Analysis of the role of *POC1A* in the development and progression of hepatocellular carcinoma

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Background: Hepatocellular carcinoma (HCC) is one of the most common and deadly cancers worldwide. POC1 centriolar protein A (*POC1A*) is a gene encoding a protein that plays a key role in the centrosome, and is one of the two isoforms of POC1. To date, the expression of *POC1A* in HCC and its potential as a biomarker and tumor therapeutic target have not been examined. This study aimed to explore the effect of *POC1A* on patients with HCC and its potential mechanism.

Methods: This study investigated the role of *POC1A* in the occurrence and development of HCC. It analyzed the expression of *POC1A* in various types of HCC patients and its effect on survival using HCC patient information from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), the Human Protein Atlas (HPA), and the Hepatocellular Carcinoma Cell DataBase (HCCDB). It then explored the major enrichment pathways and gene functions of *POC1A* in HCC using the gene set enrichment analysis (GSEA) method and examined its protein-protein interactions (PPIs). Finally, it predicted the potential transcription factors (TFs) and target microRNAs (miRNAs) of *POC1A*, and analyzed the single nucleotide variation (SNV) and copy number variation (CNV) mutations of *POC1A* and the related genes in HCC, as well as their effects on immune cells.

Results: The results showed that *POC1A* was significantly overexpressed in HCC and was significantly associated with a poor prognosis. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses indicated that *POC1A* was mainly involved in the regulation of cell-cycle pathways and chromosome segregation functions. *POC1A* showed significant interactions with NUDC and PPARG, and they both had different numbers of SNV and CNV mutations in the HCC samples. In relation to immunity, the high expression of *POC1A* and its reciprocal genes may play an important role in B cells and macrophages.

Conclusions: In general, our findings suggest that *POC1A* overexpression could have an important effect on the development of HCC by regulating cell-cycle pathways, and that it could serve as a novel prognostic biomarker and a potential therapeutic target for HCC.

Keywords: Hepatocellular carcinoma (HCC); POC1 centriolar protein A (*POC1A*); bioinformatic analysis; cell cycle

Introduction

There are various types of liver cancer, including hepatocellular carcinoma (HCC), cholangiocarcinoma, and mixed cell carcinoma, among which, HCC is the most common histological type (1). HCC is also the most common type of cancer (1). Numerous studies have shown that the occurrence and development of HCC is a complex multi-step process that involves sustained inflammatory damage, including liver cell necrosis and regeneration, and fibrotic deposition, and is not related to the absolute regulation of a single gene. After their activation in the immune system, inflammatory cells release a large amount of free radicals, such as reactive oxygen species and nitric oxide, which can also cause DNA damage and mutations in oncogenes (2,3).

Depending on the stage of HCC, the treatment methods differ; however, currently, there are insufficient biomarkers for the identification and diagnosis of HCC. Choosing the optimal treatment method can maximize the therapeutic effect and improve the survival rate. Presently, the widely used biomarkers for diagnosing HCC include serum alpha-fetoprotein (AFP), core-fused AFP, and deprotection-γ-carboxyprothrombin (4). These biomarkers play a certain role in the diagnosis of HCC, but their sensitivity is limited. Thus, there is an urgent need to identify novel biomarkers with greater specificity and sensitivity.

Bioinformatics is an emerging interdisciplinary area in the fields of biology, computer science, and informatics that can acquire, store, process, and interpret biological information. In recent years, the development of high-throughput sequencing has enabled this technology to more efficiently show the significance of a large and complex amount of biological data. Bioinformatics analysis can improve our understanding of the molecular mechanisms underlying cancer pathogenesis. Combining bioinformatics analysis with various omics approaches enables more detailed research in genomics, transcriptomics, proteomics, and other fields, and the identification of key genes through differential gene analysis. The identification of significant biomarkers provides new methods for tumor diagnosis, the prediction of clinical outcomes, and targeted therapy (5). Liang *et al.* conducted a bioinformatics analysis to examine the role of *HELLS* in promoting, inhibiting, and treating different cancers, and showed that *HELLS* had immune, targeted, and cytotoxic therapeutic value (6).

Bioinformatics analysis can also provide insights into drug treatment mechanisms. Yang *et al.* adopted a network pharmacology and multi-omics approach to investigate the active pharmaceutical ingredients and their mechanism of action in the treatment of sepsis (7). A fundamental goal of cancer research is to understand the mechanisms of cell transformation. The ability to identify all the genes that drive mutations in tumors plays an important role in cancer research. Carefully designed bioinformatics methods provide an effective means for identifying driver genes (8). In addition, bioinformatics technology can also help decipher the cancer epigenome to study cancer immune evasion (9).

POC1 centriolar protein A (*POC1A*), also known as WDR51, is a gene that encodes a protein that plays a critical role in the centrosome, and is one of the two subtypes of POC1. The POC1A protein has a WD40 protein domain at the N-terminus and a coiled-coil domain at the C-terminus. Mutations in this gene may lead to the occurrence of human fibrosis (10). POC1 is related to centromere replication, length control, and cilia formation. POC1A interacts with POC1B, and is also related to centromere integrity. The loss of one POC1 protein does not significantly disrupt mitosis, but the depletion of both

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Highlight box

Key findings

- This study was the first to identify a gene [i.e., POC1 centriolar protein A (*POC1A*)] associated with the progression of hepatocellular carcinoma (HCC).
- Through a multidimensional validation analysis and a correlation analysis of the clinical parameters, this study showed that this gene significantly affects the prognosis of patients with HCC through the regulation of cell-cycle pathways and chromosome segregation functions.
- In terms of immunity, the high expression of *POC1A* and its interacting genes may play an important role in B cells and macrophages.

What is known, and what is new?

- Gene signatures and biomarkers play a crucial role in HCC prognosis and personalized medicine.
- The identification of this new potential therapeutic target provides a theoretical basis for the development of new drugs.

What is the implication, and what should change now?

- Our findings provide a rationale for the treatment of HCC and the development of new drugs against target genes.
- Further validation studies need to be conducted to assess the specific molecular mechanisms by which genes influence the prognosis of HCC patients.

proteins can lead to spindle tissue defects, resulting in inequality or unipolar spindle formation (11). POC1A plays an important role in the formation and maintenance of centrosomes, and the integrity and replication of centrosomes are crucial for the cell cycle. Thus, mutations in this gene may have adverse effects on the formation of mitotic spindles (12). In a study on the mechanism of gastric cancer, *POC1A* was identified as a significantly differentially expressed gene, and it was hypothesized that POC1A may act as a tumor suppressor in gastric cancer by regulating the cell cycle and cell growth (13). Kasavi *et al.* conducted a meta-analysis and differential analysis on a transcriptome dataset related to ovarian cancer, and identified *POC1A* as a novel candidate prognostic biomarker for ovarian cancer (14). Zhao *et al.* also found that *POC1A* was overexpressed in almost 33 types of tumors, and a gene set enrichment analysis (GSEA) showed that *POC1A* was associated with the cell cycle and immune-related pathways (15). However, there is currently no research on the role of *POC1A* in HCC.

