC relative to normal tissues (Fig. 3C), and linear regression analysis suggested a negative relationship between PWP1 methylation degree and its transcriptional expression (Pearson's $r = -0.17$, $P < 0.001$) (Fig. 3D). To clarify the influence of PWP1 CpG methylation status on clinical outcomes, the MethSurv portal was accessed. Among 12 CpG methylation sites identified in the PWP1 gene, cg06874334 and cg24830367 presented the highest degree of methylation (Fig. 3E). In turn, hypermethylation at six PWP1 CpG sites, namely cg06874334, cg02011789, cg08744461, cg11328140, cg04112431, and cg13830393, was associated with worsened OS in HCC patiens ($p < 0.05$) (Table 1). The above results suggest that genetic and epigenetic alterations in the PWP1 gene have marked effects on hepatocarcinoma development and prognosis.

3.3. Co-interacting partners of PWP1 in LIHC

To assess possible mechanisms underlying potential PWP1 effects in HCC, we generated a PPI network of PWP1 based on TCGA-LIHC information in the STRING database and selected the top ten functional partner genes by degree of connectivity (Fig. 4A). These genes were EBNA1BP2, FTSJ3, PDCD11, RBM34, MYBBP1A, DDX24, GTPBP4, RPF2, DDX10, and RSL1D1 (Fig. 4B). Notably, the mRNA levels of PWP1 were positively correlated with those of the above ten interacting genes in patients with HCC (Fig. 4C). Moreover, the overexpression of PWP1 and EBNA1BP2, FTSJ3, PDCD11, RBM34, GTPBP4, and RPF2 was negatively associated with overall survival in LIHC patients (Fig. 4D). The above results may indicate that PWP1 and the above set of PWP1-related genes have additive or synergistic effects on liver dysplasia and/or tumorigenesis.

3.4. Functional enrichment of PWP1-associated genes in HCC

Functional enrichment analyses were next performed based on the DEGs detected in association with high and low PWP1 expression groups. We noticed that significant PWP1 partner genes were especially enriched in GO BP and KEGG terms related to 'DNA replication', 'histone modification', 'cell cycle', and 'mismatch repair'. In turn, PWP1 was mainly enriched in the 'GTPase binding' and 'single-stranded DNA binding' GO MF terms, and in 'nuclear envelope' and 'ubiquitin ligase complex' within the GO CC category

