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PHF21A expression as a biomarker of hepatocellular carcinoma progression and prognosis

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Previous studies have shown that PHF21A is associated with the initiation and progression of various tumors. However, its role in hepatocellular carcinoma (HCC) is still unclear. Thus, this study aimed to determine the expression and clinical significance of PHF21A in HCC. PHF21A expression in 201 liver cancer samples and 129 adjacent normal tissues was detected by immunohistochemistry. The correlation between PHF21A expression and the clinicopathological features and prognosis of HCC was verified in 70 other liver tissue microarray samples. The relationship between PHF21A expression and HCC immune cell infiltration was explored via the Tumor Immune Estimation Resource (TIMER). The mechanism underlying the effect of PHF21A on HCC progression was analyzed by gene set enrichment analysis (GSEA) and protein-protein interaction (PPI) network analysis. Immunohistochemical staining showed that PHF21A expression in HCC tissue was significantly lower than that in adjacent nontumor liver tissue and was associated with patient sex, tumor size, metastasis, and Edmondson grade ($p<0.05$). Kaplan-Meier analysis demonstrated that low PHF21A expression was associated with a poor prognosis, and Cox regression analysis showed that PHF21A was an independent predictor of prognosis. TIMER analysis showed that PHF21A is positively correlated with tumor immune cell infiltration levels. Functional annotation indicated that PHF21A is involved in important pathways, including transcriptional deregulation pathways in cancer. Finally, in vitro experiments confirmed the low expression of PHF21A in HCC cells. PHF21A affects the progression and prognosis of HCC, suggesting that PHF21A may play an important role in monitoring and preventing the development of HCC.

Key words: hepatocellular carcinoma, PHF21A, prognosis, immune cell infiltration, immunotherapy

Hepatocellular carcinoma (HCC) is the fifth most common cancer, the third most frequent cause of cancer-related death, and the fastest-growing cause of death due to cancer in the United States [1]. HCC remains a global health challenge, with an expected incidence of more than 1 million cases by 2025 [2]. Currently, there is no consensus on the best drugs or other therapeutic options to treat HCC. In recent years, significant progress has been made in the fields of molecularly targeted therapy and immunotherapy, and pathway-specific targeted inhibitors and immunotherapeutics have initially shown promising clinical outcomes, bringing new hope to patients with advanced HCC [3, 4]. However, despite improvements in the surveillance and treatment of HCC, many patients with unresectable or advanced tumors require systemic therapy. Therefore, it is critical to further pursue the identification of molecular mechanisms and novel therapeutic targets for HCC [5].

Histone methylation is a crucial regulator of chromatin structure, gene transcription, and the epigenetic state of cells and is involved in tumorigenesis and tumor progression [6]. PHD finger protein 21A (PHF21A) inhibits the demethylation of KDM1A-mediated histone H3 'Lys-4' *in vitro* [7, 8]. Previous studies have demonstrated an important physiological role of PHF21A in embryogenesis and tumor development, which stimulates cell proliferation and tumor progression. Bioinformatics and multimer atlas-microarray screening have identified that PHF21A mRNA expression is modulated at the translational level by GAIT-dependent hnRNP L-directed RNA switches, thereby directing multiple hypoxia-induced RNA switches and regulating the expres-

sion of certain oncogenes [6]. Functional PHF21A can be obtained through alternative RNA splicing and is localized in the cytoplasm of cancer cells where it enhances the RNA stability of CCL2, CXCL10, and TNFα through the MyD88-p38-TTP pathway, stimulating the immune response of tumor cells [7]. Importantly, the expression of PHF21A was shown to be associated with tumorigenesis and the prognosis of cancer patients. Splicing of PHF21A mRNA contributes to the progression of neuroendocrine prostate cancer and has an important role in the prognosis and treatment of gliomas [9, 10].

However, the correlation between PHF21A protein expression and the clinical features of HCC has not been investigated. In the present study, the expression of PHF21A protein in human HCC tissues was detected by immunohistochemistry, and the relationship between PHF21A expression and the clinical parameters of HCC was analyzed. Additionally, bioinformatics analyses were conducted to detect the molecular mechanism of PHF21A in HCC.

Patients and methods

Patient population and samples. The study analyzed 400 patients who underwent radical hepatic resection at Zhejiang Provincial People's Hospital from 2008 to 2015. In total, 330 cases were used to detect the difference in PHF21A expression between cancer and adjacent cancer, and 70 cases were included in the validation cohort. Adjacent normal tissues were obtained from the edge of the tumor and confirmed by pathology. The overall survival time was defined from the date of diagnosis to the end of follow-up (December 2015) or the time of death. The leading causes of death were HCC metastasis and recurrence. All specimens were fixed in formaldehyde and paraffin-embedded, and tissue microarrays (TMAs) containing 4 μm sections were prepared by Shanghai Outdo Biotech Company (Shanghai, China).

Immunohistochemistry. Immunohistochemical detection of PHF21A expression was carried out using standard protocols. TMA slides were placed in an oven for two hours at 65 °C, deparaffinized with xylene two times, and dehydrated with graded ethanol. For antigen retrieval, the slides were immersed in 0.01 M sodium citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was inhibited with 3% hydrogen peroxide for 15 min, and nonspecific binding was blocked with 1% bovine serum albumin for 15 min at room temperature. Subsequently, the slides were incubated overnight at 4 °C with anti-PHF21A rabbit monoclonal antibody (ab224612, Abcam, Cambridge, UK). The samples were then washed with PBS 3 times and incubated with biotin-labeled secondary antibody for 20 min at room temperature. Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline. Finally, the sections were counterstained with hematoxylin and dehydrated, and coverslips were mounted [11].

