Journal of Hepatocellular Carcinoma Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

# PPIH Expression Correlates with Tumor Aggressiveness and Immune Dysregulation in Hepatocellular Carcinoma

Jiaxin Bei[1,2,*], Zihao Sun[1,2,*], Rongdang Fu[3,*], Xinkun Huang[4], Jiabai Huang[4], Yongyou Luo[5], Yihu Li[6], Ye Chen[4], Zhisheng Wei[7]

[1]Department of Immuno-Oncology, First School of Clinical Medicine, the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510080, People's Republic of China; [2]Guangdong Provincial Engineering Research Center for Esophageal Cancer Precision Therapy, the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510080, People's Republic of China; [3]Department of Hepatic Surgery, the First People' S Hospital of FoShan, Guangdong, 528000, People's Republic of China; [4]Laboratory of Interventional Radiology, Department of Minimally Invasive Interventional Radiology and Department of Radiology, The Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 510260, People's Republic of China; [5]Department of Pathology, First School of Clinical Medicine, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510080, People's Republic of China; [6]Department of Hepatobiliary Surgery, the Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 510260, People's Republic of China; [7]Department of Neurology, Neurological Research Institute of Integrated Traditional Chinese and Western Medicine, First School of Clinical Medicine, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510080, People's Republic of China

*These authors contributed equally to this work

Correspondence: Ye Chen, Laboratory of Interventional Radiology, Department of Minimally Invasive Interventional Radiology and Department of Radiology, the Second Affiliated Hospital, Guangzhou Medical University, Guangzhou, Guangdong, 510260, People's Republic of China, Email onenine@126.com; Zhisheng Wei, Department of Neurology, Neurological Research Institute of Integrated Traditional Chinese and Western Medicine, First School of Clinical Medicine, the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510080, People's Republic of China, Email weizs084@gdpu.edu.cn



**Purpose:** Hepatocellular Carcinoma (HCC) features a complex pathophysiology and unpredictable immunosuppressive microenvironment, which limit the effectiveness of traditional therapies and lead to poor patient outcomes. Understanding the immune characteristics of HCC is essential for elucidating the immune microenvironment and developing more effective treatments. This study investigates the role of Peptidyl-prolyl isomerase H (PPIH) in HCC by analyzing its expression, prognosis, methylation levels, and relationship with immune cell infiltration.

**Methods:** We utilized bulk sequencing and clinical data from UCSC Xena and the GTEx database for preprocessing and subsequent differential expression analysis of PPIH in tumor and adjacent normal tissues, evaluating prognostic parameters like overall survival and disease-free interval between low and high PPIH expression groups. Immune infiltration was analyzed via CIBERSORT and ssGSEA, while DNA methylation and somatic mutation analyses were performed using MExpress and "maftools", respectively, alongside in vitro and in vivo experiments to assess PPIH's functional roles.

**Results:** Our findings indicated that PPIH is significantly upregulated in various cancer types, correlating with poor patient prognosis, increased somatic mutations, and altered gene methylation patterns. High PPIH levels were linked to enhanced T regulatory (Treg) cell infiltration and a decline in Th17 cell populations, impacting vital pathways related to DNA damage repair and tumor proliferation. Furthermore, PPIH knockdown in vitro led to reduced cell viability, proliferation, and invasion while promoting apoptosis. In vivo, PPIH knockdown repressed tumor growth and modified the immune microenvironment by attenuating Th17 cell infiltration and potentially increasing Treg cell accumulation.

**Conclusion:** This study emphasizes PPIH's critical role in HCC progression by facilitating tumor growth and survival while modulating the immune landscape, thereby positioning PPIH as a potential therapeutic target for HCC management.

**Keywords:** hepatocellular carcinoma, PPIH, bioinformatics analysis, immune infiltration, Th17/Treg cell

Received: 5 September 2024
Accepted: 28 November 2024
Published: 11 December 2024

## Introduction

Hepatocellular carcinoma (HCC) is the major histologic type of primary liver cancer and accounts for approximately 90% of all primary liver cancers.[1] With developments in early detection, oncological prevention and treatment, the mortality of most cancers is decreasing, except for HCC. Currently, HCC is one of several cancers with a 5-year survival rate under 20% and an incidence-to-mortality ratio close to one.[2–4] Although local ablation, surgical resection, and liver transplantation may cure early-stage HCC, due to its insidious onset and rapid progression, the majority of HCC patients are diagnosed at an advanced stage and lose the chance to receive radical treatments.[5] In addition, the exceedingly complex pathophysiology and difficult-to-predict immunosuppressive microenvironment of HCC severely limit the potency of traditional radiotherapy and chemotherapy, targeted therapies and even immunotherapies.[6–9] Therefore, more efforts are needed to identify novel biomarkers and targets and guide personalized HCC therapy.

Proteins called cyclophilins (CYPs) can catalyse the cis-trans conversion of proline-containing peptides, facilitating protein folding by catalysing the cis- to trans-conversion of proline-containing peptides.[10] PPIases, such as PPIA (also called CYPA) and PPIB (also called CYPB), are abnormally expressed in multiple cancer types and are often associated with malignant progression.[11,12] For example, in colorectal cancer (CRC), PPIA decreases cellular reactive oxygen species levels and promotes survival during chemotherapy through PPIA-PRDX2-mediated antioxidant signalling.[13] Among the human CRC and HCC tissues, PPIB was overexpressed in 78% and 91% of the patients, respectively. The upregulated expression of PPIB was suggested to increase invasiveness in HCC cells by stimulating matrix metalloproteinase-2 (MMP-2) and MMP-9.[14,15] Many CYP inhibitors have been developed in response to these findings, helping us investigate the potential benefits of targeting PPIases during cancer treatment.[12,16,17] However, despite the considerable amount of accumulated knowledge on PPIA and PPIB, the biological functions of other members of the PPIases and their potential roles in cancer remain largely unclear. Recently, one of the PPIase family members, PPIH (also known as CYPH), was found to be highly expressed in HCC, indicating a poor prognosis.[18,19] RNA-binding proteins (RBPs), including PPIH, are strongly associated with the progression of hepatitis B virus (HBV)-related HCC.[20] Conversely, another group reported that increased expression of PPIH in stomach adenocarcinoma (STAD) might lead to longer overall survival (OS).[21] These inconsistent findings prompted us to speculate that the role of PPIH in cancer progression might be two fold. Therefore, there is a need to investigate the distinct biological functions of PPIH more comprehensively and further define the diagnostic and prognostic value of PPIH in HCC.

In recent years, as bioinformatics has advanced and large-scale genomic data have become available, the application of bioinformatics to gain insight into cancer development and progression has increased. By integrating gene expression data from various cancer types and clinical information, we will conduct a comprehensive bioinformatic analysis to explore the expression patterns and potential clinical significance of PPIH in HCC. In this study, RNA-Seq analysis was performed on data from the Cancer Genome Atlas (TCGA) database, with more patients included. We investigated the biological function, prognostic value, survival, regression, immune cell infiltration and global methylation of PPIH. PPIH expression significantly affects the clinical outcomes of 33 cancers, including HCC, and may play an immunosuppressive role in the tumor microenvironment. Importantly, our experiments demonstrated that decreasing the expression of PPIH can impair tumor cell proliferation, migration, and invasion and improve the immunosuppressive microenvironment. In summary, our study indicates that PPIH is expected to be a novel marker for the prognostic assessment of HCC.

## Materials and Methods

### Data Source and Preprocessing

The study protocol is depicted in Figure 1, employing bulk-seq and clinical data from UCSC Xena and transcriptome data of normal tissues from the GTEx database. Preprocessing was conducted using the "limma" package, with gene sets sourced from MSigDB and further data obtained from TCGA, including CNV, SNV, and DNA methylation data.

### Identification of PPIH Expression in Pancancer

PPIH expression in tumor and adjacent normal tissues was determined using the TIME2 database and differential expression was compared in TCGA tumor specimens with GTEx normal tissues. Protein expression profiles were

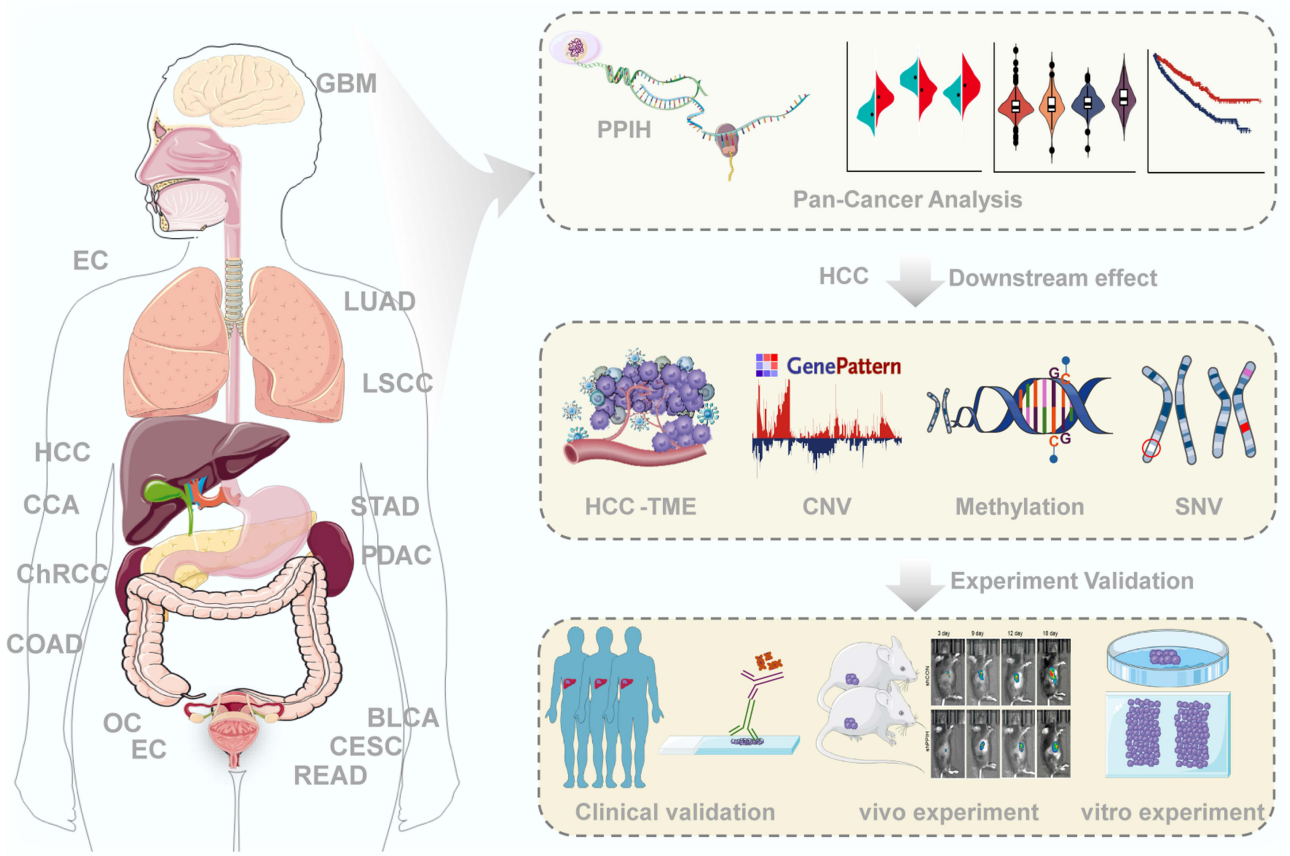


**Figure 1** Study flowchart.

analysed with the CPTAC, and the TCGA cohort was classified into low- and high-PPIH groups based on median expression values.