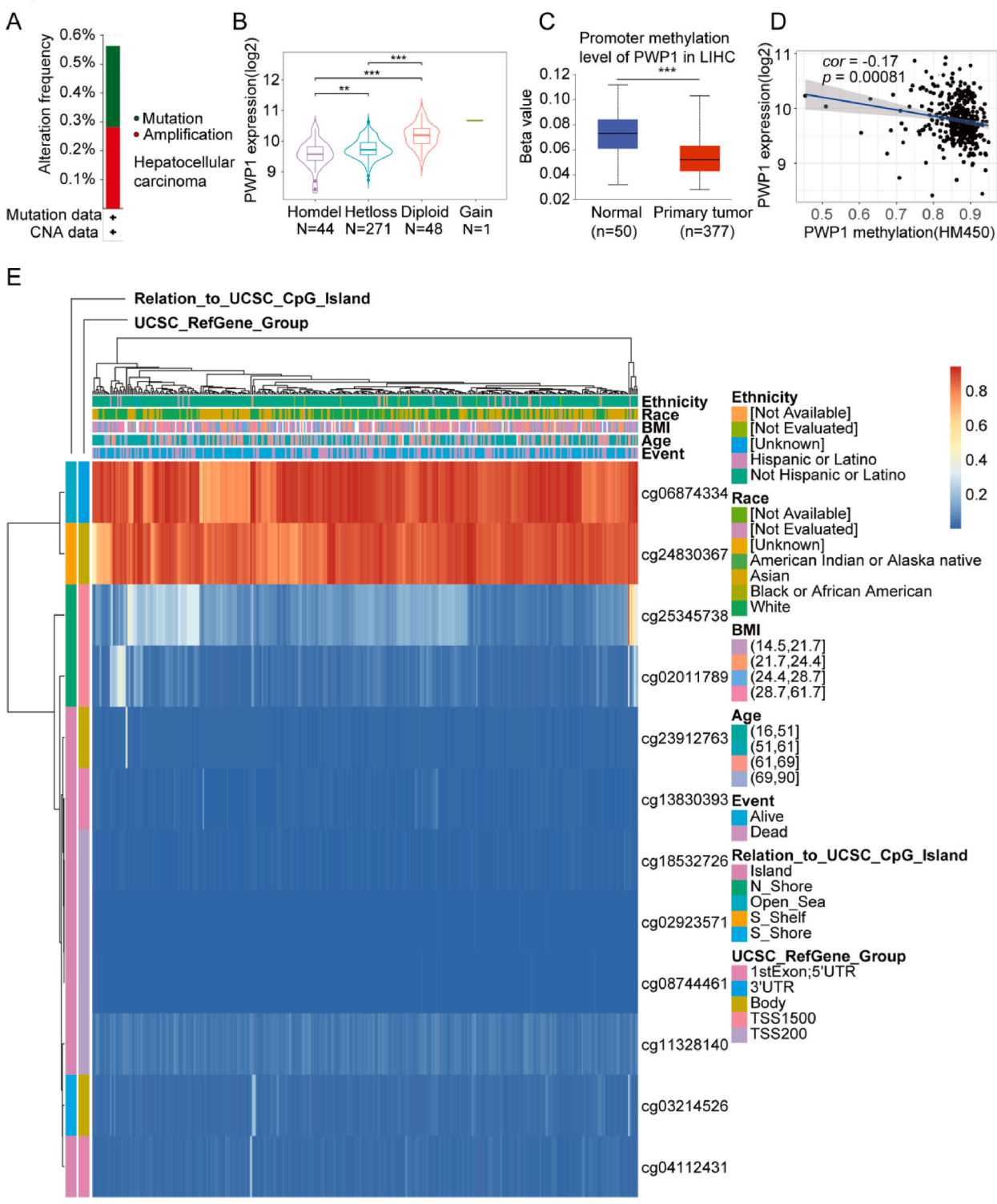


Fig. 3. Analysis of PWP1 gene copy number alterations and DNA methylation in LIHC. **A** Distribution and frequency of PWP1 gene alterations in LIHC (Firehose Legacy dataset). **B** Violin plots for comparative analysis of PWP1 mRNA expression levels in LIHC tissues with different PWP1 gene variations. **C** Comparison of PWP1 promoter methylation levels in primary liver tumors and control samples. **D** Correlation between PWP1 transcriptional expression and DNA methylation. **E** Heatmap of PWP1 methylation levels within single CpG sites. *p < 0.05; **p < 0.01; ***p < 0.001.

Table 1
Effect of PWP1 CpG hypermethylation on survival in LIHC.

CpG	HR	*P*-value
TSS1500-N_Shore-cg02011789	**1.744**	**0.0018**
TSS1500-N_Shore-cg25345738	0.778	0.22
TSS200−Island−cg08744461	**1.547**	**0.014**
TSS200−Island−cg11328140	**1.74**	**0.0022**
TSS200−Island−cg18532726	1.21	0.33
TSS200−Island−cg02923571	0.649	0.022
Body−Island−cg23912763	0.797	0.19
3′UTR−Open_Sea−cg06874334	**2.407**	**1e−04**
Body−S_Shelf−cg24830367	0.93	0.72
Body−S_Shore−cg03214526	1.248	0.29
1stExon;5′UTR−Island−cg04112431	**1.561**	**0.032**
1stExon;5′UTR−Island−cg13830393	**2.566**	**3.9e−05**

Note: CpG sites that influence survival of patients ($P < 0.05$) when hypermethylated are presented in bold font.

(Fig. 5A). To further elaborate on the signaling pathways potentially influenced by PWP1 in HCC, GSEA was conducted on TCGA-LIHC datasets. Results showed that gene sets significantly correlated with tumorigenesis in the PWP1-high group were mainly associated with "kegg_spliceosome", "kegg_DNA repair", "kegg_cell cycle", "kegg_mismatch_repair", "kegg_homologous_recombination", and "kegg_base_excision_repair" (Fig. 5B). To verify the above analysis, we knocked down PWP1 in HCC cell lines and observed that the expression of CCND1 and CDK4 were decreased compared with the control group (Fig. 6A & Fig. S1). Furthermore, when PWP1 was silenced in PLC, a greater percentage of cells were in the S phase (Fig. 6B), indicating that PWP1 may regulate the cell cycle.