In this study, we investigated the role of *POC1A* in the occurrence and development of HCC. First, we analyzed the expression of *POC1A* in various types of HCC patients and its effect on survival using HCC patient information from the Gene Expression Omnibus (GEO), The Cancer Genome Atlas (TCGA), the Human Protein Atlas (HPA), and Hepatocellular Carcinoma Cell DataBase (HCCDB). We then explored the major enrichment pathways and gene functions of *POC1A* in HCC using the GSEA method and examined its protein-protein interactions (PPIs). Finally, we predicted the potential transcription factors (TFs) and target microRNAs (miRNAs) of *POC1A* and analyzed the single nucleotide variation (SNV) and copy number variation (CNV) mutations of *POC1A* and related genes in HCC, as well as their effects on immune cells. We present this article in accordance with the REMARK reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2398/rc).

Methods

Analysis of POC1A pan-cancer expression

The differential expression of the *POC1A* gene between pan-cancerous tumor tissues and adjacent normal tissues was analyzed using the DiffExp module in the Tumor Immune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer/) database. The statistical significance of the differential

expression was assessed using the Wilcoxon test.

Expression and prognostic analysis of POC1A in HCC

GEO2R was used to analyze the expression of POC1A in the liver cancer datasets GSE159220 and GSE55758. GSE159220 comprised three HCC samples and three normal samples, while GSE55758 comprised eight HCC samples and eight adjacent samples. A log fold change ≥|0.5|, and a P value <0.05 were set as the analysis criteria. The expression and prognosis of the *POC1A* gene in liver hepatocellular carcinoma (LIHC) samples from TCGA database were analyzed and validated using the Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/) database. The normal samples were matched with LIHC sample data and Genotype-Tissue Expression (GTEx) sample data. At the prognostic level, the overall survival (OS) and disease-free survival (DFS) rates were analyzed using the quartile cut-off method.

Analysis of POC1A expression and prognosis in the HCCDB

The HCCDB (http://lifeome.net/database/hccdb/about.html), which is a liver cancer–specific analysis database, was used to analyze the expression of *POC1A* in liver cancer patient samples. The HCCDB contains 15 common liver cancer expression datasets, with up to 4,000 clinical samples, and was developed as a one-stop online resource to explore the expression of liver cancer genes through a user-friendly interface. It can also show the specific role of genes in liver cancer patients.

Expression characterization of POC1A in LIHC patient samples

The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN; http://ualcan.path.uab.edu/) database was used to analyze the expression of *POC1A* in liver cancer patients of different ages, genders, races, and cancer stages. It enabled us to collect potentially valuable information and data for further targeted analysis and problem solving. We used the Sangerbox database (http://vip.sangerbox.com/home.html) to download the unified and standardized LIHC dataset from the University of California, Santa Cruz (UCSC; https://xenabrowser.net/) database, and further extracted the expression data of the ENSG00000164087 (*POC1A*) gene in each sample. We

screened the samples from the Primary Blood Derived Cancer – Peripheral Blood and Primary Tumor database, and performed $\log_2(x+0.001)$ transformation for each expression value to investigate the expression of *POC1A* at different tumor stages.

Expression confirmation of POC1A at the protein level

To analyze the expression of *POC1A* in normal liver samples and liver cancer samples, we used the HPA (https://www.proteinatlas.org/) database to analyze the staining degree of *POC1A* in tissue sections.

Cell culture, RNA extraction, and qRT-PCR

The human normal hepatocyte cell line LO2 (Servicebio Technology Co., Ltd., Wuhan, China) was cultured in Roswell Park Memorial Institute 1640 medium, and the hepatoma cell line HepG2 (Procell Life Science & Technology Co., Ltd., Wuhan, China) was cultured in Dulbecco's Modified Eagle Medium (Hyclone, Thermo Fisher Scientific Inc., Waltham, MA), supplemented with 10% fetal bovine serum and antibiotics (10,000 U/mL penicillin and 10 mg/mL streptomycin) (Procell Life Science & Technology Co., Ltd.). The cells were incubated in a humidified atmosphere containing 5% carbon dioxide at 37 ℃. The total RNA was isolated from the intestinal tissues using the TRIzol Reagent Kit (ComWin Biotech Co., Ltd., Beijing, China) and then subjected to quantitative real-time polymerase chain reaction (qRT-PCR). RNA (1 μg) was reverse transcribed using the PrimeScript™ RT reagent Kit with genomic DNA Eraser (Takara Bio Inc., Ltd., Japan). For the messenger RNA (mRNA) analyses, the resulting complementary DNAs were analyzed in triplicate using SYBR® Premix Ex Taq™ II (Takara Bio Inc., Ltd.) on the Applied Biosystems 7500 qRT-PCR system. The relative mRNA expression level was calculated by $2^{-\triangle\triangle Ct}$, and the expression values were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers. The following qRT-PCR primers were used: GAPDH 5'-CAGGAGGCATTGCTGATGAT-3', 3'-GAAGGCTGGGGGCTCATTT-5'; and *POC1A* 5'-GCATATCAACTGGGTCCGCT-3', 3'-CACATAGGTGACAAAGCCGC-5'.

HCC-specific GO and KEGG analyses of POC1A

The LinkedOmics (http://www.linkedomics.org/login.

php) database was used for the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses and the related GSEA of *POC1A*. The patient sample was taken using the LIHC RNA sequencing sample from the University of North Carolina at Chapel Hill (UNC) institution. The LinkFinder module and a Pearson correlation test were used to analyze the genes that were significantly correlated with *POC1A*. The LinkInterpreter module and the GSEA algorithm was used for the GO and KEGG analyses. A false discovery rate (FDR) P value ≤0.05 was considered statistically significant.

POC1A major enrichment pathway-related genes for the PPI, miRNA, and TF prediction analyses

The analysis of POC1A-related PPIs, miRNAs targets and TFs were performed using the Network Analyst 3.0 (https://www.networkAnalyst.ca/) database. The significantly correlated *POC1A* pathway genes obtained from the KEGG analysis were included as predictive genes in the PPI analysis. The PPIs of POC1A were analyzed using liver samples from the DifferentialNet database, and the *POC1A*-associated target miRNAs were analyzed using the miRTarBase8.0 database. The *POC1A*-associated TFs were analyzed using the JASPER database. The website defaults were selected as the parameters.