## Prognostic Analysis of PPIH

Overall survival, progression-free interval, disease-specific survival, and disease-free interval were compared between low- and high-PPIH groups. The survival analysis results were visualised using "survminer" and "forestplot" packages.

## Annotation of Biological Characterization

Three algorithms (CIBERSORT, Quanti-seq, ssGSEA) were applied to infer immunoinfiltration scores, assessing the abundance of 24 immune cell types, including 18 T-cell subtypes. GSEA used the "c2.cp.kegg_legacy.v2023.2.Hs.entrez.gmt" reference gene set (Supplementary Table 1), and enrichment scores were correlated with PPIH expression (Supplementary Table 2, Supplementary Table 3), alongside CNV analysis with Gistic 2.0 for the TCGA-HCC cohort.

## Integrated Analysis of DNA Methylation Data and SNV Data

Differences in methylation data for the TCGA-HCC cohort were analysed with a significance threshold ($P < 0.05$ and $|logFC| > 2$). PPIH methylation was assessed using MExpress, while somatic mutations were evaluated with the "maftools" R package.

## RNA Isolation and qRT–PCR

Total RNA was extracted from transfected cells using the EasyPure RNA Kit, followed by RT–PCR with EasyTaq PCR SuperMix. PPIH primers for human and mouse were specified for amplification.

## Cell Transfection

Lentivirus transduction was conducted for 24 hours, followed by puromycin selection, with varying multiplicities of infection for different liver cell lines. Expression stability was confirmed via PCR and Western blotting, with shRNA sequences detailed for target sequences.

## Western Blotting

Cells were extracted with RIPA buffer containing proteasome and phosphatase inhibitors, and total protein concentration was measured using the Lowry assay. Western blotting was performed with anti-PPIH and anti-beta-actin antibodies.

## Wound Healing Assay

Cells were plated in a six-well plate and a scratch was created to observe migration, with micrographs captured at 0, 24, and 48 hours. The healing process was assessed by analysing the extent of cell migration into the scratched area.

## Transwell Assays

Transwell migration and invasion assays were executed with HCC cells in serum-free conditions, and a 10% fetal bovine serum solution was added to the lower chamber. The cells were then fixed, stained, and counted microscopically to determine migration and invasion capabilities.

## Apoptosis Assay

Apoptosis was measured using a FITC-Annexin V Apoptosis Detection Kit, with cells harvested, rinsed, and resuspended in the solution for analysis. Data were processed using FlowJo software on a BD LSRFortessa, assessing at least 10,000 cells.

## Immunofluorescence and Immunohistochemistry

Immunofluorescence and immunohistochemistry were conducted with tissue fixation, embedding, antigen retrieval, and blocking procedures. Various primary and secondary antibodies were utilised for detection and imaging of PPIH and interleukin-17 (IL-17) expressions.

## Live Animal Imaging

Tumor growth was monitored using an IVIS Spectrum In Vivo Imaging System, with luciferin injections for imaging on specified days. The mice were weighed and tumor dimensions were measured every six days, while survival was recorded daily.

## Flow Cytometry

Tumor tissue from sacrificed mice was processed for flow cytometry to obtain tumor-infiltrating lymphocytes, using enzymatic hydrolysate medium. The percentages of different lymphocyte populations were determined through flow cytometric analysis with appropriate antibodies.

## Cell Line Culture and Animals

Human HCC cell lines (HepG2, MHCC97h, and HCCLM3) and a luciferase-transduced mouse HCC cell line (Hepa1-6-Luci) were obtained from IMMOCELL (Xiamen, Fujian, China). The cells were maintained in a humidified incubator at 37 °C with 5% $CO_2$ in DMEM supplemented with 10% foetal bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Invitrogen, USA). Four-week-old male C57BL/6 mice were purchased from Zhuhai BesTest Bio-Tech Co. Ltd. and housed at the Laboratory Animal Centre of the First Affiliated Hospital of Guangdong Pharmaceutical University, with free access to food and water. A total of 1×10^6 hepa1-6 cells, either with or without PPIH knockdown, were injected subcutaneously for further studies. The animal experiments received ethical approval from the Laboratory Animal Ethics Committee at the First Affiliated Hospital of Guangdong Pharmaceutical University. All the animal

experiments in this study followed the guidelines for Ethical Review of Laboratory Animal Welfare in China (GB / T35892-2018).

## Clinical Samples

HCC specimen and corresponding nontumorous liver tissues were collected from 15 patients who underwent hepatectomy at the First Affiliated Hospital of Guangdong Pharmaceutical University between December 2020 and January 2024. Patients who had previously received any other therapies before the surgery were excluded. Adjacent healthy tissue from the same resected liver specimen served as normal tissue. All the tissue samples were remaining specimens from clinical examinations. The First Affiliated Hospital of Guangdong Pharmaceutical University ethical committee approved the study, and informed consent was obtained from all participants (2024 IIT NO.40). Our study complies with the Declaration of Helsinki.

## Statistical Analysis

Data analysis and visualisation were performed using R software (version 4.3.1). The Wilcoxon test assessed significant differences between two subgroups, while Spearman's rank test was used for correlations among non-normally distributed datasets. The Log rank test compared survival analysis results. Data between two groups were analysed with the Student's *t*-test, while one-way ANOVA was employed for comparisons among three groups, with a significance level of $P > 0.05$ considered significant for survival analysis.

Detailed information can be found in Supplementary Material.

# Results

## PPIH Expression in Pancancer

The workflow for this study is shown in Figure 1. PPIH mRNA expression was examined across 33 cancers using TIMER2. The results revealed that PPIH mRNA expression was significantly upregulated in adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), invasive breast carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CCA), colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), oesophageal carcinoma (EC), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSCC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), brain lower grade glioma (LGG), HCC, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LSCC), ovarian serous cystadenocarcinoma (OC), pancreatic ductal adenocarcinoma (PDAC), prostate cancer (PC), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), testicular germ cell tumors (TGCT), thymoma (THYM), Endometrial carcinoma (EC) and uterine carcinosarcoma (UCS) and was significantly upregulated, whereas this gene was downregulated in Chromophobe renal cell carcinoma (ChRCC) and acute myeloid leukaemia (AML) (Figure 2A). The PPIH expression pattern was further evaluated in the TCGA and GTEx databases, which revealed that PPIH expression was markedly elevated in BLCA, CESC, COAD, HCC, OC, PDAC and STAD but dramatically downregulated in ACC, BRCA, EC, SKCM, TGCT and THCA (Figure 2B). Furthermore, low PPIH protein expression was detected in breast cancer and EC, whereas high expression was detected in colon cancer, ovarian cancer, clear-cell RCC, lung cancer and liver cancer, as analysed using the CPTAC database (Figure 2C). We subsequently analysed PPIH expression patterns across various clinical stages in patients with tumors. Our findings revealed notable variations in PPIH expression among different clinical stages of ACC, EC, KIRC, HCC, ChRCC and CCA (Figure 2D), whereas no differences between other tumor types were observed (Supplementary Figure 1).

## Relationships Between PPIH Expression and Patient Prognosis

Afterwards, we performed univariate Cox regression analysis to assess the correlation between PPIH expression levels and patient survival. Kaplan–Meier curves were analysed to determine the associations between PPIH expression and prognosis in different types of cancer patients. Univariate Cox regression analysis revealed that PPIH expression was significantly associated