Evaluation of immunostaining score. The immunostaining score, reflecting staining intensity and the fraction of stained cells, was assigned independently by two pathologists under double-blind conditions. Stain intensity grading used the following criteria: 0 points for no staining, 1 point for weak or light-yellow staining, 2 points for moderate staining, and 3 points for strong or brownish-yellow staining. Slides were scored on a 0–3+ standard scoring scale with 0 being 0–5% staining, +1 being 6–25% staining, +2 being 26–50% staining, and +3 being 50% or greater staining. The total PHF21A immunostaining score was calculated by multiplying the staining intensity and percentage of positive cells and varied from 0 to 9. A staining score ≤4 was considered a low PHF21A expression level, while a staining score >4 was considered a high PHF21A expression level.

Cell culture. The American Type Culture Collection (ATCC, Manassas, VA, United States) is a supplier of hepatocellular carcinoma cell lines (MHCC97H, HCCLM3, and SMMC-7721) and human normal liver cell lines LO2 (control cells). All cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) containing 10% fetal bovine serum (FBS) and placed in an incubator at 37 °C with 5% CO_2.

Quantitative reverse transcription-polymerase chain reaction. The total RNA in the cells was extracted using the RNA-Quick Purification Kit (YISHAN Biotechnology, Shanghai, China). By using Hifair III 1st Strand cDNA Synthesis SuperMix for qPCR (gDNA Digester Plus) (Yeasen, Shanghai, China) during reverse transcription, cDNA was generated from isolated RNA and stored at −80 °C until further use. Subsequently, Hieff UNICON Universal Blue qPCR SYBR Green Master Mix (Yeasen, Shanghai, China) was used to perform quantitative fluorescence analysis of the synthesized cDNA using real-time polymerase chain reaction (RT-PCR). Fold changes of target genes were calculated by the 2−ΔΔCT method. The primer sequences were as follows: 5'-AGCTACGGAAGaACCTGATAGT-3' (forward) and 5'-GCTTTCAAGACGAGAGgTAGTGT-3' (reverse); GAPDH 5'-GGAGCGAGATCCCTCCAAAAT-3' and 5'-GGCTGTTGTCATACTTCTCATGG-3'.

Western blot analysis. The expression of PHF21A in each cell line was confirmed by western blotting. Total protein was isolated from each cell using RIPA buffer containing protease inhibitors (FUDE, Hangzhou, China), and protein concentrations were checked by using a Bradford assay kit (Ddbio Science) with BSA as a standard. Electrophoresis was then performed on SDS-PAGE gels (APPLYGEN, Beijing), and the separated proteins were electrotransferred onto PVDF membranes (Merck Millipore, USA). After blocking with WB blocking liquid (Yeasen, China) for 20 min, the cells were incubated with a primary antibody (ab224612, Abcam, Cambridge, UK), followed by a secondary antibody (ZSGB-Bio, Beijing). Finally, the results were determined by enhanced chemiluminescence (ECL).

The Cancer Genome Atlas (TCGA) database. The TCGA (https://cancergenome.nih.gov/) is a tool that improves diagnostic methods and treatment standards for cancer prevention. TCGA contains genomic, epigenetic, transcription, and proteomics data for more than 20 different cancer types. TCGA database can provide broad insights into the potential genetic abnormalities present in multiple cancer types and correlate them with specific clinical information, such as histopathology and clinical staging [12].

Functional enrichment analysis. The STRING v11 database (https://string-db.org/) was used to identify 50 genes frequently adjacent to PHF21A. The functions of PHF21A and the 50 genes significantly associated with PHF21A were then analyzed using the DAVID database (https://david.ncifcrf.gov/summary.jsp), and the biological process (BP), cell component (CC), molecular function (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) data were visualized as Scott plots using the "ggplot2" R package.

TIMER. TIMER (https://cistrome.shinyapps.io/timer/) is an online dataset server that can be used to assess the abundance of 6 types of immune infiltrates (CD8 T cells, neutrophils, CD4 T cells, dendritic cells, B cells, and macrophages) to provide a comprehensive analysis and visualization of tumor-infiltrating immune cells. TIMER can analyze and visualize the immune response to the tumor and its association with the molecular and clinical features of the tumor, helping to reveal associations between immune infiltration, gene expression, mutations, and survival characteristics in TCGA cohort [13].

Statistical analysis. The R package v3.6.1 (https://www.r-project.org/) and SPSS 16.0 software (SPSS, Chicago, IL, USA) were used for all statistical analyses. The correlation between PHF21A expression and clinicopathological features was determined using the χ^2 test. The survival curve was evaluated using the Kaplan-Meier method. To explore the relationship between PHF21A expression and the prognosis of HCC patients, univariate and multivariate analyses were carried out using Cox proportional risk regression models. A p-value <0.05 was considered to indicate statistical significance.

Results

Expression of PHF21A in HCC and normal tissues. The protein level of PHF21A in HCC and matched normal tissues was assessed by immunohistochemistry. The results showed that PHF21A was mainly located in the cytoplasm and that its expression was significantly lower in HCC tissue than in nontumor tissue ($p<0.05$, Figure 1).

Relationship between PHF21A expression and clinical features. Analysis of the correlation between PHF21A expression levels and clinicopathological parameters of HCC was then performed. The expression of PHF21A protein was significantly correlated with patient sex (p=0.032), tumor