3.5. PWP1 knockdown inhibits HCC cell growth in vitro

To directly assess the pro-tumorigenic role of PWP1 in HCC, PWP1 knockdown experiments were conducted in human HCC (PLC and SMMC7721) cells. RT-qPCR and Western blot detection demonstrated that the knockdown efficiency of PWP1-targeted siRNAs reached approximately 80 % (Fig. 7A and B & Fig. S1). As shown in the cell growth curves and colony formation assays depicted in Fig. 7C and D, PWP1 silencing greatly slowed down the in vitro proliferation rate and tumorigenic potential of PLC and SMMC7721 cells. In summary, the data presented suggests an oncogenic role for PWP1 in HCC.

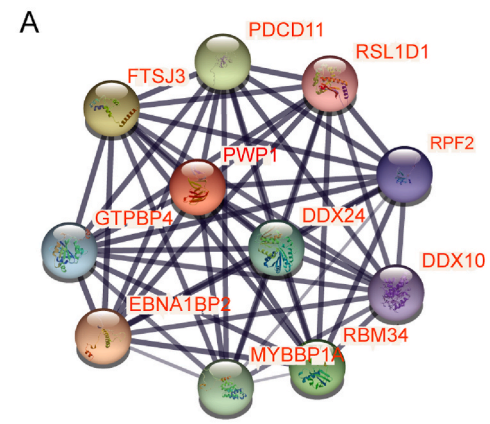
4. Discussion

Hepatocellular carcinoma (HCC) is a pervasive disease with high incidence and morbidity. It is induced by multiple genetic and environmental elements and poses a challenge to both precise diagnosis and individualized therapy. Thus, identifying suitable markers for early detection and targeted therapy will help extend the lifetime of HCC patients. PWP1 is a member of the Transducin/WD40-repeat protein superfamily located on chromosome 12q23.3. Prior studies have described the regulatory effects of PWP1 on numerous biological processes such as rRNA biogenesis, cell signaling, vesicle transport, cytoskeleton assembling, and cell cycle transition [7,11,28,29]. Nevertheless, the expression profile and potential regulatory activity of PWP1 in HCC remain essentially unexplored.

In this study, we initially confirmed that PWP1 was overexpressed in multiple cancers, and downregulated instead in KICH, PRAD, and THCA. These results suggested that PWP1 participates in the tumorigenesis of a wide range of cancers. To further determine the role of PWP1 on cancer patient prognosis, survival curves were plotted based on differential PWP1 expression in cancer tissues. Results demonstrated that the correlation between PWP1 expression and overall survival was positive for LIHC and LUAD and negative for KIRC, three tumor types with characteristically high PWP1 expression. Based on these findings, we speculate that PWP1 influences tumor cell dynamics in a cancer-specific fashion. In turn, analysis of GEO datasets indicated that PWP1 was upregulated in liver cancer compared to normal paracancerous tissues, as well as in liver cancer compared to normal liver cell lines. This common expression pattern suggests that PWP1 may serve as a useful biomarker for diagnosis and personalized therapy in LIHC.

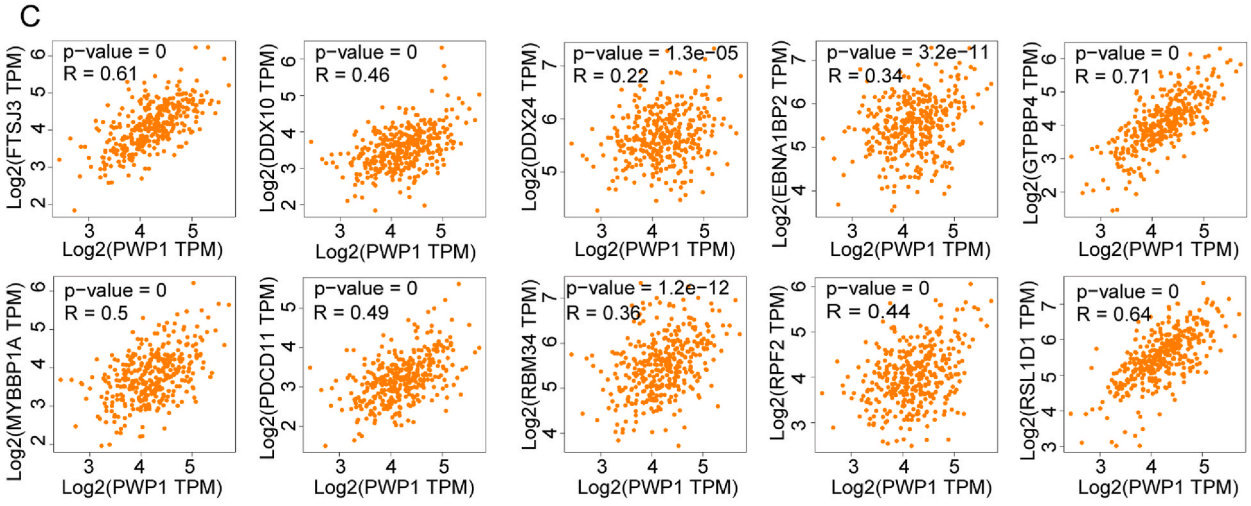
Genetic and epigenetic alterations may alter organs' functions and are connected with recurrence and metastasis of malignant tumors. In the present study, a low rate of genetic mutation (approximately 0.5 %) was detected for the PWP1 gene in HCC. As expected, PWP1 DNA methylation correlated negatively with its mRNA expression. Furthermore, our analysis revealed that 2/12 CpG sites (cg06874334 and cg24830367) in the PWP1 gene showed the highest methylation levels, while 6 CpG sites presented prognostic significance. Although the specific mechanisms require further exploration, these findings indicated that the methylation status of the PWP1 gene may influence HCC development.