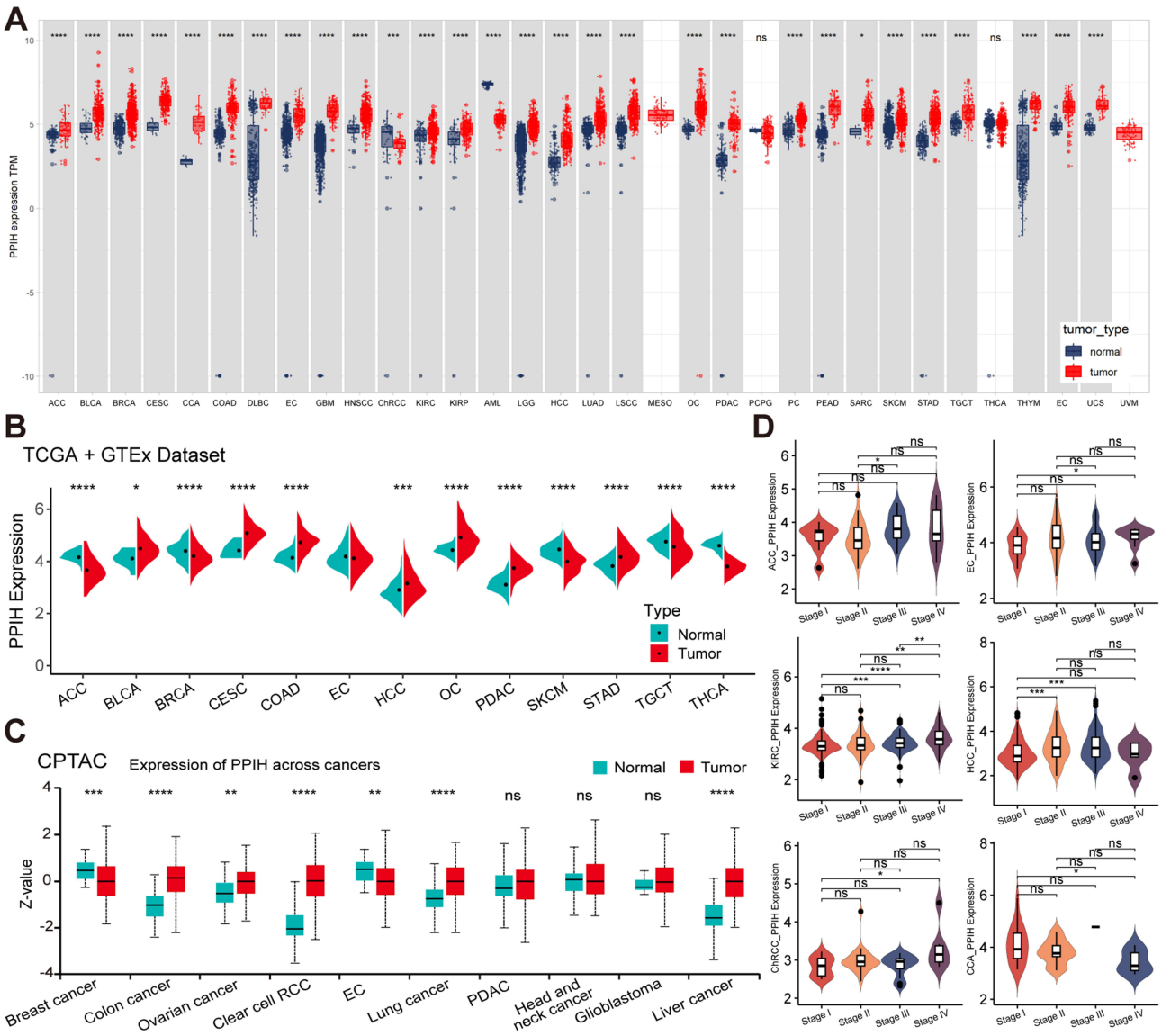


**Figure 2** Expression profiles of PPIH across various cancer types and pathological stages. (**A**) Analysis of PPIH gene expression levels in both paracancer tissues and tumor tissues from the TCGA cohort using TIMER2. (**B**) Comparison of PPIH expression levels between tumor tissues in the TCGA database and normal tissues in the GTEx database. (**C**) Assessment of differences in PPIH protein expression levels between normal and tumor tissues based on data from the CPTAC database. (**D**). Evaluation of variations in PPIH expression levels across different pathological stages within the ACC, EC, KIRC, HCC, KICH and CHOL cohorts. "ns": not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

with poorer overall survival (OS) in ACC, ChRCC, KIRC, AML, LGG, HCC, PDAC, and SARC patients, but not in OC patients (Figure 3A). Kaplan–Meier survival analysis revealed that LGG, HCC, SARC, KIRC, AML and ACC patients had shorter OS with high PPIH expression, but BRCA and STAD had protective effects (Figure 3B). Furthermore, PPIH was associated with poorer PFIs in the ACC, ChRCC, KIRC, KIRP, LGG, HCC, PDAC, and PC, with longer PFIs observed in the UVM (Figure 3C). Survival curves revealed high PPIH expression was associated with a worse PFI in ACC, HCC, PC, KIRC, KIRP, and LGG (Figure 3D).

Additionally, elevated PPIH expression was correlated with adverse DFIs in KIRP, HCC, and PC (Supplementary Figure 2A). Survival analysis revealed a shorter DFI in patients with high expression of PPIH in KIRP, HCC, and PC (Supplementary Figure 2B). Univariate Cox regression analysis revealed associations of PPIH expression with poorer DSS in ACC, ChRCC, KIRC, LGG, HCC, PC, and SARC (Supplementary Figure 2C). Survival analysis further

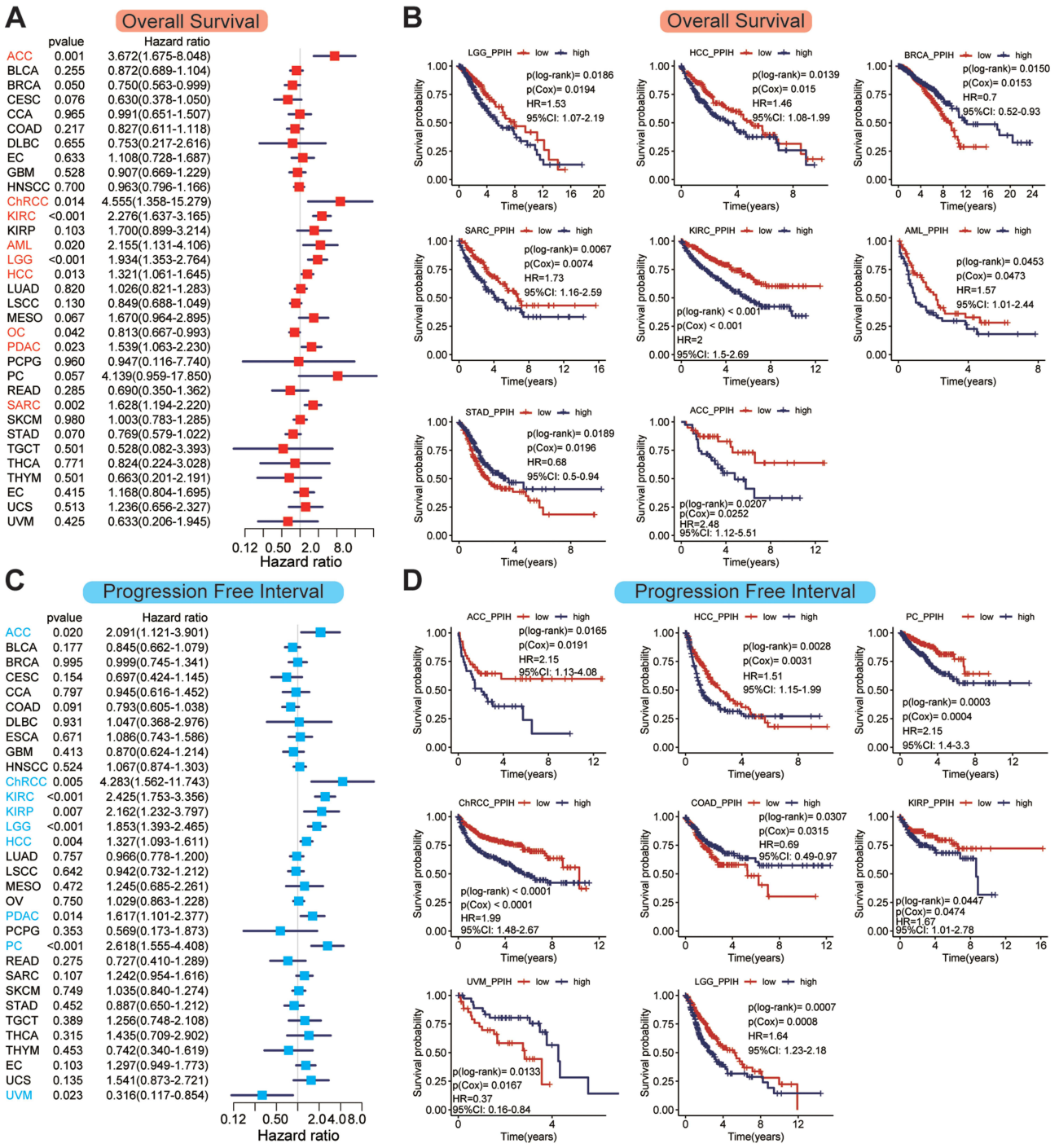


**Figure 3** Associations between PPIH expression levels and patient survival in the TCGA cohort. (**A**) Univariate Cox regression analysis showing the impact of PPIH expression levels on overall survival (OS) in the TCGA cohort. (**B**) Correlations between low and high PPIH expression levels and OS in the TCGA cohort. (**C**) Forest plot depicting univariate Cox regression analysis of PPIH expression levels and the progression-free interval (DFI). (**D**) Association between low and high PPIH expression levels and DFI in the TCGA cohort.

confirmed that high PPIH expression was significantly associated with worse DSS in ACC, HCC, SARC, LGG, THCA, and KIRC patients but showed the opposite trend in COAD patients (Supplementary Figure 2D).

## Biological Characteristics and Somatic Mutation Profile of PPIH

Further analysis of PPIH expression in HCC patients was performed, and CIBERSORT, quantiseq and ssGSEA algorithms were applied to estimate TME components. Surprisingly, Treg infiltration increased with increasing PPIH