Characterization of POC1A-interacting gene alterations

The SNV and CNV mutations of the *POC1A* gene in LIHC were analyzed using the Gene Set Cancer Analysis (GSCA; http://bioinfo.life.hust.edu.cn/GSCA/#/) database.

Prognostic analysis of the POC1A reciprocal genes in patients with HCC

The GEPIA2 (http://gepia2.cancer-pku.cn/#index) database was used to draw a prognostic heatmap of the *POC1A* interaction genes in the liver cancer patient samples. The median was chosen as the grouping threshold, and a P value <0.05 indicated that the gene had a significant effect on patient prognosis.

mRNA correlation analysis of the POC1A interaction genes in patients with HCC

The GEPIA2 database was used to analyze the correlation between the mRNA and the POC1A interaction genes in

the LIHC samples. The Spearman algorithm was used to perform the paired-gene expression correlation analysis of a given TCGA and GTEx expression dataset. A P value <0.05 indicated a significant correlation between two genes.

Analysis of POC1A and the interaction genes in various immune cells

The uniformly standardized LIHC dataset was downloaded from the UCSC (https://xenabrowser.net/) database, and the samples were screened from the Primary Blood Derived Cancer-TCGA-Acute Myeloid Leukemia, Primary Tumor, Metastatic-TCGA-Skin Cutaneous Melanoma, Primary Blood Derived Cancer-Bone Marrow, Primary Solid Tumor, and Recurrent Blood Derived Cancer-Bone Marrow datasets. We performed a $\log_2(x+0.001)$ transformation for each expression value, and we extracted the gene expression profile of each tumor separately from these datasets, mapped each expression profile to GeneSymbol, and used the R package ESTIMATE to calculate the stromal, immune, and ESTIMATE scores for each patient with LIHC tumors based on gene expression (16). The relationship between the expression of *POC1A*, the major interacting genes, and the grade of immune infiltration of various immune cell types in the HCC samples was analyzed by the TIMER database. The results showed the Rho values and statistical significance of some Spearman correlations corrected for purity. A P value <0.05 indicated a significant correlation between two genes. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Statistical analysis

The *t*-test was used for the intergroup analysis. Survival curves were generated using the Kaplan-Meier method. A P value <0.05 was considered statistically significant.

Results

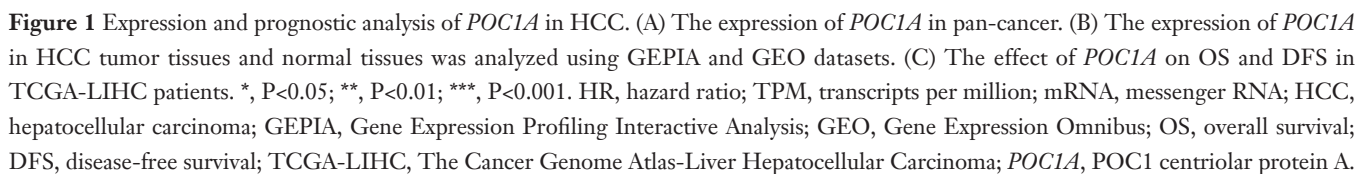### POC1A expression was significantly upregulated in multiple cancers

First, we examined the expression of *POC1A* in the pan-cancer samples. The TIMER database was used to perform the differential gene analysis of the *POC1A* gene in pan-cancer samples (*Figure 1A*). The results revealed that the *POC1A* gene was highly expressed in various cancer tissues compared to normal tissues.

POC1A was highly expressed in HCC and significantly correlated with a poor OS rate

We selected HCC for further study and explored the expression of *POC1A* using the GEO dataset. The results showed that the expression of *POC1A* was significantly higher in TCGA-LIHC tumor samples than the normal samples (*Figure 1B*). Next, we analyzed the OS and DFS rates of LIHC patients based on *POC1A* expression. The Kaplan-Meier analysis showed that the LIHC patients with high *POC1A* expression had a significantly lower OS rate (log-rank P=0.001) and DFS rate (log-rank P=0.03) than those with low *POC1A* expression. The hazard ratio (HR) results showed that the risk of the *POC1A* high-expression group was significantly higher than that of the *POC1A* low-expression group in terms of both OS (HR =2.3, P=0.002) and DFS (HR =1.6, P=0.03). Thus, *POC1A* could serve as an independent prognostic risk factor for HCC (*Figure 1C*). *Table 1* sets out the results for the analysis of the relationship between *POC1A* expression in the LIHC tissues and the clinicopathologic characteristics of the patients. The high expression of *POC1A* significantly affected the AFP level, histologic grade, and pathologic tumor (T) stage of the patients.

Cross-validation in the HCC-specific HCCDB

To cross-validate the accuracy of the conclusion that *POC1A* is highly expressed in HCC and significantly correlated with a poor OS rate, the HCCDB, a HCC-specific database, was used to further analyze the differential expression levels of the *POC1A* gene. The results of 10 different HCC datasets showed that in all datasets, the expression levels of the *POC1A* gene in the HCC tissue was significantly higher compared to that in the adjacent liver tissue (*Figure 2A*). In HCCDB18, there was a significant difference between the high and low expression of *POC1A* in the LIHC and adjacent tissues. The observation graph (*Figure 2B*) clearly showed that the survival rate of patients with low *POC1A* expression was higher than that of patients with high expression for the follow-up period, and the difference in the survival rate between the groups gradually increased as the survival years increased. However, in the HCCDB15 dataset, the effects of the *POC1A* expression on the prognosis of the diseased patients were the same, but the difference was not statistically significant (P=0.17). Even in the adjacent tissues with a long-term follow-up period of 2 years, the survival rate of patients with high *POC1A*

Figure 1 Expression and prognostic analysis of *POC1A* in HCC. (A) The expression of *POC1A* in pan-cancer. (B) The expression of *POC1A* in HCC tumor tissues and normal tissues was analyzed using GEPIA and GEO datasets. (C) The effect of *POC1A* on OS and DFS in TCGA-LIHC patients. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. HR, hazard ratio; TPM, transcripts per million; mRNA, messenger RNA; HCC, hepatocellular carcinoma; GEPIA, Gene Expression Profiling Interactive Analysis; GEO, Gene Expression Omnibus; OS, overall survival; DFS, disease-free survival; TCGA-LIHC, The Cancer Genome Atlas-Liver Hepatocellular Carcinoma; *POC1A*, POC1 centriolar protein A.