Systematic functional analysis of significant PWP1-interacting genes found that 6 of these were risk factors in HCC patients. This observation suggests that a complex PWP1-regulated network is involved in the initiation and/or development of HCC. Consistent with early findings showing that PWP1 functions as a cell cycle-associated regulator in human pancreatic adenocarcinoma, subsequent functional enrichment analysis indicated that PWP1 may promote HCC progression by regulating cell cycle pathways. Consistent also with our observation that PWP1 is an indicator of unfavorable prognosis in LUAD, a previous study revealed a contribution of PWP1 to the proliferation and invasion of human lung cancer cells via restraining the Hippo pathway and strengthening the Wnt signaling pathway [14]. This evidence further confirmed our assumption that PWP1 acts as a risk factor in both HCC and LUAD and plays a



B

Gene symbol	Annotation	Score
EBNA1BP2	Probable rRNA-processing protein EBP2	0.987
FTSJ3	pre-rRNA processing protein FTSJ3	0.978
PDCD11	Protein RRP5 homolog	0.976
RBM34	RNA-binding protein 34	0.976
MYBBP1A	Myb-binding protein 1A	0.964
DDX24	ATP-dependent RNA helicase DDX24	0.962
GTPBP4	Nucleolar GTP-binding protein 1	0.961
RPF2	Ribosome production factor 2 homolog	0.960
DDX10	Probable ATP-dependent RNA helicase DDX10	0.958
RSL1D1	Ribosomal L1 domain-containing protein 1	0.956