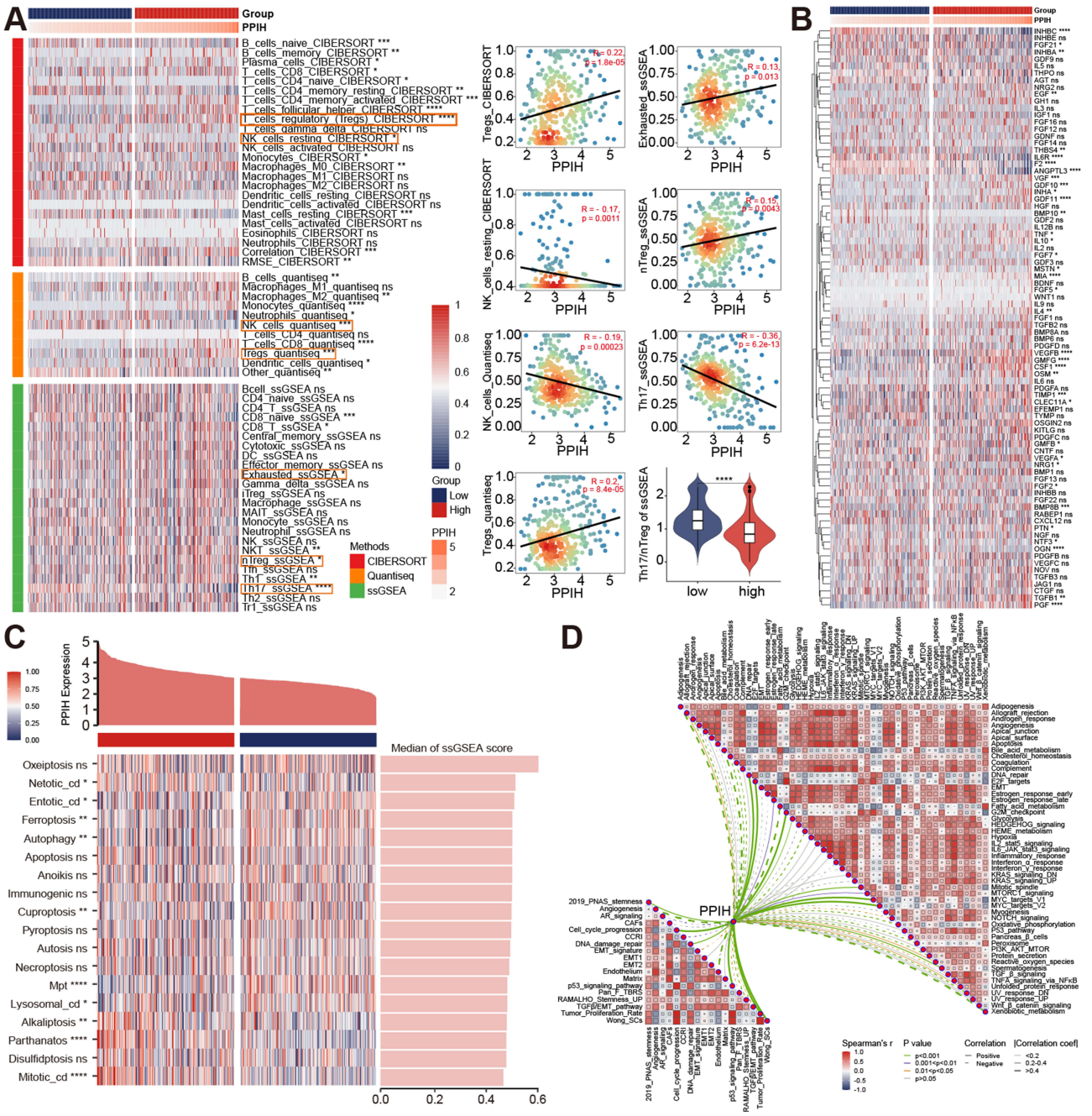


**Figure 4** Associations of PPIH with the TME and cell death pathways in HCC. (**A**) Relationships between PPIH expression levels and infiltrating abundance were calculated via three algorithms: CIBERSORT, quantiseq and ssGSEA. A redder colour in the scatter plot indicates denser points. (**B**) Relationships between the PPIH expression levels and 83 EMT-associated growth factors. (**C**) Relationships between the PPIH expression levels and enrichment scores of 18 cell death-related gene sets. (**D**) Relationships between the PPIH expression levels and specific features, including published pathways and hallmark pathways. CCRI: cytokine–cytokine receptor interaction. "ns": not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

expression, whereas natural killer (NK) cell and T helper 17 (Th17) cell infiltration decreased. Specifically, we found substantial infiltration of exhausted T cells in the high-PPIH group (Figure 4A). We assembled 83 epithelial–mesenchymal transition (EMT)-related growth factors and found that these growth factors were actively expressed in the high-PPIH group. (Figure 4B). ssGSEA of 18 published pathways regulating cell death revealed that oxeiptosis had the highest level of enrichment in HCC, while no marked difference was found between the low- and high-PPIH groups (Figure 4C). Additionally, a heatmap revealed that Netotic_cd, Alkaliptosis, Parthanatos, and Mitotic_cd were prominently active in the group with high PPIH levels. To further elucidate the immune

characteristics of PPIH, we analysed the correlations between PPIH expression and specific key features and hallmark pathways. We found that PPIH expression was positively associated with stemness, the cell cycle, DNA damage repair, P53 signalling, tumor proliferation, E2F targeting, the G2M checkpoint, glycolysis, the mitotic spindle, and the MYC targeting pathways but negatively correlated with angiogenesis, the endothelium and metabolism-associated pathways (Figure 4D).

GSEA performed using KEGG pathway analysis revealed that metabolism-related pathways were significantly enriched in low-expressing PPIH samples (Figure 5A). As a result of ssGSEA of the indicated gene sets, we found

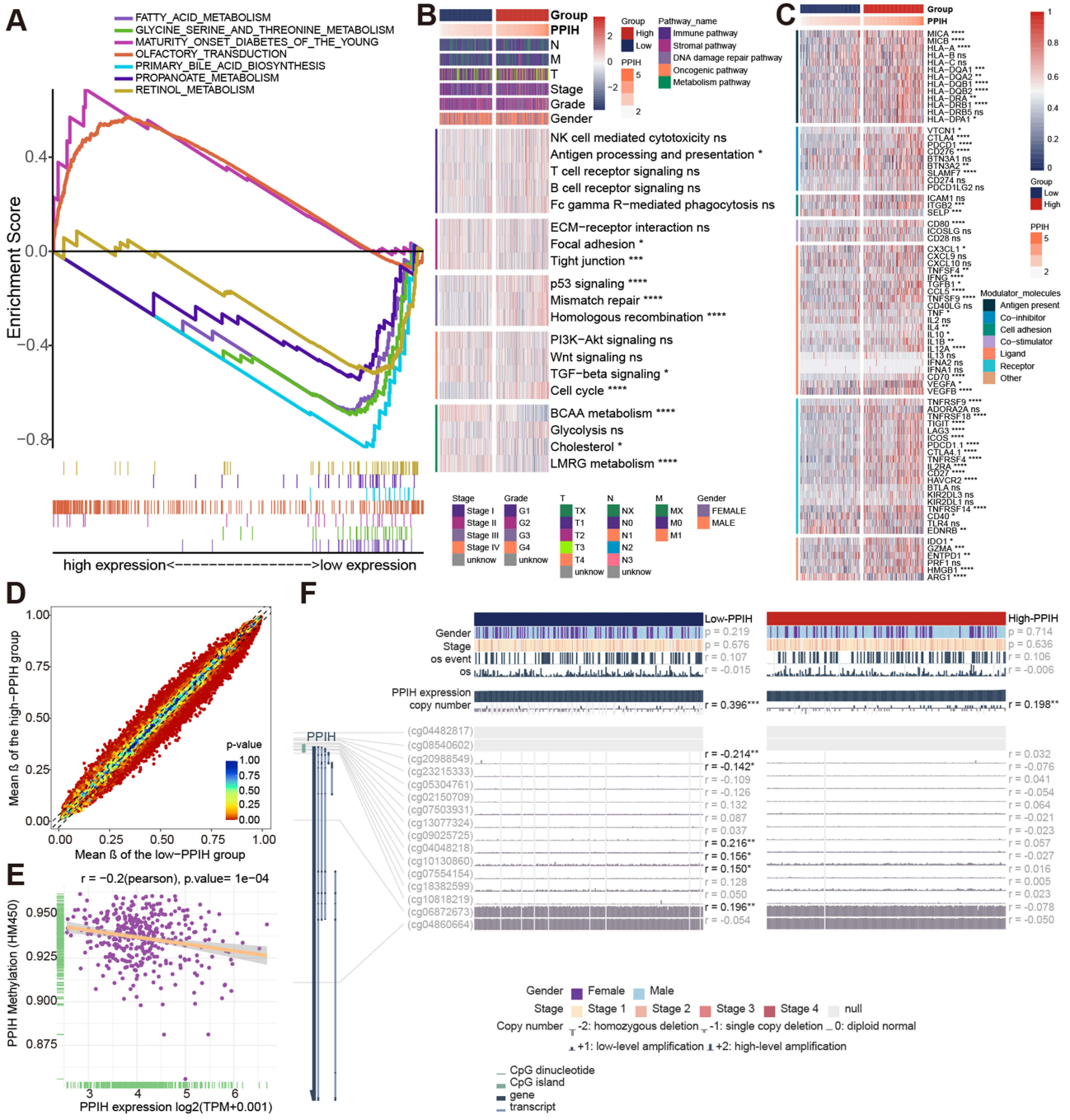


**Figure 5** Differences in immune signatures and PPIH methylation between the low- and high-PPIH subgroups in HCC. (**A**) GSEA results demonstrate pathways notably enriched in both subgroups. (**B**) Enrichment levels of immune- and metabolism-associated pathways in both subgroups. (**C**) Heatmap of immunomodulatory factor expression in both subgroups. (**D**) Visualization of genes associated with differences in methylation. Each dot represents a gene. (**E**) Scatterplot of the correlation between PPIH methylation β values and PPIH expression. (**F**) Association between PPIH expression levels and methylation in both subgroups. "ns": not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

significantly enriched DNA damage repair and cell cycle-related pathways in samples with high expression of PPIH, whereas matrix pathway, BCAA metabolism, cholesterol metabolism, and LMRG metabolism pathway activities were significantly downregulated (Figure 5B). Further analysis revealed high expression of ENTTPD1, IDO1, HMGB1, antigen presentation, coinhibitors, ligands, and receptor-associated immunomodulatory molecules in the high-expression PPIH group (Figure 5C). Given that CNV causes genomic rearrangements, the association between PPIH expression and CNV was further explored. CNV loading analysis revealed a striking increase in gene amplification and deletion in the PPIH high-expression group according to the heatmap and violin plots (Figure 5D and E). Each locus is plotted in ascending order according to GISTIC scores, illustrating the distribution of CNVs between the high-expression and low-expression groups of PPIH (Figure 5F).

## Methylation of PPIH and the Immune Landscape in HCC

Given the pivotal roles of gene methylation and mutation in tumorigenesis and progression, a detailed analysis of tumor characteristics was conducted across low- and high-PPIH subgroups. Methylation data from the TCGA-HCC cohort revealed 85,172 differentially methylated probes (Figure 6A). Interestingly, the level of PPIH methylation was negatively correlated with its mRNA expression (Figure 6B). Using the MEXPRESS method, we subsequently analysed the PPIH methylation patterns in both subgroups and detected a significant correlation between the promoter methylation of PPIH and its expression in the low-PPIH subgroup (Figure 6C). SNV data were used to detect somatic mutation profiles in both subgroups, and 20 highly mutated genes were identified (Figure 6D). Seven differentially mutated genes were highlighted by Fisher's test ($P < 0.05$) (Figure 6E). Figure 6F shows the mutation ratios of commonly mutated genes in both subgroups. High tumor mutation burden (TMB) is frequently associated with $CD8^+$ T-cell infiltration and inflamed gene signatures, which are believed to be cancer-agnostic markers of ICI activity.[22,23] Next, we examined the relationship between PPIH expression levels and TMB in the TCGA-HCC cohort. Increasing PPIH expression levels were correlated with increasing TMB, and in groups with high PPIH expression, the TMB was significantly greater than that in groups with low PPIH expression (Figure 6G).