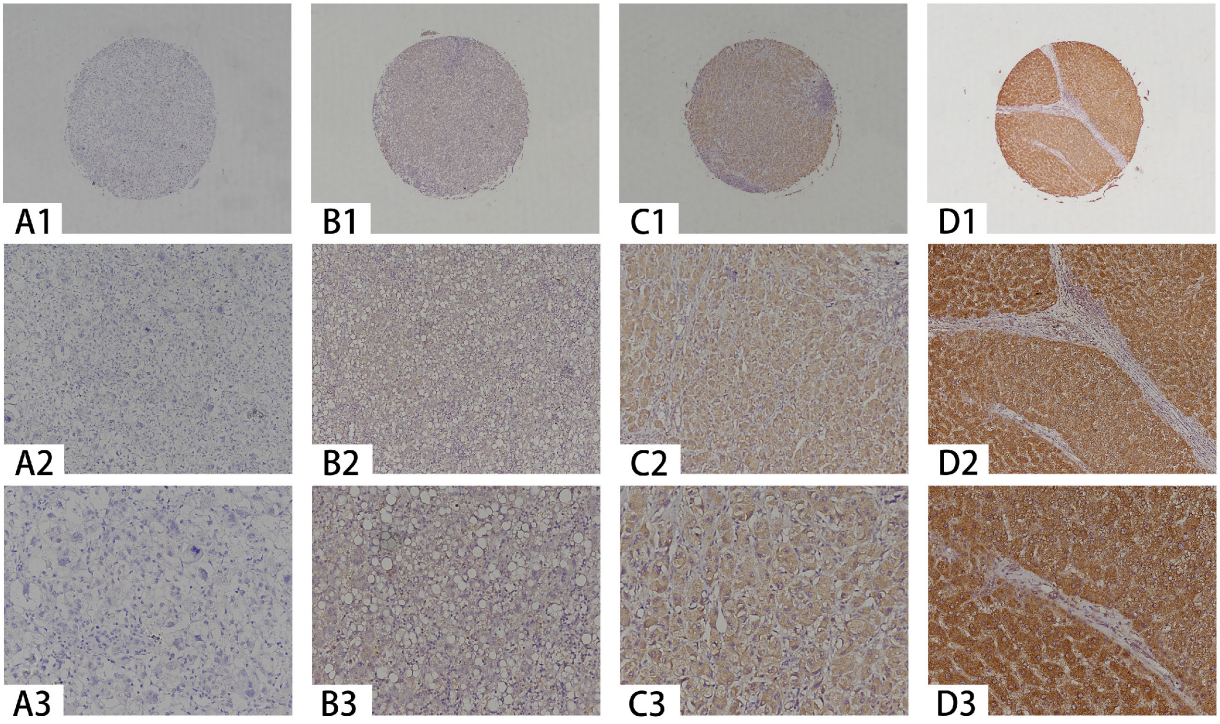

Figure 1. PHF21A immunohistochemical staining of hepatocytes adjacent to the tumor and cancerous liver tissues. A1–A3) Hepatocellular carcinoma tissue, control group (PBS). B1–B3) Low PHF21A expression in HCC tissue. C1–C3) Moderate expression of PHF21A in HCC tissue. D1–D3) High expression of PHF21A in cirrhotic tissues. Magnification: ×40 (A1–D1), ×200 (A2–D2), ×400 (A3–D3).

size (p=0.039), metastasis (p=0.026), and Edmondson grade (p=0.042) (Table 1). These findings indicated that low expression of PHF21A was associated with tumor progression in HCC.

Prognostic value of PHF21A expression in HCC. The Kaplan-Meier survival curve analysis showed that PHF21A expression was significantly correlated with the overall survival of HCC patients. The overall survival rate of patients with high PHF21A expression was markedly higher than that of patients with low PHF21A expression (p<0.01, Figure 2). Univariate Cox regression analysis of the important factors affecting the survival rate was conducted and showed that tumor size (p<0.01), Edmondson grade (p<0.01), metastasis (p<0.01), microvascular infiltration (p<0.01), and PHF21A expression (p<0.01) were correlated with the prognosis of HCC. Multivariate Cox regression analysis showed that only the Edmondson grade (p<0.01), distant metastasis (p=0.02), and PHF21A expression (p=0.03) independently predicted overall survival time (Table 2).

Western blotting and PCR of PHF21A. To verify these results, we analyzed the abnormal expression of PHF21A in HCC cells by real-time PCR and western blotting. As shown in Figures 3A and 3B, we found that the mRNA and protein expression levels of PHF21A in HCC-LM3, MHCC-97H, and SMMC-7721 cells were much lower than those in LO2 cells. These results indicated that the downregulation of PHF21A enhances the malignancy of HCC cells.

Enrichment analysis and pathway prediction of the PHF21A functional network in HCC. To elucidate the biological function of PHF21A in HCC, we used the STRING

Table 1. Relationship between PHF21A expression and clinicopathological parameters of HCC patients.

Clinical parameters	PHF21A expression			
	Low	High	χ^2	p-value
Gender			4.585	0.032*
Male	155 (77.5%)	113 (86.9%)		
Female	45 (22.5%)	17 (13.1%)		
Age (years)			1.046	0.306
<55	82 (41.0%)	46 (35.4%)		
≥55	118 (59.0%)	84 (64.6%)		
Size			4.261	0.039*
≤5 cm	108 (55.1%)	84 (66.7%)		
>5 cm	88 (44.9%)	42 (33.3%)		
Number of tumors			0.011	0.915
single	164 (82.0%)	106 (81.5%)		
multiple	36 (18.0%)	24 (18.5%)		
Metastasis			4.974	0.026*
M0	186 (94.4%)	111 (87.4%)		
M1	11 (5.6%)	16 (12.6%)		
Microvascular invasion			0.000	0.983
No	76 (50.7%)	48 (50.5%)		
Yes	74 (49.3%)	47 (49.5%)		
Edmondson grade			4.119	0.042*
I+II	132 (67.7%)	73 (56.6%)		
III	63 (32.3%)	56 (43.4%)		
Cirrhosis			0.102	0.750
negative	68 (34%)	42 (32.3%)		
positive	132 (66%)	88 (67.7%)		
HBV			0.040	0.842
absent	39 (19.8%)	24 (18.9%)		
present	158 (80.2%)	103 (81.1%)		

Notes: The total number of cases is <330 because of incomplete pathological data; bold signifies p-value <0.05.