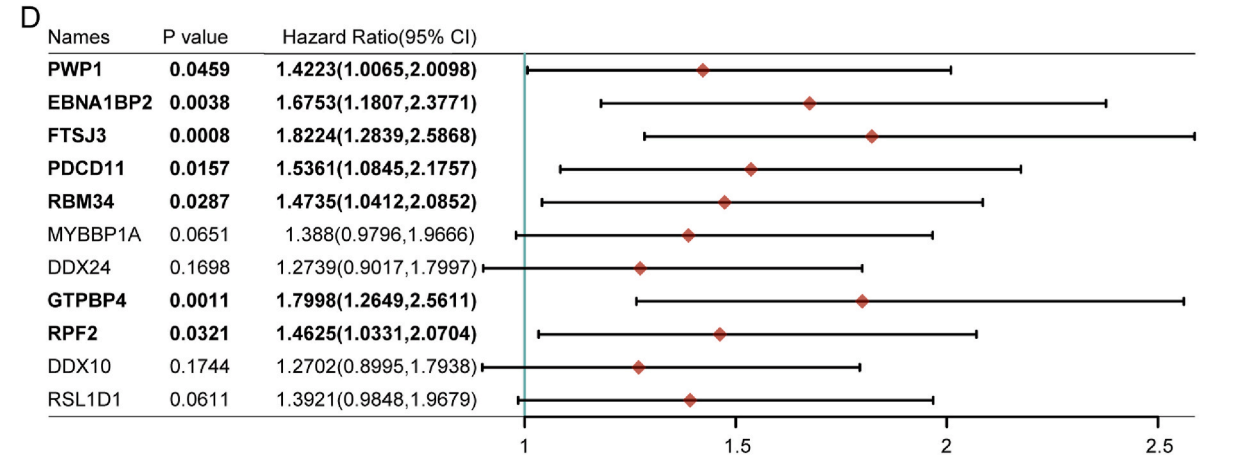
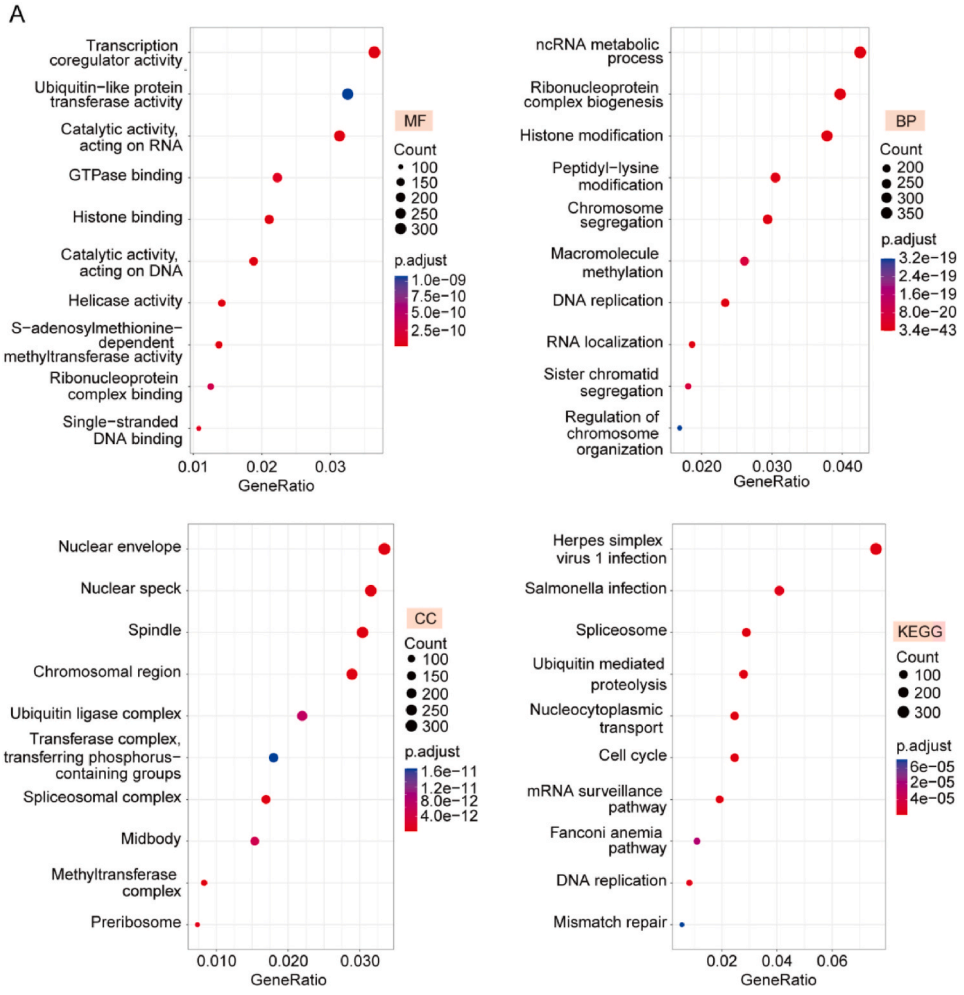


Fig. 4. Co-interacting partners of PWP1 in LIHC. **A** PPI network of PWP1 in LIHC. **B** Annotations for the top ten PWP1-interacting proteins in LIHC. **C** Correlation analyses of PWP1-related protein-protein interactions. **D** Forest plot of hazard ratios for PWP1 and partner genes in LIHC. Bold font indicates $P < 0.05$.

pro-proliferative role in HCC by driving various distinctive signaling pathways.

We also performed proliferation assays to assess the function of PWP1 in two HCC cell lines, which showed that PWP1 silencing decreased cell viability and colony formation potential. Further research aimed at defining the exact mechanism by which PWP1 overexpression induces proliferation of HCC cells will be of great value to develop PWP1-targeted inhibitors for HCC treatment.



(caption on next page)

Fig. 5. Functional enrichment of PWP1-associated genes in LIHC. **A** GO and KEGG enrichment analyses of PWP1-interacting genes. **B** Gene sets enriched in the PWP1-high LIHC cancer group.