## HCC Tissues Exhibit PPIH Upregulation

To confirm the above findings, additional HCC patient tumor samples were subjected to HE staining, IF and immunohistochemical (IHC) staining. In clinical HCC samples, PPIH expression was significantly upregulated compared with that in normal tissues (Figure 7A). The positive areas of PPIH were calculated and are shown in Figure 7B. Furthermore, to further verify the differential infiltration of Th17 cells between HCC tissues with differential PPIH expression, we used immunofluorescence staining and observed increased infiltration of Th17 cells in HCC tissues with low PPIH expression, followed by intermediate infiltration in tissues with moderate expression. In contrast, HCC tissues with high PPIH expression presented minimal infiltration of Th17 cells. Increasing PPIH expression may inhibit Th17 cell infiltration, as these findings indicate a correlation between PPIH and Th17 cell infiltration (Figure 7C). The immunofluorescence intensity was quantified using ImageJ and is shown in Figure 7D.

## PPIH Knockdown Suppresses the Viability and Proliferation of HCC Cells

To further explore the pathophysiological role of PPIH in HCC cells, we constructed Hepa1-6, HCCLM3, MHCC97H and HepG2 cells with PPIH knockdown. We performed Western blotting to confirm the PPIH knockdown efficiency (Figure 8A). Then, PPIH mRNA levels were determined via qRT–PCR and normalized to Actb mRNA levels (Figure 8B). Moreover, we used cells with stable PPIH knockdown for further proliferation and migration analyses.

To investigate whether PPIH affects the migration of HCC cells, wound healing assays were performed. All the cells were cultured under low-serum conditions after a scratch was made to eliminate the effect of cell proliferation. Compared with control cells, PPIH-knockdown cells showed slower wound closure for the first 48 hours. The same phenomenon was observed between the different types of cellular models (Figure 8C-F). Subsequently, cell apoptosis was detected via flow cytometry via Annexin V/PI staining. The results revealed that, after 48 h, PPIH-knockdown human HCC cells underwent more apoptosis than control cells (Figure 8G-J). A Transwell invasion assay examined invasion after PPIH

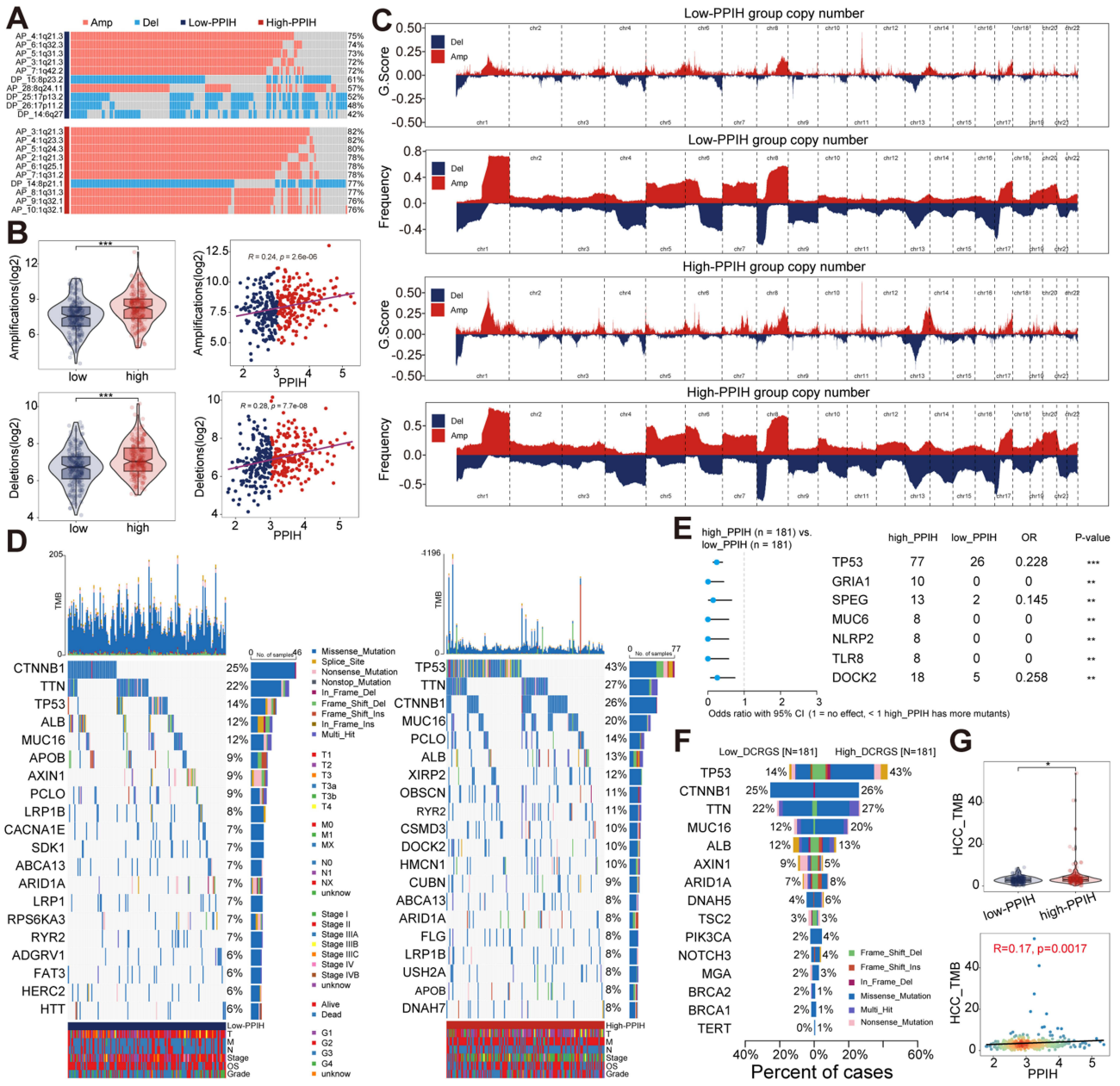


**Figure 6** Genetic alterations in the low- and high-PPIH groups in HCC. (**A**) Heatmap of the top 10 regions with the highest CNV loads in both subgroups. (**B**) Violin plots of gene amplification and deletion between the two subgroups. (**C**) CNV profiling showing the distributions of the G score and mutation frequency in the two subgroups. (**D**) The top 20 genes with the highest mutation frequency in both subgroups. (**E**) Forest plot of the differentially mutated genes in both subgroups. (**F**) Mutation types of commonly mutated genes in HCC. (**G**) Association between the HCC mutation load and PPIH expression. A redder colour in the scatterplot indicates denser points. "ns": not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

knockdown in HCC cells. The results revealed that, compared with the control, PPIH knockdown impaired cell invasion (Figure 8K).

## Knocking Down PPIH Inhibits Tumor Growth and Reprograms the Tumor Immune Microenvironment in HCC Mice

The next step was to perform an in vivo experiment to confirm the role of PPIH in tumor cell proliferation. Wild-type and PPIH-knockdown Hepa 1–6 cells were used to establish the subcutaneous tumor-bearing mouse model. Fluorescence live imaging revealed that the PPIH-knockdown tumors grew more slowly than the control tumors (Figure 9A). Compared

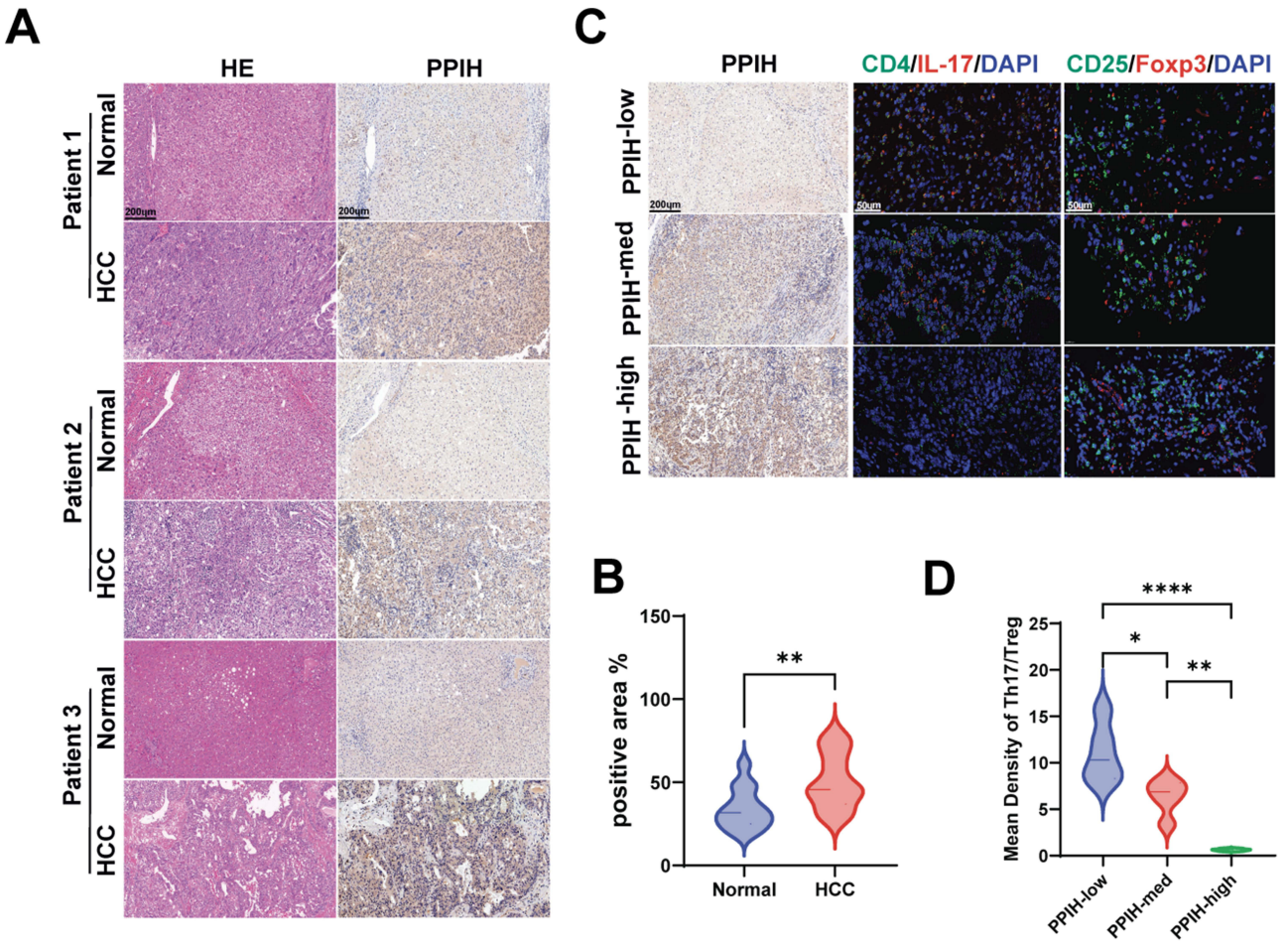


**Figure 7** The levels of PPIH expression and Th17/Treg cell infiltration in clinical tissues. (**A**) Representative HE staining and PPIH IHC staining images of clinical HCC samples. n=10. (**B**) Percentage of PPIH-positive area over total area. (**C**) Representative images of $CD4^+$ $IL\text{-}17^+$ T cells and $CD25^+$ $Foxp3^+$ T cells IF staining in clinical HCC samples. n=5. (**D**) Quantification of the mean immunofluorescence intensity of Th17/Treg. n=5. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$ indicates statistical significance compared with the control.

with the control, PPIH knockdown significantly reduced the tumor volume (Figure 9B). Furthermore, the mice with PPIH-knockdown tumors showed prolonged survival (Figure 9C). Tumor weights were measured after sacrifice and are presented as the mean tumor weight ± SD in (Figure 9D). The tumor weight was obviously reduced after PPIH knockdown, suggesting that the tumor proliferation ability decreased (Figure 9E).