Table 1 Relationship between expression of *POC1A* and clinicopathological features in 374 patients with liver hepatocellular carcinoma

Characteristics	Low expression of *POC1A* (n=187)	High expression of *POC1A* (n=187)	P
Gender, n (%)			0.22
Female	66 (17.6)	55 (14.7)	
Male	121 (32.4)	132 (35.3)	
Age (years), n (%)			0.38
≤60	84 (22.5)	93 (24.9)	
>60	102 (27.3)	94 (25.1)	
Alpha-fetoprotein, n (%)			0.009
≤400 ng/mL	116 (41.4)	99 (35.4)	
>400 ng/mL	23 (8.2)	42 (15)	
Grade, n (%)			0.009
G1	29 (7.9)	26 (7)	
G2	98 (26.6)	80 (21.7)	
G3	56 (15.2)	68 (18.4)	
G4	1 (0.3)	11 (3)	
Pathologic T stage, n (%)			0.03
T1	101 (27.2)	82 (22.1)	
T2–T4	83 (22.4)	105 (28.3)	
Overall survival event, n (%)			0.009
Alive	134 (35.8)	110 (29.4)	
Dead	53 (14.2)	77 (20.6)	

The statistical analyses were performed using the Chi-square test. *POC1A*, POC1 centriolar protein A.
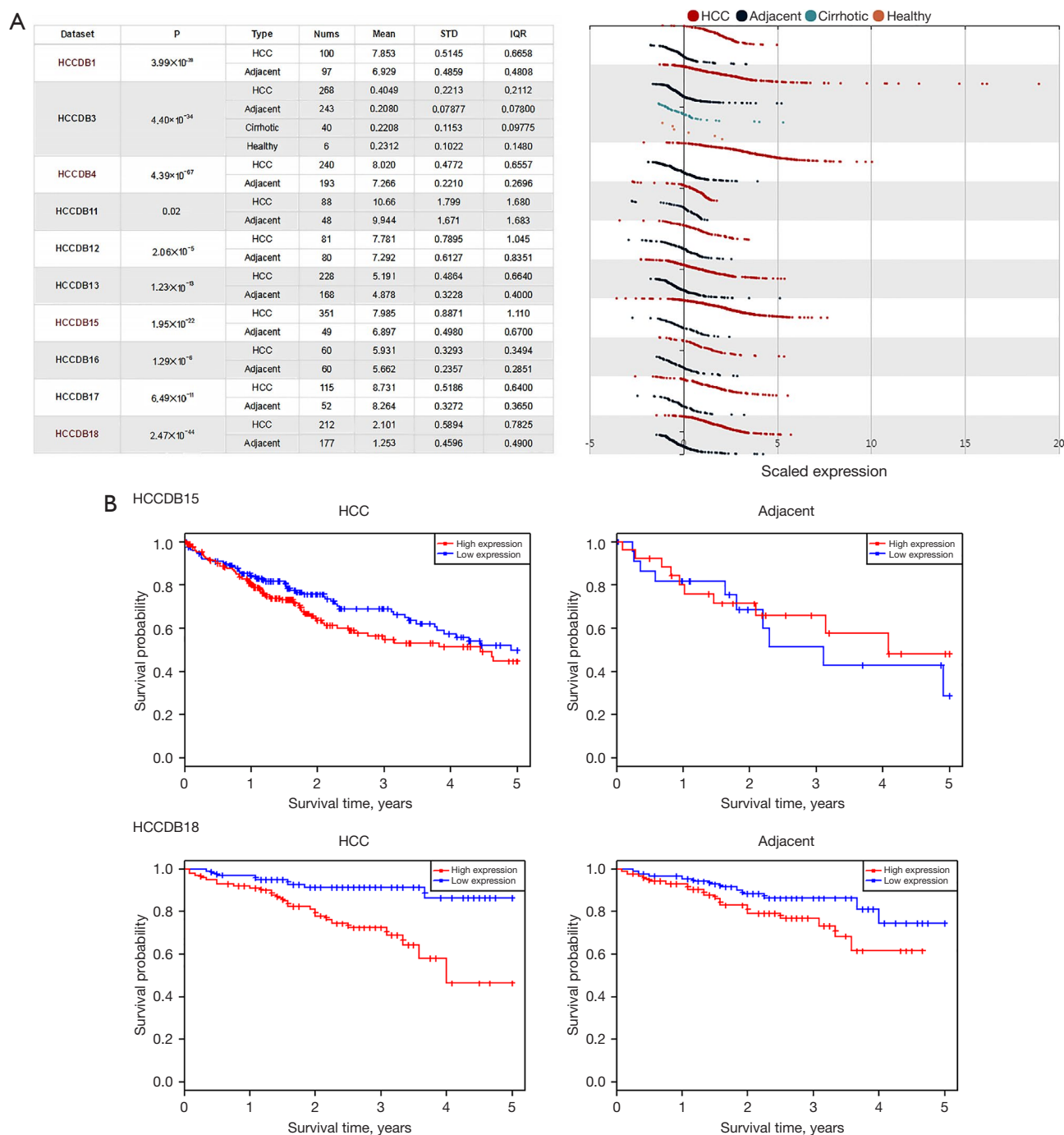
expression was higher than that of patients with low *POC1A* expression (*Figure 2B*).

### Characterization of POC1A expression in HCC

To investigate the expression of *POC1A* in different subtypes of LIHC patients, we compared the expression of *POC1A* in the LIHC tissue samples of patients with different clinical and pathological parameters. As *Figure 3A* shows, compared with the normal group, the expression level of *POC1A* was significantly increased in the HCC group. The expression levels of *POC1A* in both the male and female HCC patients were higher than those of control patients, and the expression levels were higher in males than females (*Figure 3B*). The expression level of *POC1A* in the HCC patients aged ≥21 years was generally higher than that of the control patients, and the expression

level of *POC1A* in the HCC patients aged 41–60 years was also significantly higher than that in the patients aged 61–80 years (*Figure 3C*). The expression levels of *POC1A* were all higher in the Caucasian, African-American, and Asian HCC patients than the normal patients. The expression of *POC1A* was also significantly higher in the tissue of Asian LIHC patients than the tissue of Caucasian LIHC patients (*Figure 3D*).