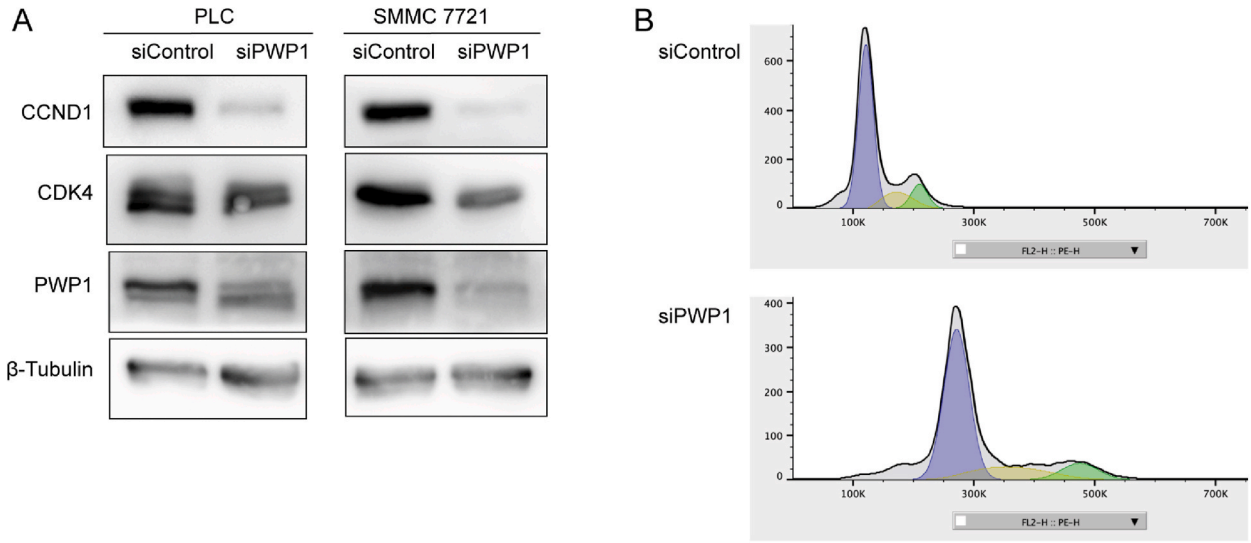


Fig. 6. Verification of PWP1-associated pathways in HCC cells. **A** Western blot detection of CCND1 and CDK4 expression in PLC and SMMC7721 cells transfected with PWP1 siRNAs. **B** Flow cytometric analysis of PLC cells transfected with PWP1 siRNAs.

5. Conclusion

Our study revealed that PWP1 is commonly overexpressed in HCC and is in association with poor survival. Further analysis suggested that differential DNA methylation patterns in the PWP1 gene, as well as its coordinated expression and interaction with several partner genes, are likely involved in the pathogenesis of HCC. Functional enrichment analyses revealed cell cycle-related pathways regulated by PWP1. In addition, we provide direct in vitro evidence that PWP1 knockdown decreases cell viability and colony formation in HCC cells. These findings therefore allow us to propose PWP1 as a prospective therapeutic target in HCC.

Funding

This research was supported by Chinese Academy of Medical Sciences (CAMS) Innovation Fund for Medical Sciences (2021-I2M-1-066), Program for Innovative Research Team in Universities of Inner Mongolia Autonomous Region (NMGIRT2225), Science and Technology Project of Inner Mongolia Autonomous Region (2023YFSH0016).

Ethics statement

Review and/or approval by an ethics committee was not needed for this study because data collection comes from publicly available data or information and all experiments in this study did not involve human samples, animal experiments, or animal samples. Informed consent was not required for this study because this study does not involve the collection, processing, or use of personal or animal information.

Data availability statement

The data and materials of this research are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Rong Huang: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Fu Xu:** Data curation. **Luying Su:** Data curation. **Yan Lu:** Data curation. **Wei Liu:** Data curation. **Shuaihu Liu:** Data curation. **Ling Yang:** Conceptualization. **Su Liya:** Writing – review & editing, Conceptualization. **Wei Song:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