To further investigate the potential anti-tumor mechanism of PPIH inhibition, RNA-sequencing (RNA-seq) was performed on wild-type and PPIH-knockdown Hepa 1–6 tumors to identify genes with altered expression in response to PPIH inhibition. As shown by the Pearson's correlation coefficient matrix (Supplementary Figure 3A), the overall gene expression patterns in samples from the same group were strongly correlated (mean Pearson correlation, 0.95), indicating high internal consistency and reliability evidence between biological replicates. Subsequently, the differentially expressed genes between shCON group and shPPIH group were identified by R package DESeq2 with a filtering criteria of fold-change $\geq$ 1.5 and $P < 0.05$ (Supplementary Figure 3B). By Gene Ontology (GO) enrichment analysis of 4598 differentially expressed genes, we found that the biological process affected by PPIH knockdown were mainly involve regulation of the immune system process and immune response, followed by response to stimulus and then cell migration (Supplementary Figure 3C). Compared with the shCON group, most of genes associated with T cell differentiation (GO:0030217), positive regulation of T cell activation (GO:0050870) and positive regulation of activated T cell proliferation (GO:0042104), were significantly up-regulated in the shPPIH group (Supplementary Figure 3D), suggesting that inhibition of PPIH may play a positive role in improving the immunosuppressive status of HCC via stimulation of

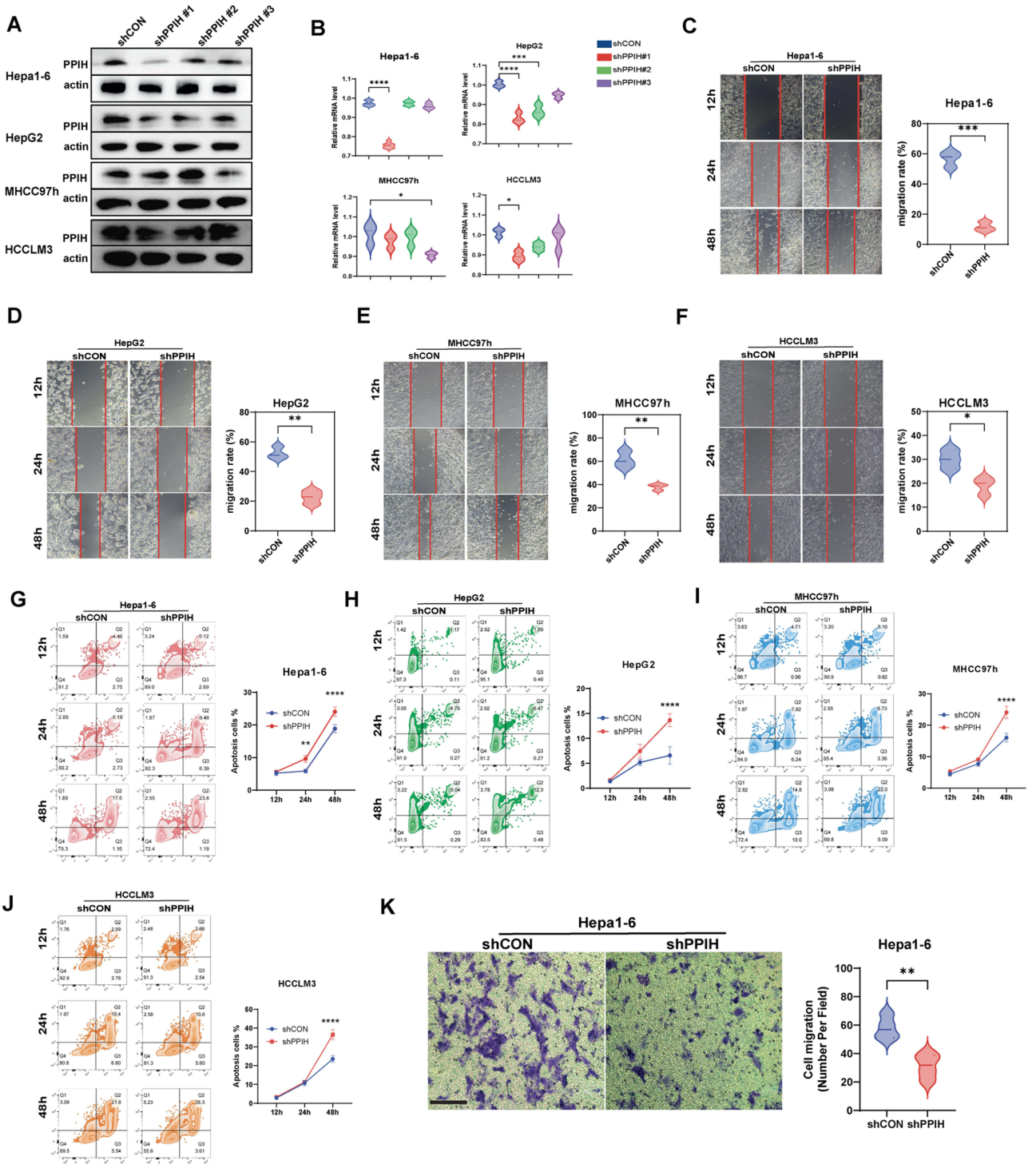


**Figure 8** PPIH knockdown inhibits HCC cell proliferation, migration and invasion. (**A**) Western blotting confirmed the successful knockdown of PPIH by PPIH-specific shRNA in Hepa1-6, HepG2, MHCC97h and HCCLM3 cells. (**B**) Quantitative PCR analysis of the efficiency of PPIH knockdown in Hepa1-6, HepG2, MHCC97h and HCCLM3 cells. n = 3. (**C-F**) Cell migration in the scratch assay. (**G-J**) PPIH knockdown induced apoptosis in different HCC cell lines, which increased with time. (**K**) Transwell invasion assay using a transwell chamber before and after PPIH knockdown. n =4, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ indicate statistical significance compared with the control.

T cell-mediated anti-tumor immunity. Consistently, KEGG pathways analysis of differentially up-regulated genes showed that the signaling pathways responsible for Th1, Th2 and Th17 cell differentiation was highly activated after PPIH inhibition (Supplementary Figure 3E). As it is well known, in addition to recruiting immune cells to tumor sites, Th17

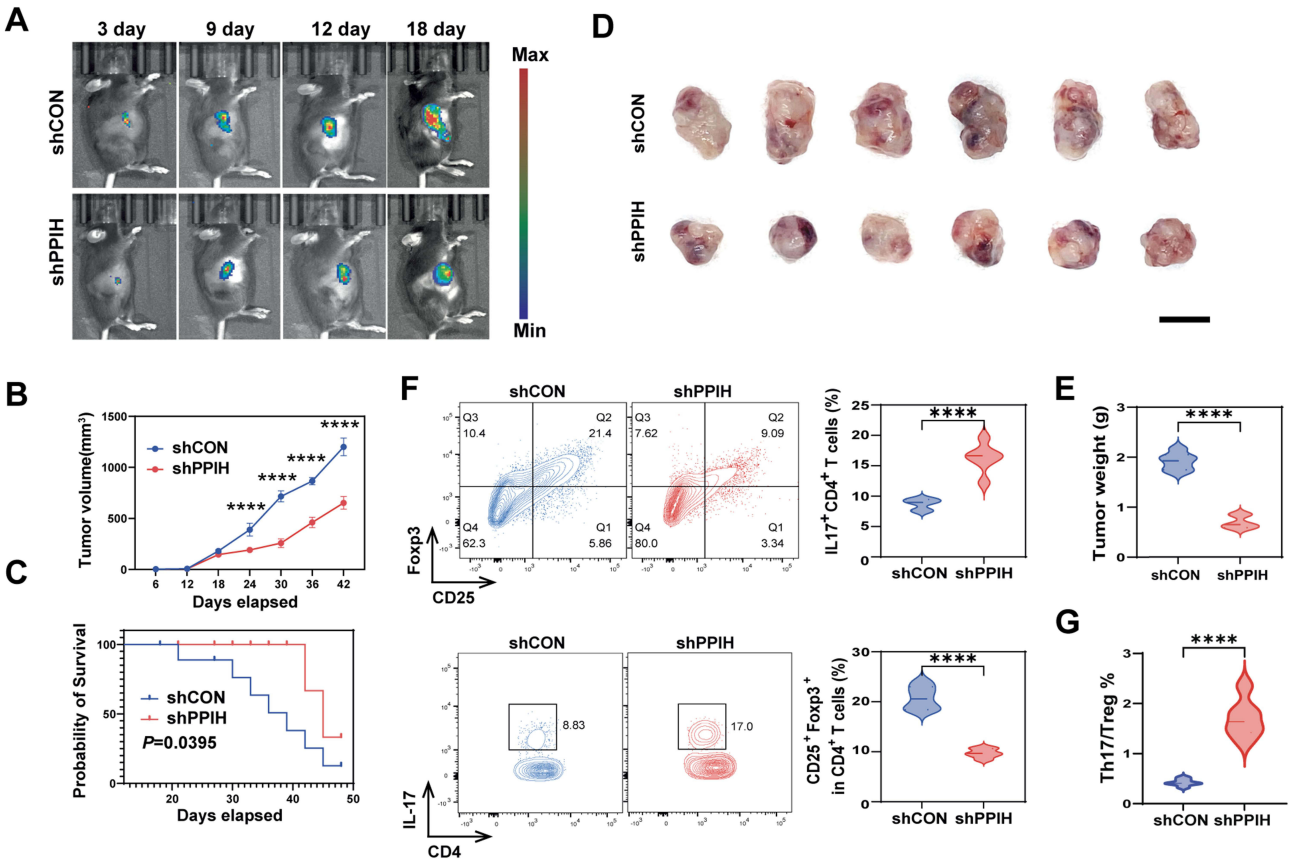


**Figure 9** PPIH promotes tumor growth in mice. (**A**) Representative live fluorescence images of tumor-bearing mice 3 days after cell inoculation. (**B**) The subcutaneous tumor volume of each group was recorded every six days (the 6th, 12th, 18th, 24th, 30th, 36th, and 42nd days). (**C**) Growth curves of the two groups. (**D**) Images of subcutaneous tumors from each group (scale bar: 1 cm). (**E**) The weight of tumor in each group. (**F**) Representative flow dot plots of Th17 T cells and Tregs in tumor tissue and quantified by flow cytometry. (**G**) The proportion of Th17/Treg in each group. n=6, ****$P < 0.0001$ indicates statistical significance compared with the control.