In terms of tumor staging, the expression level of *POC1A* in the HCC patients was positively correlated with the first three stages of the tumor, but not with the fourth stage (*Figure 3E,3F*). In the tumor, node, metastasis (TNM) staging criteria, T refers to the condition of the primary tumor, which is sequentially represented by T1–T4 as the volume of the tumor increases and the extent of adjacent tissue involvement increases. The results showed that *POC1A* was significantly correlated with the progression

A

Dataset	P	Type	Nums	Mean	STD	IQR
HCCDB1	3.99×10^{-28}	HCC	100	7.853	0.5145	0.6658
		Adjacent	97	6.929	0.4859	0.4808
HCCDB3	4.40×10^{-34}	HCC	268	0.4049	0.2213	0.2112
		Adjacent	243	0.2080	0.07877	0.07800
		Cirrhotic	40	0.2208	0.1153	0.09775
		Healthy	6	0.2312	0.1022	0.1480
HCCDB4	4.39×10^{-67}	HCC	240	8.020	0.4772	0.6557
		Adjacent	193	7.266	0.2210	0.2696
HCCDB11	0.02	HCC	88	10.66	1.799	1.680
		Adjacent	48	9.944	1.671	1.683
HCCDB12	2.06×10^{-5}	HCC	81	7.781	0.7895	1.045
		Adjacent	80	7.292	0.6127	0.8351
HCCDB13	1.23×10^{-13}	HCC	228	5.191	0.4864	0.6640
		Adjacent	168	4.878	0.3228	0.4000
HCCDB15	1.95×10^{-22}	HCC	351	7.985	0.8871	1.110
		Adjacent	49	6.897	0.4980	0.6700
HCCDB16	1.29×10^{-6}	HCC	60	5.931	0.3293	0.3494
		Adjacent	60	5.662	0.2357	0.2851
HCCDB17	6.49×10^{-11}	HCC	115	8.731	0.5186	0.6400
		Adjacent	52	8.264	0.3272	0.3650
HCCDB18	2.47×10^{-44}	HCC	212	2.101	0.5894	0.7825
		Adjacent	177	1.253	0.4596	0.4900

B HCCDB15

HCCDB18

Figure 2 Cross-validation of the prognosis and expression of *POC1A* in HCC using the HCCDB. (A) Analysis of *POC1A* expression in multiple datasets in HCCDB. (B) In the HCCDB15 and HCCDB18 datasets, the high expression of *POC1A* led to a decrease in patient survival. STD, standard deviation; IQR, interquartile range; HCC, hepatocellular carcinoma; HCCDB, Hepatocellular Carcinoma Cell DataBase; *POC1A*, POC1 centriolar protein A.
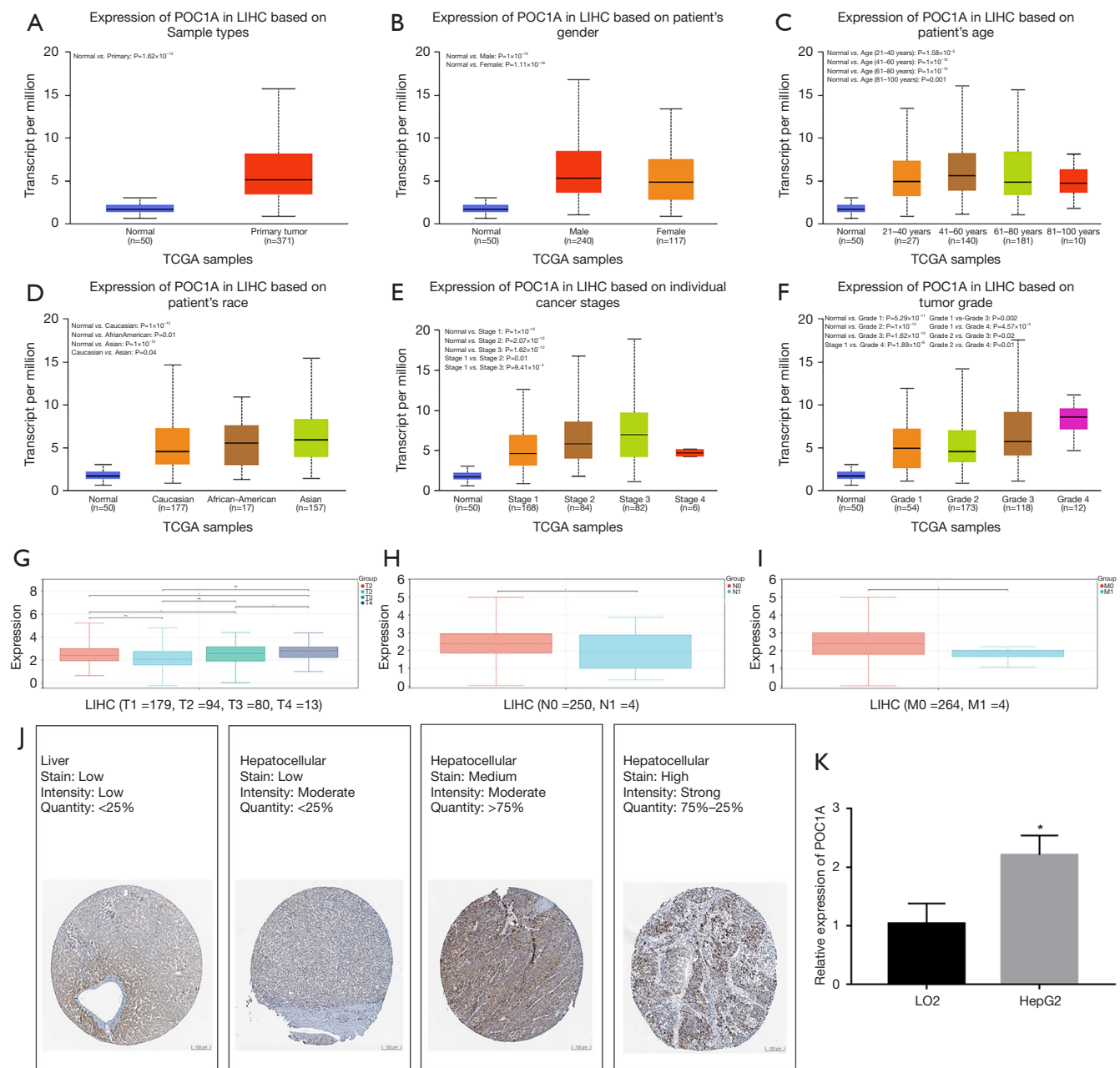
Figure 3 Analysis of the expression of *POC1A* in various classifications of HCC patients, as well as in HCC and liver cancer cell lines. (A) Comparison of *POC1A* between cancer tissue samples and normal tissue samples. (B) Comparison of the expression of *POC1A* in different genders. (C) Comparison of the expression of *POC1A* in different age groups. (D) The expression levels of *POC1A* were all higher in the Caucasian, African-American, and Asian HCC patients than the normal patients. The expression of *POC1A* was significantly higher in LIHC tissue samples from Asian patients than LIHC tissue samples from Caucasian patients. (E) The expression of *POC1A* in individual cancer stages. (F) The expression of *POC1A* in different transistor grades. (G) The expression of *POC1A* in tumor (T) stage. (H) The expression of *POC1A* in node (N) stage. (I) The expression of *POC1A* in metastasis (M) stage. (J) Analysis of POC1A expression in normal liver tissue and cancer tissue samples from patients with hepatocellular carcinoma using immunohistochemical methods in the Human Protein Atlas database. Liver sample: https://www.proteinatlas.org/ENSG00000164087-POC1A/tissue/liver#img, hepatocellular samples: https://www.proteinatlas.org/ENSG00000164087-POC1A/pathology/liver+cancer#img. Scale bar: 100 µm. (K) Analysis of *POC1A* mRNA expression in LO2 and HepG2 cell lines by qPCR assay. *, P<0.05; **, P<0.01; -: no significance. TCGA, The Cancer Genome Atlas; HCC, hepatocellular carcinoma; LIHC, liver hepatocellular carcinoma; mRNA, messenger RNA; qPCR, quantitative polymerase chain reaction; *POC1A*, POC1 centriolar protein A.