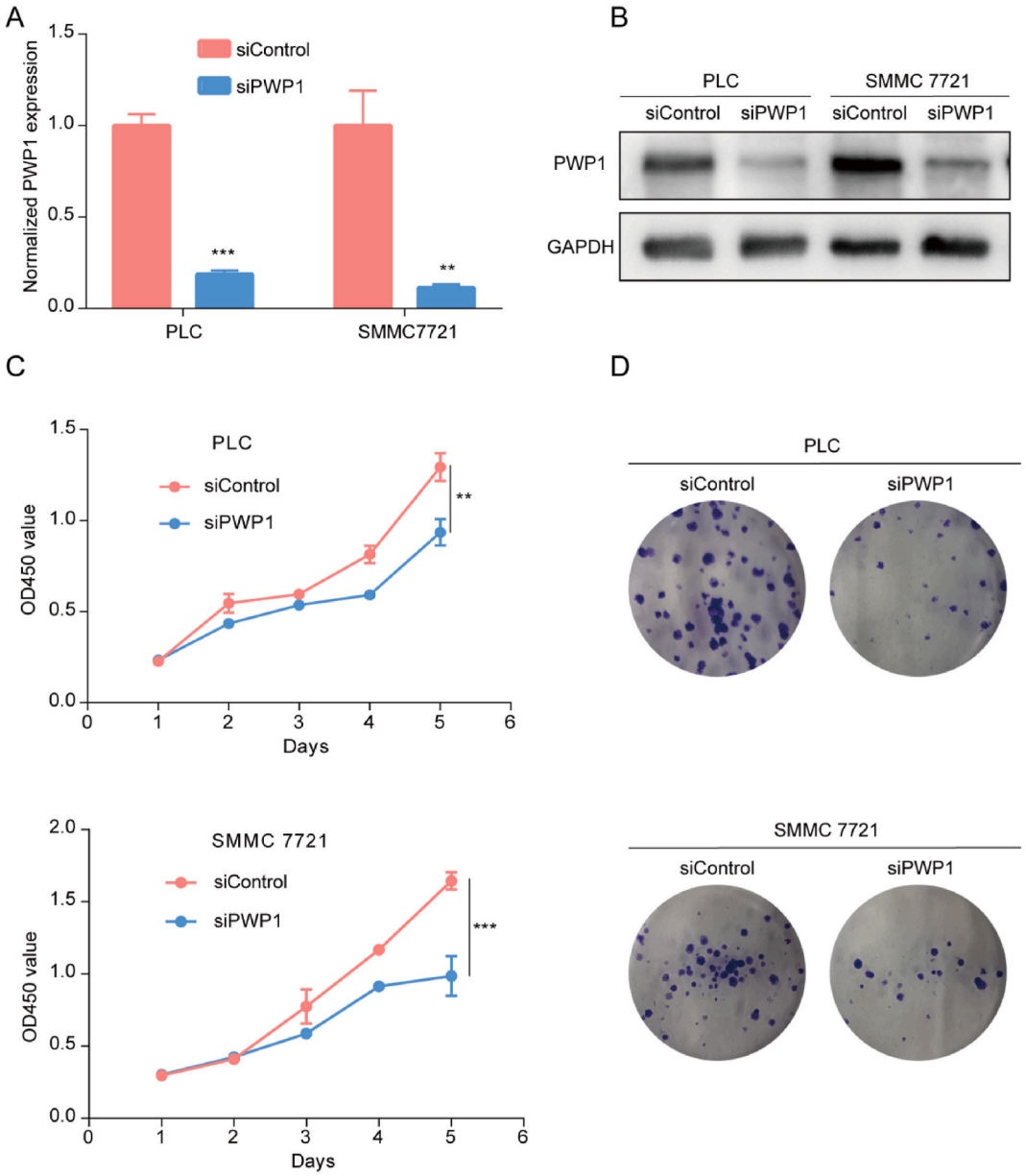


Fig. 7. PWP1 silencing inhibits the proliferation of HCC cells in vitro. **A, B** RT-qPCR and Western blot detection of PWP1 expression in liver cancer cells. **C** Results of CCK-8 proliferation assays. **D** Representative images of colony formation assays. Data are shown as mean ± SD from three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.

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.

Acknowledgements

We thank Charlesworth Author Services (https://cwauthors.com?rcode=DOVEMEDICALPRESS) for editing this manuscript.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32409.