cells could help CD8 re-expression and acquisition of cytotoxic T cell properties by producing IL-17 or converting toward Th1 phenotype.[24,25] To validate the transcriptomic results, we evaluated the proportions of infiltrating regulatory T (Treg) cells and Th17 cells in the tumors described above using flow cytometry. The results revealed that PPIH induced a decrease in Th17 cells and an increasing trend in Treg cells in the TME (Figure 9F), and the proportion of Th17/Treg in shCON group was significantly less than in shPPIH group (Figure 9G), which is also consistent with previous bioinformatic predictions. Together, These findings demonstrate that PPIH drives shifts in the tumor immune microenvironment in HCC to facilitate tumorigenesis and tumor growth.

## Conclusion

This study used bulk transcriptomes from pancancer cells, gene expression profiles associated with HCC prognosis, immune infiltration analysis, methylation analysis and in vivo/in vitro experiments to evaluate the effects of PPIH on Th17/Treg cells and provided nuanced insight into the HCC microenvironment.

## Discussion

Previous research has revealed potential associations between the PPIase family and the pathological conditions of cancers. DNA repair can be promoted directly by PPIases through interactions with NBS1.[26,27] PPIases within breast cancer cells inhibit exogenous IL-36-induced anchorage-independent growth.[28] Specifically, PPIF has been reported to be a prognostic predictor molecule for prostate cancer and is closely related to macrophage invasion.[29] Patient prognoses are negatively influenced by increased PPIA in NSLC.[30] PPIA acts as a peptidyl-prolyl cis-trans isomerase, which affects substrate protein folding and stability in addition to cell signalling, thus suppressing exosome-specific protein release.[31]

This may be how PPIase family proteins promote cancer progression. Nevertheless, in HCC, current knowledge regarding PPIH remains largely lacking. Our analysis of the TCGA, GTEx, and CPTAC databases revealed heterogeneous expression patterns of PPIH across different cancer types. It is upregulated in BLCA, CESC, COAD, HCC, OC, PDAC, and STAD and promotes tumorigenesis and progression. These findings are consistent with studies conducted by Weiqiang Lu[30] and Ye jun et al,[19] who reported elevated PPIH expression in similar cancer types.[18] We found that high levels of PPIH expression in various cancer types are related to poor progression-free survival, disease-specific survival, and overall survival according to univariate Cox regression analysis and Kaplan–Meier survival analysis. These findings suggest that PPIH may be a potential prognostic biomarker for assessing patient outcomes in these cancers. Additional studies are needed to validate these results and elucidate the underlying mechanisms.

Th17 cells are subsets of $CD4^+$ T helper cells vital to immune responses. Th17 cells have contradictory effects on tumors. On the one hand, in addition to being inflammatory cells, Th17 cells bind to proinflammatory cytokines, preventing the immune system from monitoring cancer cells.[32–35] On the other hand, Th17 cells inhibit tumor growth; promote cancer cell apoptosis; and restrain tumor angiogenesis by secreting TNFα, IFN-γ, IL-17, IL-21, and IL-22, as well as chemokines, to attract macrophages and dendritic cells in tumor tissue.[36,37] As a subset of $CD4^+$ T cells, Tregs can inhibit the immune response.[38] As part of their ability to suppress immune cells, Tregs secrete anti-inflammatory cytokines and disrupt metabolic pathways in HCC through direct cell–cell contact inhibition, cell–cell contact inhibition, and disruption of metabolic pathways.[39] In recent years, new perspectives and advancements have revealed that dysregulation of the Th17/Treg cell balance is involved in developing and progressing of several disorders, including cancer, autoimmune diseases, and inflammatory diseases.[40] It was first postulated in 1863 that tissue injury could lead to cell proliferation, leading to cancer.[41] Excess inflammation caused by Th17 cells or excessive immunosuppression caused by Treg cells may promote cancer growth. A previous study has demonstrated that oral squamous cell carcinoma patients with a low Th17/Treg ratio have a poor prognosis.[42] Pancreatic cancer patients exhibit a disordered balance between Th17 and Treg cells, with Treg cells expressing more and Th17 cells expressing less.[43] Similarly, our study highlights the complex immune characteristics and molecular pathways associated with PPIH expression in HCC. Changes in the immune microenvironment occur concomitantly with high PPIH expression. Increased infiltration of Treg cells and decreased Th17 cells were observed in the high-PPIH group, whereas exhausted T cells were substantially present in the high-PPIH group, with a low Th17/Treg ratio, indicating potential immune dysfunction. Moreover, the high-PPIH group exhibited active expression of EMT-related growth factors, which may contribute to tumor progression and metastasis. Thus, the presence of PPIH promotes both direct tumor growth and the formation of an immunosuppressive microenvironment in HCC. Additionally, this study revealed that HCC patients with high PPIH levels seem to have a higher TMB but are accompanied by an immunosuppressive tumor microenvironment, indicating that the TMB could not serve as a better predictor of immunotherapy responses in HCC. As shown in a study by Huang RJ et al,[44] TMB-H mutation failed to support its use as a biomarker for ICB treatment in all solid cancer types. However, further tumor type-specific studies are needed. Since TMB thresholds differ widely across cancer types, determining the benefit of anti-PD-1/L1 therapy based on a single TMB cut-off is not feasible.

Research has shown that when Th17 cells enter and expand in the tumor microenvironment, they also produce CXCL9 and CXCL10, which enhance the operation and maintenance of effector T cells and natural killer cells.[45] The transferred Th17 cells induce antitumor effects by altering the microenvironment.[46] Our findings suggest that PPIH, which is expressed in tumor cells, affects the degree of Th17 and Treg cell infiltration. Specifically, high PPIH expression induces the infiltration of Tregs but hinders the differentiation of Th17 cells. It is possible that PPIH regulates protein folding and consequently mediates some tumor-associated antigens, ultimately interfering with the differentiation of Th17 cells. Tumor-associated antigens, which may be mutated, misfolded, degraded, proteolytically cleaved, or overexpressed, trigger the immune response against a tumor.[47] As shown by Rika Aizawa et al, PPIA, as part of the complex, can predict the efficacy of immunotherapy for non-small cell carcinomas.[48] Additionally, it has been reported to affect the lesion microenvironment in other types of diseases. PPIases trigger pathogenic chronic inflammation in multiple sclerosis models, including upregulated Th1 and Th17 cells.[49] In renal fibroblasts transitioning towards fibrosis, PPIA is upregulated and secreted upon cytokine stimulation, suggesting an important role for the protein.[50] Moreover, our result point out that PPIH knockdown helps to regulate immune system processes, immune response, response to stimulus and

cell migration. According to KEGG enrichment analysis, PPIH may affect the immune microenvironment through interactions between cytokines. In summary, in our comprehensive study of PPIH, we observed its overexpression in various cancer types and its association with unfavourable clinical outcomes, subsequent genetic alterations, and immunological circumstances. Especially in HCC, PPIH plays a vital role in tumorigenesis and reshapes Th17/Treg cell infiltration. We performed molecular and animal experiments to verify our conclusions. Therefore, this study provides insight into immune-related mechanisms in HCC and suggests a new biomarker.

## Ethics Statement Compulsory

Approval of the research protocol by an Institutional Reviewer Board. The studies involving patients were reviewed and approved by the First Affiliated Hospital of Guangdong Pharmaceutical University (2024-NO.40). Informed Consent. Informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article. Registry and the Registration No. of the study/trial. N/A.

Animal Studies. Animal studies were carried out in accordance with the protocols approved by the Institutional Animal Care and Use Committee at the First Affiliated Hospital of Guangdong Pharmaceutical University.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This study was supported by the Guangzhou Basic and Applied Basic Research Foundation (Grant No. 2024A04J4811), Hospital Fund of the First Affiliated Hospital of Guangdong Pharmaceutical University (Grant No. ZXYXTQJ202304) and the National Natural Science Foundation of China (Grant No. 82202273).

## Disclosure

The authors declare no competing interests.

## References

1. Dopazo C, Soreide K, Rangelova E, et al. Hepatocellular carcinoma. *Eur J Surg Oncol*. 2024;50(1):107313. doi:10.1016/j.ejso.2023.107313
2. Singal AG, Kanwal F, Llovet JM. Global trends in hepatocellular carcinoma epidemiology: implications for screening, prevention and therapy. *Nat Rev Clin Oncol*. 2023;20(12):864–884. doi:10.1038/s41571-023-00825-3
3. Llovet JM, Pinyol R, Yarchoan M, et al. Adjuvant and neoadjuvant immunotherapies in hepatocellular carcinoma. *Nat Rev Clin Oncol*. 2024;21(4):294–311. doi:10.1038/s41571-024-00868-0
4. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. doi:10.3322/caac.21834
5. Brown ZJ, Tsilimigras DI, Ruff SM, et al. Management of Hepatocellular Carcinoma: a review. *JAMA Surg*. 2023;158(4):410–420. doi:10.1001/jamasurg.2022.7989
6. Liu J, Park K, Shen Z, et al. Immunotherapy, targeted therapy, and their cross talks in hepatocellular carcinoma. *Front Immunol*. 2023;14:1285370. doi:10.3389/fimmu.2023.1285370
7. Rimassa L, Personeni N, Czauderna C, et al. Systemic treatment of HCC in special populations. *J Hepatol*. 2021;74(4):931–943. doi:10.1016/j.jhep.2020.11.026
8. Finn RS, Qin S, Ikeda M, et al. Atezolizumab plus Bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med*. 2020;382(20):1894–1905. doi:10.1056/NEJMoa1915745
9. Cheng AL, Qin S, Ikeda M, et al. Updated efficacy and safety data from IMbrave150: atezolizumab plus bevacizumab vs. sorafenib for unresectable hepatocellular carcinoma. *J Hepatol*. 2022;76(4):862–873. doi:10.1016/j.jhep.2021.11.030
10. Gallardo-Flores CE, Colpitts CC. Cyclophilins and their roles in Hepatitis C virus and flavivirus infections: perspectives for novel antiviral approaches. *Pathogens*. 2021;10(7):902. doi:10.3390/pathogens10070902
11. Lavin PT, Mc GM. Cyclophilin function in Cancer; lessons from virus replication. *Curr Mol Pharmacol*. 2015;9(2):148–164. doi:10.2174/1874467208666150519115443
12. Naoumov NV. Cyclophilin inhibition as potential therapy for liver diseases. *J Hepatol*. 2014;61(5):1166–1174. doi:10.1016/j.jhep.2014.07.008
13. Peng L, Jiang J, Chen HN, et al. Redox-sensitive cyclophilin A elicits chemoresistance through realigning cellular oxidative status in colorectal cancer. *Cell Rep*. 2021;37(9):110069. doi:10.1016/j.celrep.2021.110069