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of staging, but there was no such significant correlation in terms of the N and M staging (*Figure 3G-3I*).

Immunohistochemical (IHC) staining data obtained from the HPA database showed the protein expression of POC1A (*Figure 3J*). Notably, the expression level of the POC1A protein was higher in the HCC tissues than the normal liver tissues. The mRNA expression levels of *POC1A* were examined using the normal hepatocyte cell line LO2 and the hepatoma cell line HepG2. As *Figure 3K* shows, the mRNA expression of *POC1A* in the HepG2 cell line was significantly higher than that in the LO2 cell line, which was consistent with the results of our analysis.

GO and KEGG analyses of POC1A in HCC

To explore the functions and possible regulatory role of *POC1A* in HCC, the LinkedOmics database was used to explore the co-expressed genes of *POC1A*. The results showed that there were 19,922 co-expressed genes with FDR values ≤0.05 and 500 times statistical analysis simulations were conducted. The co-expressed genes of *POC1A* is presented in the form of a volcanic map (*Figure 4A*). The top 50 genes that were positively or negatively correlated with *POC1A* were selected and presented in a heat map (*Figure 4B*). The heat map used the Z-score to standardize the gene expression values and displays the gene expression patterns clustered by sample grouping. GO and KEGG enrichment analyses were conducted of the co-expressed genes. The GO analysis showed that the related genes were mainly involved in chromosome separation, non-coding RNA processing, ribonucleoprotein complex biogenesis, ribosomal RNA metabolism, organelle division, DNA replication, and cell-cycle checkpoints. The KEGG analysis showed that the related genes were mainly enriched in ribosome, the cell cycle, splicing bodies, DNA replication, pyrimidine metabolism, and other processes (*Figure 4C*). These results suggest that POC1A may act as a part of the centrosome to affect DNA replication, regulate the cell cycle, and thus affect HCC cell proliferation, promoting tumor occurrence and development.

Prediction of interacting proteins of POC1A and potential miRNAs and TFs

We selected the cell-cycle pathway for further analysis. *Figure 5A* shows the GSEA enrichment map of *POC1A* in the cell-cycle pathway. We then conducted a PPI analysis of the enriched genes of this pathway. The results are shown in

Figure 5B-5D. In this pathway, POC1A interacted with the NUDC protein. Next, we further predicted the potential miRNAs and TFs of POC1A, and the results showed that *POC1A* may be regulated by miRNAs such as *hsa-mir-148a-3p*, *hsa-mir-10a-5p*, and *hsa-mir-10b-5p*. Genes such as *FOXC1*, *YY1*, and *NFIC* may be the TFs of *POC1A*. The specific results are provided in the Figure S1.

Expression and prognostic mapping of POC1A and interacting genes in HCC

To preliminarily explore the possible role of the *POC1A* interaction genes in HCC, we first used TCGA-LIHC patient samples from the GEPIA database to examine the expression of the POC1A interaction genes and to predict the *POC1A* TFs. We then plotted a prognosis map. The results are shown in *Figure 6A,6B*. Notably, the expression of the *PPARG*, *NUDC*, *YY1*, and *HNF4A* genes was found to be increased in the LIHC samples, but there was no significant difference between normal samples and tumor samples. The prognosis map showed that the high expression of *PPARG* and *NUDC* significantly affected the OS rate of the LICH patients. We then analyzed the correlation between the *POC1A* interacting genes and patient prognosis in the LIHC patient samples, and the results are shown in *Figure 6C*. Notably, *POC1A* was significantly correlated with *NUDC* (P=5.8×10⁻¹³) and *PPARG* (P=8.7×10⁻⁹). *Figure 6D* shows the expression of different genotypes of *POC1A*, *NUDC*, and *PPARG* in the LIHC samples.

Analysis of genetic changes in POC1A and interacting genes, and analysis of multiple immune cell types in LIHC samples

To analyze the SNV and CNV changes in *POC1A* and the genes interacting with *POC1A* in the LIHC samples, we used the GSCA database. In the LIHC patient samples, *POC1A*, *NUDC*, and *PPARG* each had SNV mutations of varying degrees. Among the SNVs, missense mutations and single nucleotide polymorphisms, and Cytosine > Thymine were the main types of variants (*Figure 7A,7B*). Among the CNVs, homozygous CNVs and heterozygous CNVs were also present in *POC1A*, *NUDC*, and *PPARG*. Among the heterozygous CNVs, *POC1A*, *NUDC*, and *PPARG* were found to have different levels of deletion and amplification (*Figure 7C,7D*).