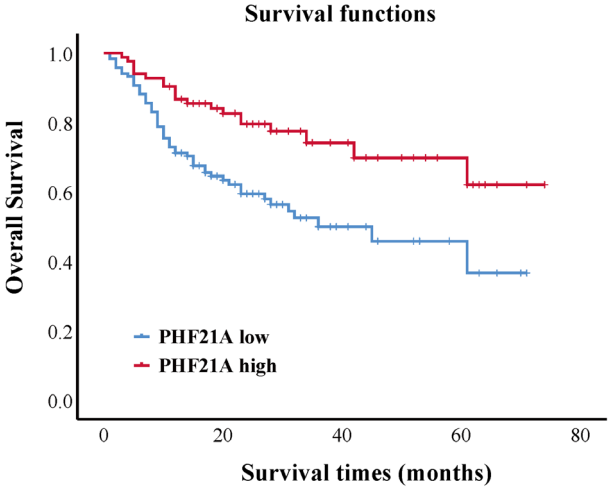

Figure 2. Kaplan-Meier survival curves showed a significant difference in prognosis between high and low PHF21A expression (p<0.05).

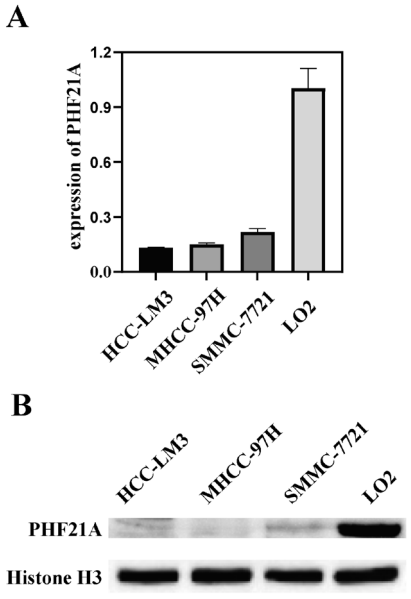

Figure 3. PHF21A mRNA and protein expression in cells. A) qRT-PCR results showed that PHF21A mRNA was low in HCC-LM3, MHCC-97H, and SMMC-7721 cells. B) The protein expression level of PHF21A in HCC-LM3, MHCC-97H, and SMMC-7721 cells was much lower than that in LO2 cells as shown in western blotting.

Table 2. Univariate and multivariate Cox regression survival analysis of clinicopathological parameters and PHF21A expression in hepatocellular carcinoma patients.

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Age	0.65	0.41–1.04	0.07		NA	
Gender	1.58	0.92–2.70	0.10		NA	
Tumor size	2.09	1.31–3.33	<0.01**	1.50	0.71–3.17	0.29
Number of tumors	1.24	0.68–2.26	0.49		NA	
Edmondson grade	2.78	1.74–4.46	<0.01**	3.51	1.61–7.66	<0.01**
Metastasis	4.82	2.55–9.11	<0.01**	3.11	1.21–7.98	0.02*
Microvascular invasion	2.11	1.25–3.56	<0.01**	0.87	0.38–1.99	0.73
HBs antigen	1.17	0.65–2.10	0.60		NA	
Cirrhosis	1.15	0.70–1.91	0.58		NA	
AFP level	2.51	1.39–4.51	<0.01**	1.83	0.86–3.90	0.12
PHF21A expression	0.43	0.26–0.73	<0.01**	1.29	0.13–2.63	0.03*

Note: bold signifies p-value <0.05. Abbreviations: HBs antigen-hepatitis B surface antigen; AFP-alpha-fetoprotein; HR-hazard ratio; CI-confidence interval

Table 3. Cox analysis of the relation between six immune cells and PHF21A mRNA expression in HCC patients' prognosis.

Variable	Coefficient	HR	95% CI	p-value	Significance
Age	0.014	1.014	0.997–1.032	0.109	
Gender Male	–0.022	0.978	0.597–1.603	0.931	
Race Black	1.143	3.136	1.149–8.559	0.026	*
Race White	–0.082	0.921	0.558–1.520	0.747	
Stage 2	0.206	1.229	0.720–2.096	0.450	
Stage 3	0.760	2.139	1.306–3.504	0.003	**
Stage 4	1.456	4.289	1.233–14.920	0.022	*
Purity	0.861	2.366	0.724–7.732	0.154	
B cell	–6.697	0.001	0.000–2.179	0.079	
CD8 T cell	–5.275	0.005	0.000–0.838	0.043	*
CD4 T cell	–8.348	0.000	0.000–0.487	0.032	*
Macrophage	8.519	5008.647	14.086–1780930.5	0.004	**
Neutrophil	–3.317	0.036	0.000–6231.247	0.590	
Dendritic	4.876	131.123	2.627–6545.879	0.015	*
PHF21A	0.145	1.156	0.776–1.724	0.476	

Note: *p<0.05; **p<0.01

database to identify 50 genes frequently adjacent to PHF21A. The correlation analysis showed that these 50 adjacent genes were significantly associated with PHF21A and its underlying molecular mechanisms. Gene set enrichment analysis (GSEA) was performed. Cytoscape 3.7.2 was used to visualize the PPI results revealing intergenic connections (Figure 4). The KEGG analysis of the DAVID database indicated that most of the relevant pathways included transcriptional misregulation in cancer, RNA polymerase, pyrimidine metabolism, cell cycle, basal transcription factors, and alcoholism (Figure 5A). In addition, Gene Ontology analysis, including analyses of the CC, BP, and MF categories, was used for functional annotation, and KEGG analysis and the ggplot2 package of R software were used for pathway enrichment analysis. "Nucleoplasm", "DNA-template transcription", and "protein binding" were the most common terms in cellular composition, biological processes, and molecular functions (Figures 5B–5D). These results suggest that PHF21A expression is associated with excessive activation of numerous oncogenes signaling pathways in HCC, particularly those controlling gene transcription.