14. Kim Y, Jang M, Lim S, et al. Role of cyclophilin B in tumorigenesis and cisplatin resistance in hepatocellular carcinoma in humans. *Hepatology*. 2011;54(5):1661–1678. doi:10.1002/hep.24539
15. Lee YS, Jeong S, Kim KY, et al. Honokiol inhibits hepatoma carcinoma cell migration through downregulated Cyclophilin B expression. *Biochem Biophys Res Commun*. 2021;552:44–51. doi:10.1016/j.bbrc.2021.03.011
16. Lee J. Cyclophilin A as a new therapeutic target for Hepatitis C virus-induced hepatocellular carcinoma. *Korean J Physiol Pharmacol*. 2013;17(5):375–383. doi:10.4196/kjpp.2013.17.5.375
17. Schiene-Fischer C, Fischer G, Braun M. Non-immunosuppressive cyclophilin inhibitors. *Angew Chem Int Ed Engl*. 2022;61(39):e202201597. doi:10.1002/anie.202201597
18. Ye J, Pang Y, Yang X, et al. PPIH gene regulation system and its prognostic significance in hepatocellular carcinoma: a comprehensive analysis. *Aging (Albany NY)*. 2023;15(20):11448–11470. doi:10.18632/aging.205134
19. Ye J, Ying J, Chen H, et al. PPIH acts as a potential predictive biomarker for patients with common solid tumors. *BMC Cancer*. 2024;24(1):681. doi:10.1186/s12885-024-12446-9
20. Li M, Liu Z, Wang J, et al. Systematic analysis identifies a specific RNA-binding protein-related gene model for prognostication and risk-adjustment in HBV-related hepatocellular carcinoma. *Front Genet*. 2021;12:707305. doi:10.3389/fgene.2021.707305
21. Li J, Zhou W, Wei J, et al. Prognostic Value and Biological Functions of RNA Binding Proteins in Stomach Adenocarcinoma. *Onco Targets Ther*. 2021;14:1689–1705. doi:10.2147/OTT.S297973
22. Li F, Li C, Cai X, et al. The association between CD8+ tumor-infiltrating lymphocytes and the clinical outcome of cancer immunotherapy: a systematic review and meta-analysis. *EClinicalMedicine*. 2021;41:101134. doi:10.1016/j.eclinm.2021.101134
23. Marcus L, Fashoyin-Aje LA, Donoghue M, et al. FDA approval summary: pembrolizumab for the treatment of tumor mutational burden-high solid tumors. *Clin Cancer Res*. 2021;27(17):4685–4689. doi:10.1158/1078-0432.CCR-21-0327
24. Issuree PD, Day K, Au C, et al. Stage-specific epigenetic regulation of CD4 expression by coordinated enhancer elements during T cell development. *Nat Commun*. 2018;9(1):3594. doi:10.1038/s41467-018-05834-w
25. Sun R, Zhang PP, Weng XQ, et al. Therapeutic targeting miR130b counteracts diffuse large B-cell lymphoma progression via OX40/OX40L-mediated interaction with Th17 cells. *Signal Transduct Target Ther*. 2022;7(1):80. doi:10.1038/s41392-022-00895-2
26. Bedir M, Outwin E, Colnaghi R, et al. A novel role for the peptidyl-prolyl cis-trans isomerase Cyclophilin A in DNA-repair following replication fork stalling via the MRE11-RAD50-NBS1 complex. *EMBO Rep*. 2024;25(8):3432–3455. doi:10.1038/s44319-024-00184-9
27. Guan B, Huang C, Meng L, et al. Dissecting prostate cancer: single-cell insight into macrophage diversity, molecular prognosticators, and the role of Peptidylprolyl Isomerase F. *Int Immunopharmacol*. 2024;138:112599. doi:10.1016/j.intimp.2024.112599
28. Poudel M, Bhattarai PY, Shrestha P, et al. Regulation of Interleukin-36gamma/IL-36R signaling axis by PIN1 in epithelial cell transformation and breast tumorigenesis. *Cancers (Basel)*. 2022;14(15):3654. doi:10.3390/cancers14153654
29. Lin CH, Tariq MJ, Ullah F, et al. Current novel targeted therapeutic strategies in multiple myeloma. *Int J Mol Sci*. 2024;25(11):6192. doi:10.3390/ijms25116192
30. Lu W, Cui J, Wang W, et al. PPIA dictates NRF2 stability to promote lung cancer progression. *Nat Commun*. 2024;15(1):4703. doi:10.1038/s41467-024-48364-4
31. Maneix L, Iakova P, Lee CG, et al. Cyclophilin A supports translation of intrinsically disordered proteins and affects haematopoietic stem cell ageing. *Nat Cell Biol*. 2024;26(4):593–603. doi:10.1038/s41556-024-01387-x
32. Salazar Y, Zheng X, Brunn D, et al. Microenvironmental Th9 and Th17 lymphocytes induce metastatic spreading in lung cancer. *J Clin Invest*. 2020;130(7):3560–3575. doi:10.1172/JCI124037
33. Sharp SP, Avram D, Stain SC, et al. Local and systemic Th17 immune response associated with advanced stage colon cancer. *J Surg Res*. 2017;208:180–186. doi:10.1016/j.jss.2016.09.038
34. Bi L, Wu J, Ye A, et al. Increased Th17 cells and IL-17A exist in patients with B cell acute lymphoblastic leukemia and promote proliferation and resistance to daunorubicin through activation of Akt signaling. *J Transl Med*. 2016;14(1):132. doi:10.1186/s12967-016-0894-9
35. Marshall EA, Ng KW, Kung SH, et al. Emerging roles of T helper 17 and regulatory T cells in lung cancer progression and metastasis. *Mol Cancer*. 2016;15(1):67. doi:10.1186/s12943-016-0551-1
36. Fu LQ, Yang X, Cai MH, et al. Role of Treg/Th17 Imbalance, Microbiota and miRNAs in pancreatic cancer: therapeutic options. *Crit Rev Immunol*. 2020;40(1):75–92. doi:10.1615/CritRevImmunol.2020033631
37. Muranski P, Borman ZA, Kerkar SP, et al. Th17 cells are long lived and retain a stem cell-like molecular signature. *Immunity*. 2011;35(6):972–985. doi:10.1016/j.immuni.2011.09.019
38. Woodward DA, Roozen HN, Dufort MJ, et al. The human tissue-resident CCR5(+) T cell compartment maintains protective and functional properties during inflammation. *Sci Transl Med*. 2019;11(521):eaaw8718. doi:10.1126/scitranslmed.aaw8718
39. Ajith A, Merimi M, Arki MK, et al. Immune regulation and therapeutic application of T regulatory cells in liver diseases. *Front Immunol*. 2024;15:1371089. doi:10.3389/fimmu.2024.1371089
40. Kim ME, Kim DH, Lee JS. FoxO Transcription Factors: applicability as a Novel Immune Cell Regulators and Therapeutic Targets in Oxidative Stress-Related Diseases. *Int J Mol Sci*. 2022;23(19): 11877. doi:10.3390/ijms231911877
41. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860–867. doi:10.1038/nature01322
42. Liu S, Liu Z, Shan Z, et al. Skewed Th17/Treg balance during progression and malignant transformation of oral submucous fibrosis. *Oral Dis*. 2022;28(8):2119–2130. doi:10.1111/odi.13853
43. Wang X, Wang L, Mo Q, et al. Changes of Th17/Treg cell and related cytokines in pancreatic cancer patients. *Int J Clin Exp Pathol*. 2015;8(5):5702–5708.
44. Mcgrail DJ, Pilie PG, Rashid NU, et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann Oncol*. 2021;32(5):661–672. doi:10.1016/j.annonc.2021.02.006
45. Fu C, Zhang X, Zhang X, et al. Advances in IL-7 research on tumour therapy. *Pharmaceuticals (Basel)*. 2024;17(4):415. doi:10.3390/ph17040415
46. Wittling MC, Knochelmann HM, Wyatt MM, et al. Distinct host preconditioning regimens differentially impact the antitumor potency of adoptively transferred Th17 cells. *J Immunother Cancer*. 2024;12(6):e008715. doi:10.1136/jitc-2023-008715
47. Zaenker P, Ziman MR. Serologic autoantibodies as diagnostic cancer biomarkers--a review. *Cancer Epidemiol Biomarkers Prev*. 2013;22(12):2161–2181. doi:10.1158/1055-9965.EPI-13-0621

48. Aizawa R, Nakamura Y, Ikeda T, et al. Immune complexome analysis of serum samples from non-small-cell lung cancer patients identifies predictive biomarkers for nivolumab therapy. *Clin Chim Acta*. 2022;532:84–88. doi:10.1016/j.cca.2022.05.021
49. Ge ZZ, Wu YB, Xue ZY, et al. The therapeutic effects of the peptidyl-prolyl cis/trans isomerase Pin1 inhibitor juglone on animal-model experimental autoimmune encephalomyelitis. *J Physiol Pharmacol*. 2021;72(2). doi:10.26402/jpp.2021.2.05.
50. Dihazi GH, Eltoweissy M, Jahn O, et al. The secretome analysis of activated human renal fibroblasts revealed beneficial effect of the modulation of the secreted peptidyl-prolyl cis-trans isomerase A in kidney fibrosis. *Cells*. 2020;9(7):1724. doi:10.3390/cells9071724