References

[1] H. Sung, J. Ferlay, R.L. Siegel, et al., Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, Ca - Cancer J. Clin. 71 (2021) 209–249.
[2] J. Zhou, H. Sun, Z. Wang, et al., Guidelines for the diagnosis and treatment of hepatocellular carcinoma (2019 edition), Liver Cancer 9 (2020) 682–720.
[3] C. Su, Survivin in survival of hepatocellular carcinoma, Cancer Lett. 379 (2016) 184–190.
[4] R.J. Duronio, J.I. Gordon, B. Ms, Comparative analysis of the beta transducin family with identification of several new members including PWP1, a nonessential gene of Saccharomyces cerevisiae that is divergently transcribed from NMT1, Proteins 13 (1992) 41–56.
[5] B. Honoré, U. Baandrup, S. Nielsen, V. H, Endonuclein is a cell cycle regulated WD-repeat protein that is up-regulated in adenocarcinoma of the pancreas, Oncogene 21 (2002) 1123–1129.
[6] N. Suka, E. Nakashima, K. Shinmyozu, et al., The WD40-repeat protein Pwp1p associates in vivo with 25S ribosomal chromatin in a histone H4 tail-dependent manner, Nucleic Acids Res. 34 (2006) 3555–3567.
[7] J. Talkish, I.W. Campbell, A. Sahasranaman, et al., Ribosome assembly factors Pwp1 and Nop12 are important for folding of 5.8S rRNA during ribosome biogenesis in Saccharomyces cerevisiae, Mol. Cell Biol. 34 (2014) 1863–1877.
[8] Y. Yu, W. Jia, Y. Lyu, et al., Pwp1 regulates telomere length by stabilizing shelterin complex and maintaining histone H4K20 trimethylation, Cell Discovery 5 (2019).
[9] J. Shen, W. Jia, Y. Yu, et al., Pwp1 is required for the differentiation potential of mouse embryonic stem cells through regulating Stat3 signaling, Stem Cell. 33 (2015) 661–673.
[10] W. Zhang, Q.D. Morris, R. Chang, et al., The functional landscape of mouse gene expression, J. Biol. 3 (2004) 21.
[11] Y. Liu, T.I. Cerejeira Matos Rheino, H. V, PWP1 promotes nutrient-responsive expression of 5S ribosomal RNA, Biology open 1 (2018) bio037911.
[12] Y. Liu, J. Mattila, S. Ventela, et al., PWP1 mediates nutrient-dependent growth control through nucleolar regulation of ribosomal gene expression, Dev. Cell 43 (2017) 240–252 e245.
[13] A.L. Casper, K. Baxter, M. Van Doren, No child left behind encodes a novel chromatin factor required for germline stem cell maintenance in males but not females, Development 138 (2011) 3357–3366.
[14] L. Wei, P. Li, Y. Luo, et al., PWP1 promotes the malignant phenotypes of lung cancer cells by interacting with DVL2 and Merlin, OncoTargets Ther. 13 (2020) 10025–10037.
[15] T. Li, J. Fan, B. Wang, et al., TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells, Cancer Res. 77 (2017) e108–e110.
[16] Q. Lian, S. Wang, G. Zhang, et al., HCCDB: a database of hepatocellular carcinoma expression atlas, Dev. Reprod. Biol. 16 (2018) 269–275.
[17] D.S. Chandrashekar, B. Bashel, S.A.H. Balasubramanya, et al., UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses, Neoplasia 19 (2017) 649–658.
[18] B. Gyorffy, A. Lanczky, A.C. Eklund, et al., An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients, Breast Cancer Res. Treat. 123 (2010) 725–731.
[19] B. Gyorffy, A. Lanczky, Z. Szallasi, Implementing an online tool for genome-wide validation of survival-associated biomarkers in ovarian-cancer using microarray data from 1287 patients, Endocr. Relat. Cancer 19 (2012) 197–208.
[20] Z. Tang, B. Kang, C. Li, et al., GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, Nucleic Acids Res. 47 (2019) W556–W560.
[21] E. Cerami, J. Gao, U. Dogrusoz, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov. 2 (2012) 401–404.
[22] V. Modhukur, T. Iljasenko, T. Metsalu, et al., MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data, Epigenomics 10 (2018). MethSurv: a web tool to perform multivariable survival analysis using DNA methylation data.
[23] D. Szklarczyk, A.L. Gable, K.C. Nastou, et al., The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets, Nucleic Acids Res. 49 (2021) D605–D612.
[24] K. Tomczak, P. Czerwinska, M. Wiznerowicz, The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge, Contemp. Oncol. 19 (2015) A68–A77.
[25] P.D. Thomas, The gene ontology and the meaning of biological function, Methods Mol. Biol. 1446 (2017) 15–24.
[26] M. Kanehisa, M. Furumichi, M. Tanabe, et al., KEGG: new perspectives on genomes, pathways, diseases and drugs, Nucleic Acids Res. 45 (2017) D353–D361.
[27] A. Subramanian, P. Tamayo, V.K. Mootha, et al., Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 15545–15550.
[28] C. Zhang, J. Lin, W. Liu, et al., Structure of Utp21 tandem WD domain provides insight into the organization of the UTPB complex involved in ribosome synthesis, PLoS One 9 (2014) e86540.
[29] E.J. Neer, C.J. Schmidt, R. Nambudripad, S. Tf, The ancient regulatory-protein family of WD-repeat proteins, Nature 371 (1994) 297–300.