Relationship between PHF21A expression and immune cell infiltration. For the correlation analysis of the PHF21A expression level versus (vs.) the immune cell infiltration of hepatocellular carcinoma, TIMER was used to assess the infiltration of immune cells. The relationship between immune cell infiltration in the tumor and PHF21A expression was also explored by Cox analysis, revealing the clinical relevance of HCC tumor immune subsets. The level of PHF21A expression in HCC patients was significantly associated with tumor stage 3 and macrophage infiltration (Table 3). The expression of PHF21A was positively correlated with the infiltra-

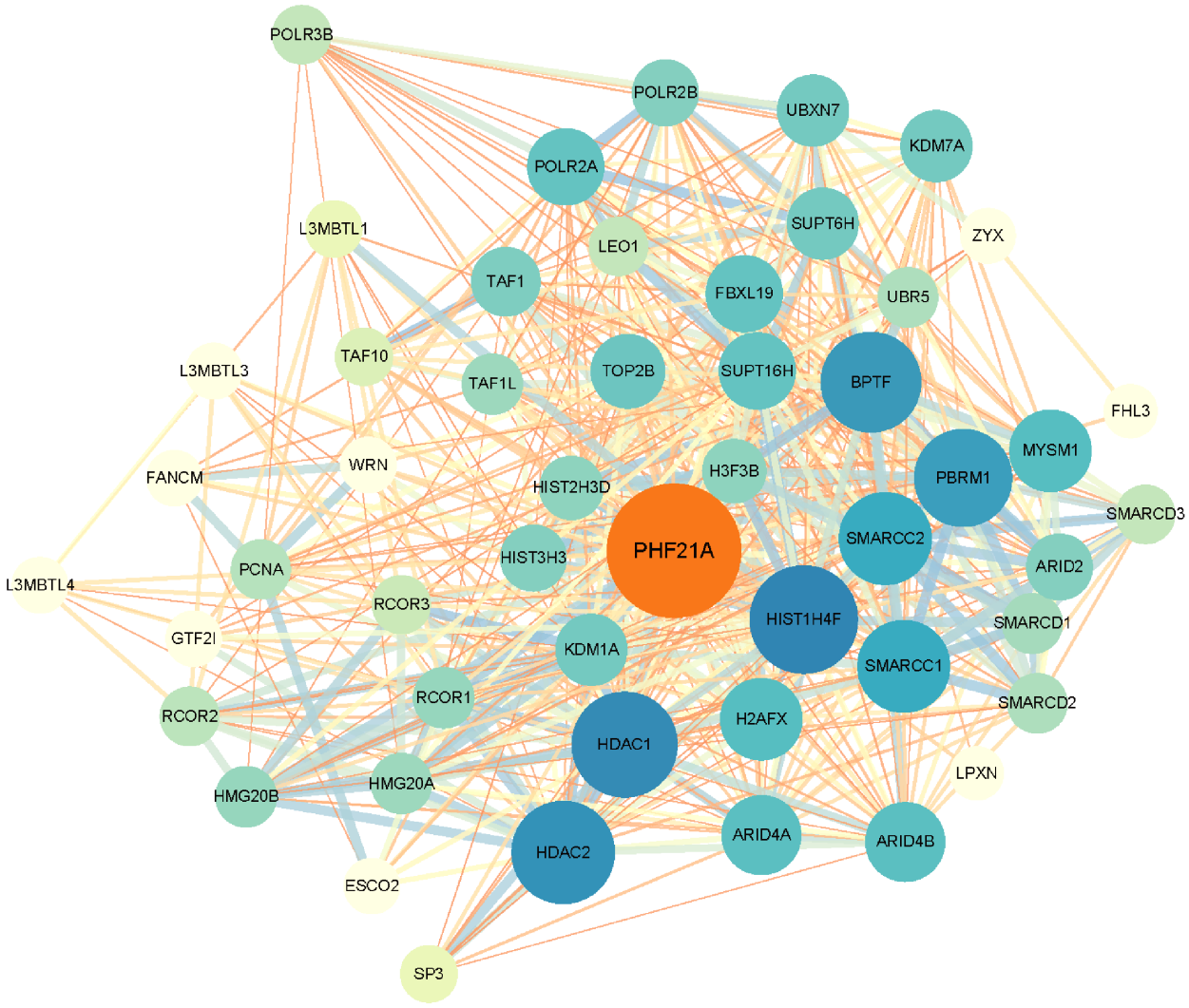

Figure 4. Protein-protein interaction network of PHF21A-related genes. PPI analysis was performed using Cytoscape 3.7.2 software.

tion of macrophages (cor=0.583, p=2.16e-32), neutrophils (cor=0.542, p=1.03e-27), and dendritic cells (cor=0.478, p=8.01e-21) (Figure 6A). Further comparison of the level of tumor immune cell infiltration with the PHF21A gene copy number showed a correlation between PHF21A copy number and B cell infiltration ($p<0.05$; Figure 6B). These results suggest that PHF21A is closely related to the activation of the immune response by HCC. Finally, TIMER analysis indicated that PHF21A is positively correlated with the expression level of PDCD1 (cor=0.354, p=2.14e-12) and CTLA4 (cor=0.309, p=1.16e-09) in HCC, indicating that PHF21A may serve as an effector/activator of the T-cell signature (Figure 6C).

Discussion

HCC is a major cause of morbidity and mortality worldwide and represents a serious public health threat [14]. In the absence of treatment, the prognosis of advanced HCC is bleak. Therefore, breakthroughs in therapeutic strategies are required [15]. In recent years, the clinical management of HCC has undergone significant changes. Although the overall prognosis of patients with this type of cancer remains poor, due to immunotherapies, the survival rate is significantly improved, especially in patients with advanced stages of the disease [16, 17]. Furthermore, even targeted therapy in the treatment of HCC patients has frequently yielded disappointing results in clinical trials due to a lack of prognostic biomarkers. Biomarkers have become powerful tools for diagnosis, prognosis evaluation, and treatment response prediction, and their use can improve patient stratification to maximize clinical benefits [18].