To explore *POC1A* and the related genes in the immune cells of the LIHC patients, we first calculated the Pearson
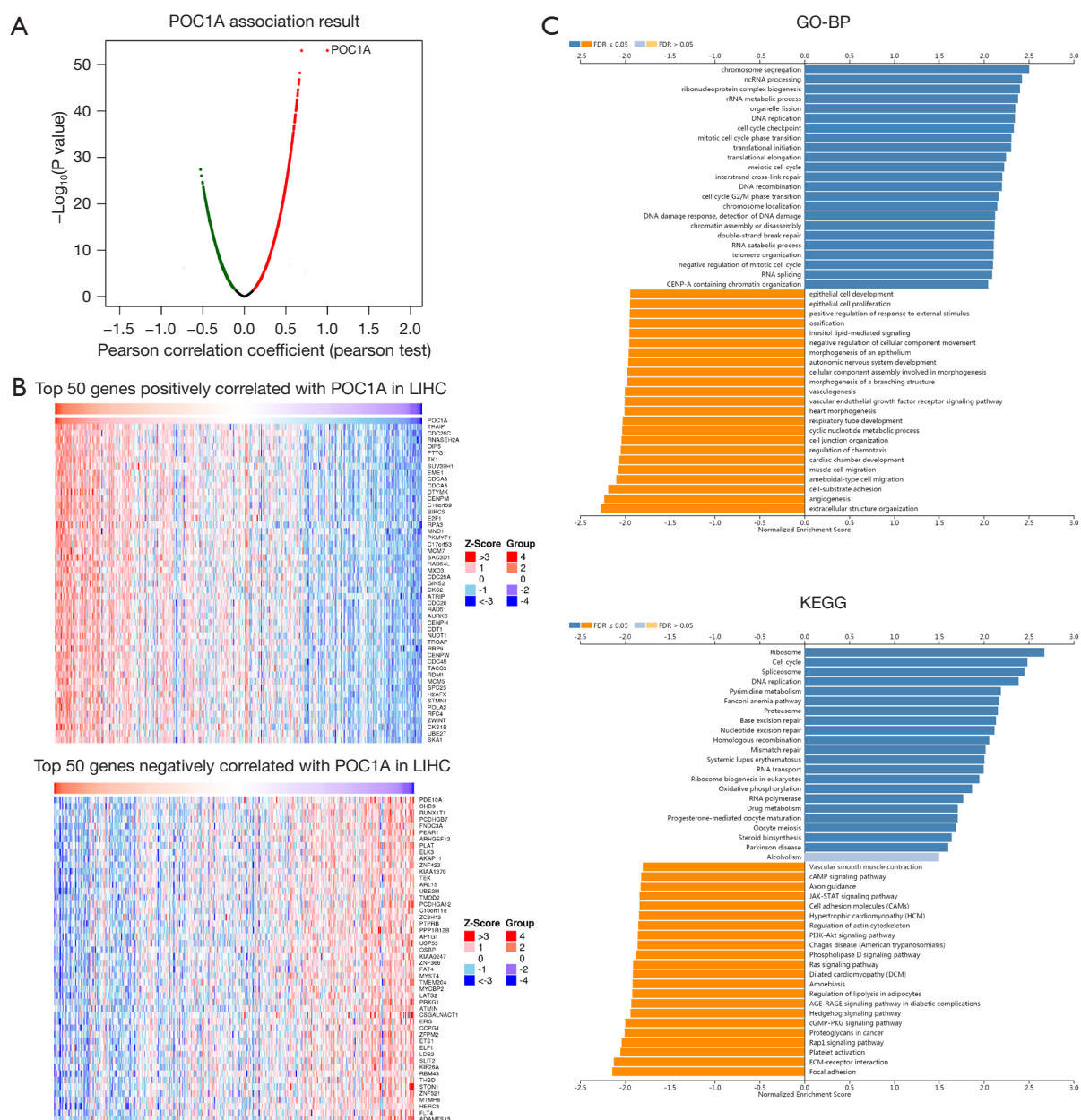
Figure 4 Analysis of the cellular functions and enrichment pathways affected by *POC1A* in liver cancer samples. (A) The gene maps related to *POC1A*; the red dots represent significantly upregulated genes, while the green dots represent significantly downregulated genes, and the black dots represent the genes that are not differentially expressed. (B) The gene heatmap ranking the top 50 genes correlated with *POC1A* in LIHC. (C) LIHC samples were used in the analysis of the GO-BPs and the KEGG analysis of *POC1A*. LIHC, liver hepatocellular carcinoma; GO, Gene Ontology; BP, biological process; KEGG, Kyoto Encyclopedia of Genes and Genomes; *POC1A*, POC1 centriolar protein A.

correlation coefficients between the genes and immune infiltration scores in individual tumors using the "corr.test" function of the R package psyche (version 2.1.6) to identify the significantly correlated immune infiltration scores. We found that both *POC1A* (P=3.2×10^{-9}) and *PPARG* (P=0.02)

were significantly correlated with the immune infiltration of the LIHC samples (*Figure 8A*). We then used the TIMER database to analyze the possible roles of core interacting genes with *POC1A* in immune cells. *Figure 8B* shows the relationship between the infiltration levels of *POC1A* and
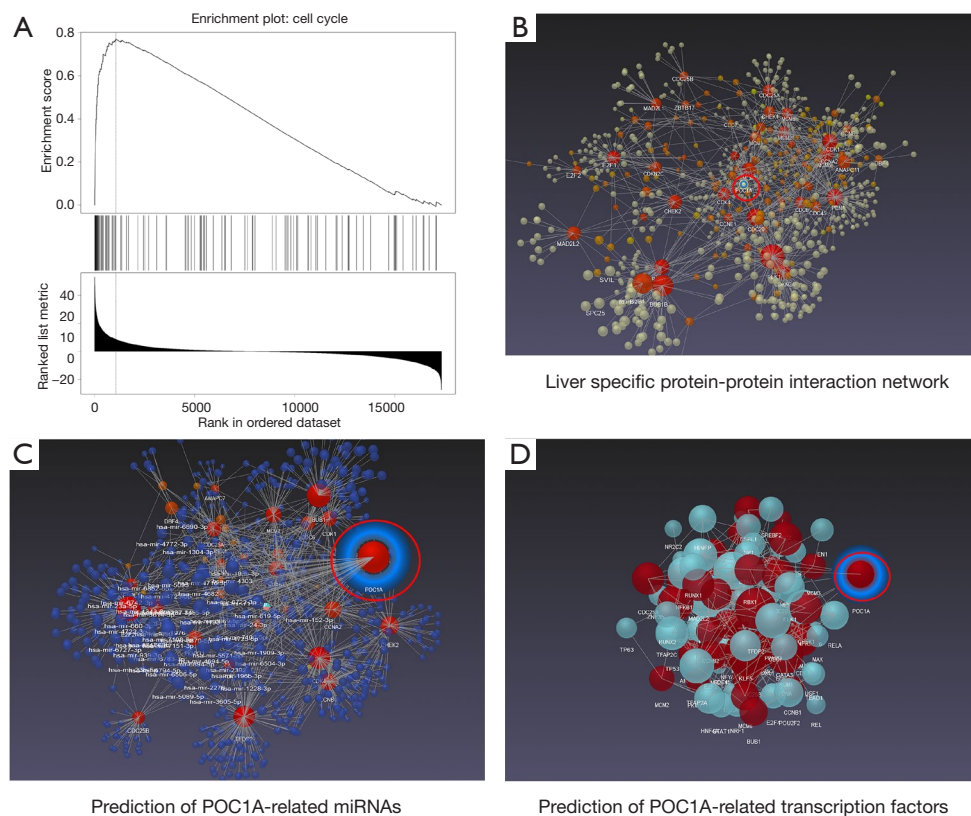
Figure 5 Analysis of the possible interacting proteins and TFs acting on the *POC1A* gene. (A) GSEA enrichment map of *POC1A* in the cell-cycle pathway. (B) The predicted liver-specific PPIs of POC1A in the cell-cycle pathway; the red circle highlights the POC1A protein. (C) The predicted miRNAs targeting POC1A; the red circle highlights the POC1A protein. (D) The predicted TFs that act on POC1A; the red circle highlights the POC1A protein. TFs, transcription factors; GSEA, gene set enrichment analysis; PPIs, protein-protein interactions; miRNAs, microRNAs; *POC1A*, POC1 centriolar protein A.