PHF21A is one of the core subunit proteins in the human six-subunit core-BRAF complex that has the ability to deacetylate histones. Its structure contains a finger protein

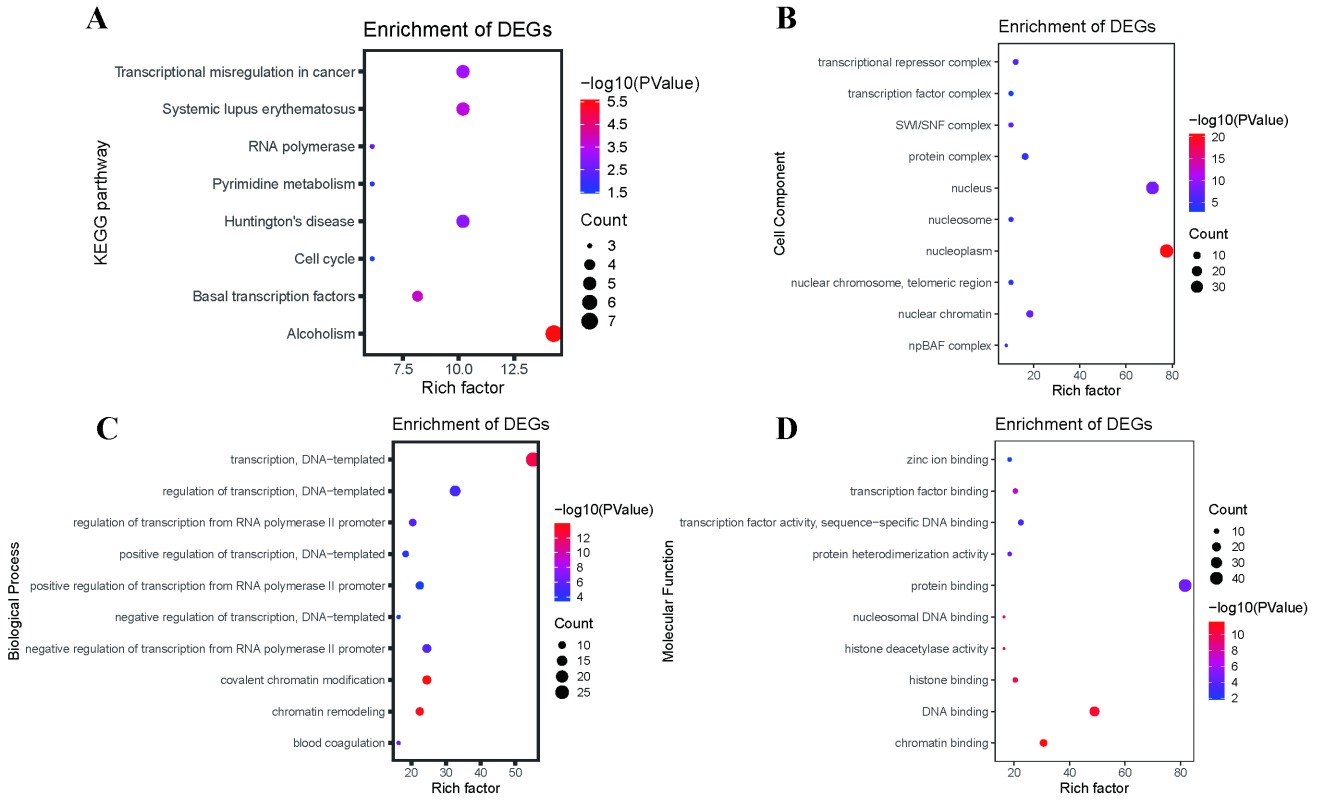

Figure 5. Genes co-expressed with PHF21A in HCC. A) KEGG enrichment analysis of PHF21A. B) Gene Ontology cell component (CC) results. C) Gene Ontology-biological process (BP) results. D) Gene Ontology molecular function (MF) results.

in the plant homolog domain, which is an essential factor for epigenetic regulation and genome maintenance [19, 20]. The C-terminal region of human PHF21A containing PHD zinc finger domains can directly bind to each of the other five components of BHC and prevent BHC-mediated transcriptional inhibition; this interaction can affect the development of HCC [21–23]. Additionally, PHF21A, as a component of the braF-histone deacetylase complex, can induce REST-dependent transcription inhibition, suppressing the transcription of target genes [24]. Chromatin immunoprecipitation assays demonstrated that PHF21A has histone deacetylation activity and can be recruited to the RE1/NRSE site of the type II sodium channel occupied by REST and synaptophysin I, thus limiting access to chromatin suppressor genes by removing acetyl groups, and this pathway implicated is in tumorigenesis [7, 25].

In the current study, we evaluated the expression of PHF21A protein in tumor tissues and adjacent normal liver tissues of up to 330 HCC patients by immunohistochemistry. The analysis of the correlation between the immunohistochemical score and clinicopathological and genetic characteristics demonstrated that PHF21A may play an important role in HCC tumorigenesis and progression. Moreover, high expression of PHF21A was negatively correlated with tumor size ($p<0.05$), metastasis ($p<0.05$), and Edmondson grade ($p<0.05$), and its low expression was closely related to a higher tumor grade in HCC patients. Patient sex also affected the expression of PHF21A in the tumor, possibly reflecting lifestyle differences between male and female patients, such as alcohol consumption. In addition, the Kaplan-Meier survival analysis documented that HCC patients with low PHF21A expression had a worse prognosis than those with high PHF21A expression. In addition, clinical samples from 70 HCC patients were used to verify the expression of PHF21A protein again. Compared with adjacent normal liver tissues, PHF21A protein expression in HCC tissues was significantly decreased (Supplementary Figure S1A), which was consistent with the Kaplan-Meier analysis results (Supplementary Figure S1B). Similarly, the PCR and WB results showed that the expression of PHF21A in HCC-LM3, MHCC-97H, and SMMC-7721 cells was lower than that in normal liver LO2 cells. These results suggest that PHF21A may act as a tumor suppressor to inhibit the occurrence and development of HCC. These results revealed that PHF21A might play an important role as a tumor suppressor, inhibiting the initiation, and development of HCC.

New prognostic biomarkers may improve the monitoring of the progression of HCC [26]. The data obtained in our

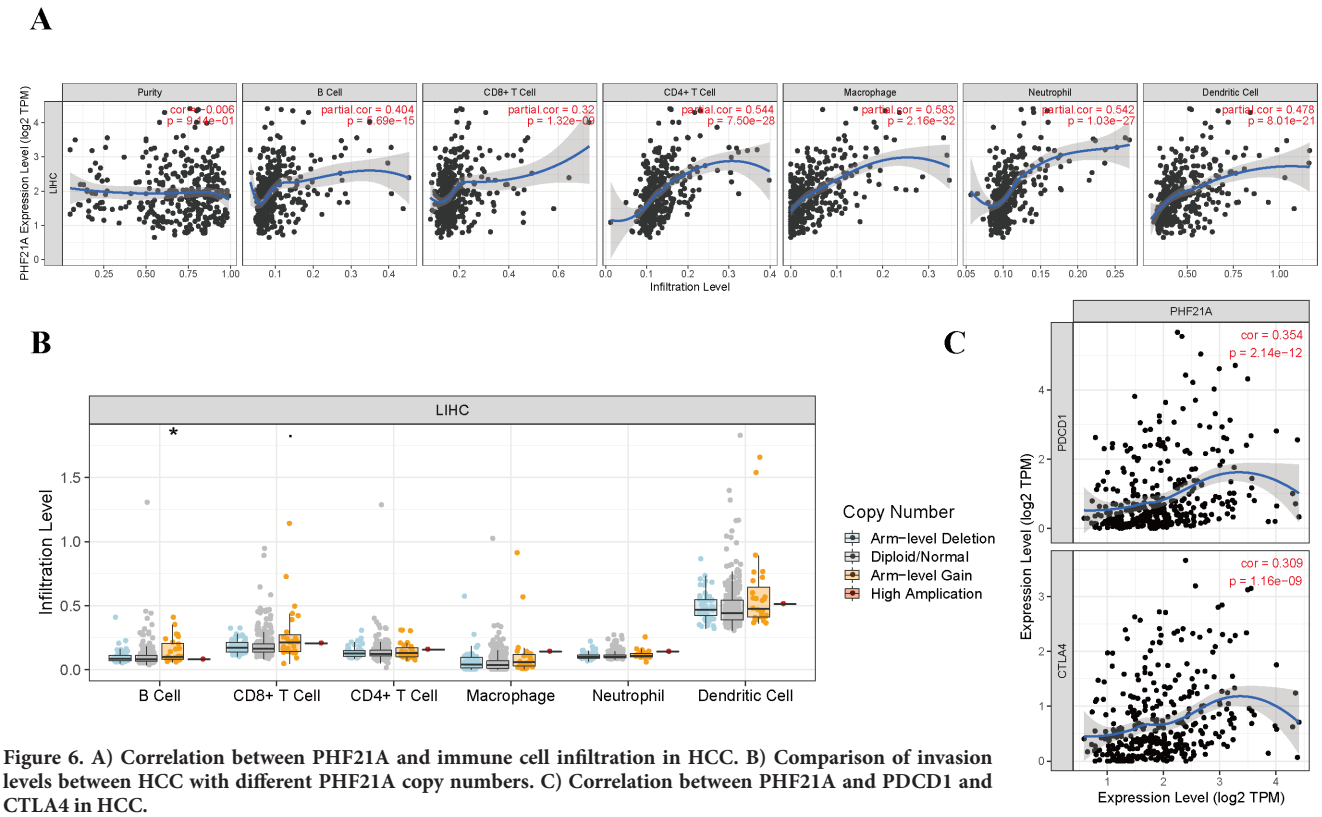

Figure 6. A) Correlation between PHF21A and immune cell infiltration in HCC. B) Comparison of invasion levels between HCC with different PHF21A copy numbers. C) Correlation between PHF21A and PDCD1 and CTLA4 in HCC.

study demonstrated that PHF21A may not only play a crucial role in inhibiting the progression of HCC but can also serve as an important molecular marker of the onset and development of HCC. However, the specific molecular mechanism by which PHF21A protein affects HCC remains to be explored. To explore the possible mechanisms that regulate the abnormal expression of PHF21A, we generated a gene-protein interaction network of PHF21A and explored it by GO and KEGG enrichment analyses. The results demonstrated that the main functions of PHF21A included protein binding, DNA binding, and chromatin binding. Importantly, PHF21A is closely related to transcriptional misregulation in cancer, affecting transcription factors and controlling nuclear and cytoplasmic transcription. The molecular pathways responsible for HCC carcinogenesis are closely related to these functions of PHF21A. However, its actual expression and clinical significance in cancer, especially liver cancer, have not been thoroughly investigated. To fill this gap, we explored multiple public datasets through bioinformatics analysis, providing a direction for studying the potential biological mechanisms of PHF21A in HCC.

The current investigation also documented that the expression of PHF21A is related to the infiltration of HCC by immune cells. The expression of PHF21A in HCC was highly positively correlated with the presence of B cells, CD8+ T cells, CD4+ T cells, macrophages, neutrophils, and dendritic cells. These results suggest that PHF21A plays an important role in regulating the tumor-induced immune response and thus can affect the prognosis of HCC. Moreover, PHF21A expression was positively correlated with the levels of PDCD1 and CTLA4 in HCC. Based on this finding, we hypothesized that low PHF21A expression in the tumor microenvironment reduces PDCD1 and CTLA4 expression on the tumor cell surface. These changes then suppress the activity of the immune system in the tumor microenvironment, resulting in a poor prognosis of HCC [27]. Although this hypothesis needs further investigation, our results strongly suggest that PHF21A is an important regulator of tumor invasion by immune cells in HCC.

Certain limitations of this study should be acknowledged. Only one hospital center was included, the number of specimens was rather restricted, and the cases might not be representative of the diverse population of HCC patients. However, the entire investigation also has a certain reference value, and we are committed to further research to establish the basis for the biological function of PHF21A in liver cancer. To verify and expand current findings, we will conduct *in vitro* and animal experiments to confirm the role of PHF21A in the occurrence and progression of HCC and its relationship with immune cell infiltration into the tumor microenvironment in the near future. We will also explore the specific mechanism and role of PHF21A in other types of cancer.

In conclusion, our study reveals the important role of PHF21A in patients with HCC. Decreased PHF21A protein levels are associated with more aggressive tumor progression and a poor prognosis of HCC, suggesting that PHF21A can be used as an important molecular marker for the development of HCC. Further analysis revealed that PHF21A may be involved in the progression of HCC through multiple signaling pathways, including those related to transcriptional misregulation and immune cell infiltration. However, precise identification of the biological function of PHF21A in HCC requires additional studies, and more in-depth research could lead to the application of PHF21A as a clinical biomarker or therapeutic target of HCC.

Supplementary information is available in the online version of the paper.

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PHF21A expression as a biomarker of hepatocellular carcinoma progression and prognosis

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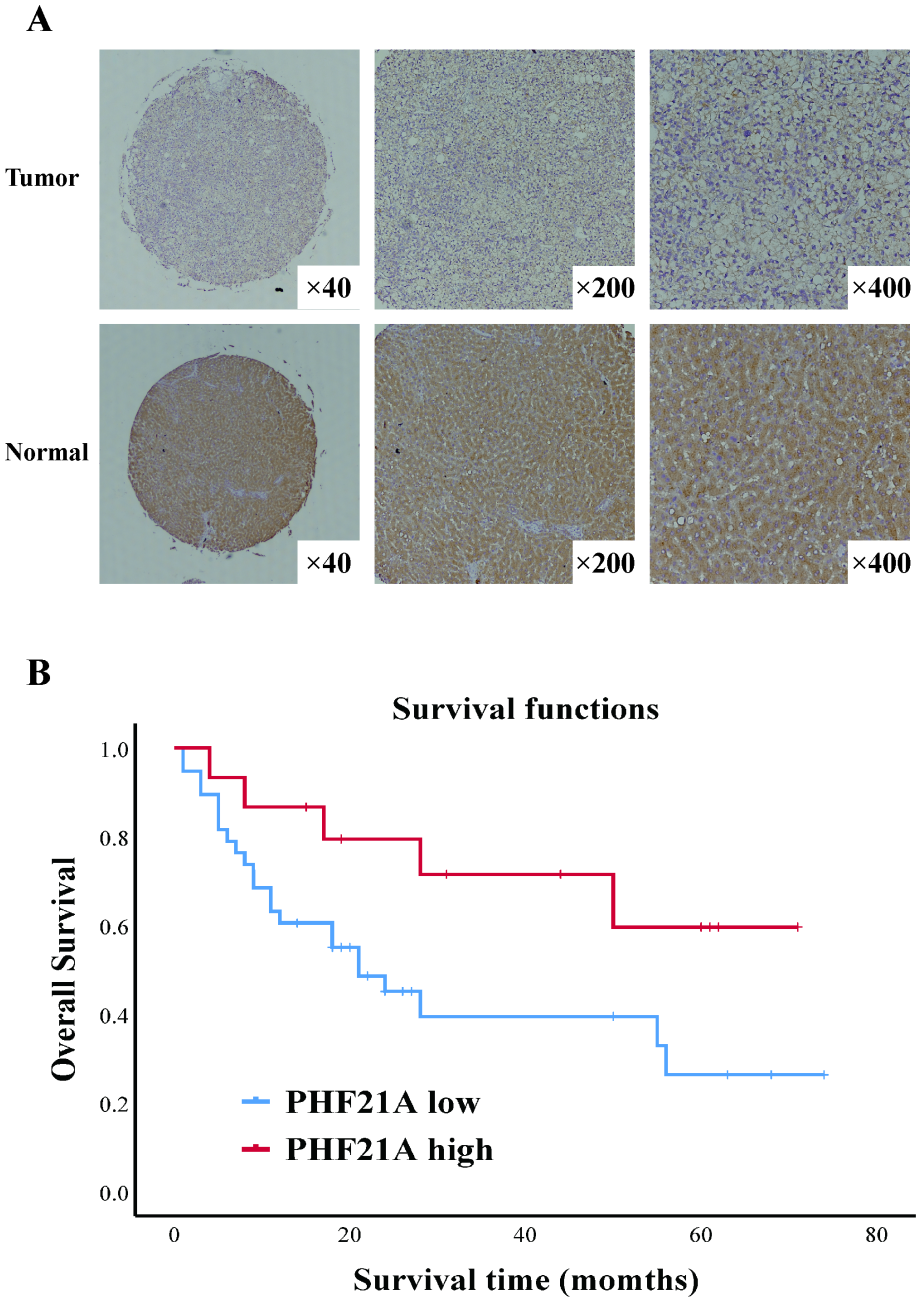
Supplementary Information



Supplementary Figure S1. PHF21A protein expression and prognostic significance in HCC TMA. A) IHC staining showed that PHF21A expression was significantly decreased in HCC tissues compared with adjacent normal liver tissues in the TMA analysis. B) Kaplan-Meier survival analysis showed that patients with low PHF21A expression had shorter overall survival ($p < 0.05$).