six types of immune cells under different somatic copy number alterations levels in the LIHC samples. There was a significant difference in the infiltration level of high amplification between the B cells and macrophages. *Figure 8C* shows that the high expression of *POC1A* and its interacting genes play a significant role in various immune cell types. These genes and the way they interacted with POC1A warrant further analysis. Taken together, we can conclude that *POC1A* is significantly overexpressed in HCC, and promotes the occurrence and development of HCC by affecting the cell cycle. The use of *POC1A* as a potential molecular marker may play an important role in the treatment of HCC.

Discussion

Causes of HCC include chronic liver disease caused by

hepatitis B virus and hepatitis C virus, long-term alcohol consumption, and non-alcoholic fatty liver disease (17). HCC has an extremely high mortality rate. This study aimed to explore whether *POC1A* could serve as a new therapeutic target for HCC. The analyses in this study were mainly conducted using a number of public databases and bioinformatics methods. First, the TIMER database was used to show that the expression level of *POC1A* was significantly upregulated in pan-cancer. Next, the effect of *POC1A* on LIHC tissue was closely explored. The results showed that the expression level was significantly elevated in patients with LIHC, and high gene expression was significantly negatively correlated with survival prognosis. High levels of *POC1A* were associated with lower OS rates in HCC patients, and a further correlation was found between *POC1A* and some clinical and pathological features, including gender, age, race, and tumor stage. GO
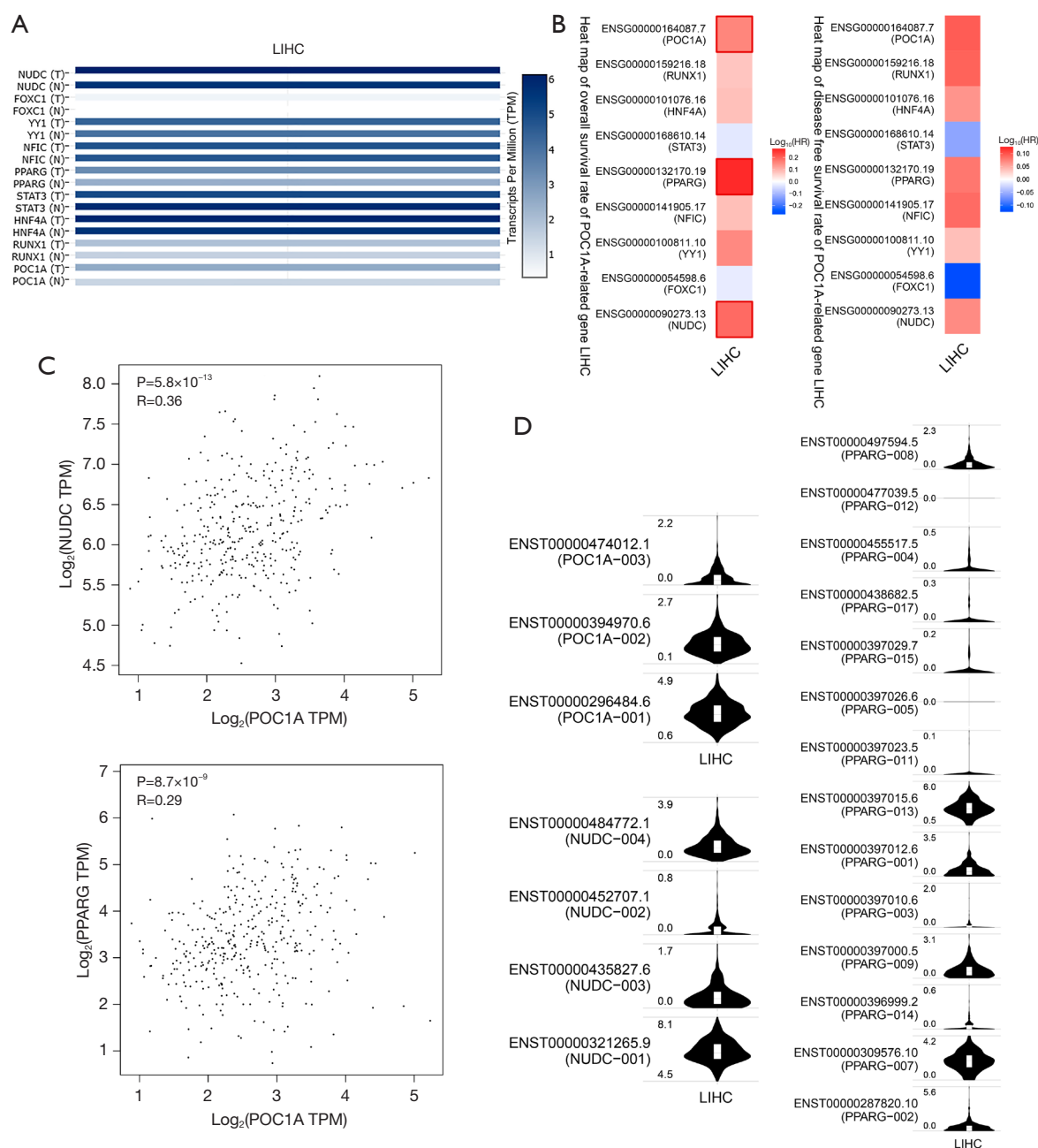
Figure 6 Analysis of the predicted gene changes and expression prognosis of *POC1A*-related genes in LIHC. (A) The expression of the *POC1A*-related genes in LIHC samples. (B) Heatmap showing the effect of the *POC1A*-related genes on the OS and DFS of LIHC patients. (C) The correlation between *POC1A* and *NUDC* and *PPARG*. (D) The expression of different genotypes of *POC1A*, *NUDC*, and *PPARG* in LIHC samples. TPM, transcripts per million; T, tumor; N, normal; LIHC, liver hepatocellular carcinoma; OS, overall survival; DFS, disease-free survival; *POC1A*, POC1 centriolar protein A.
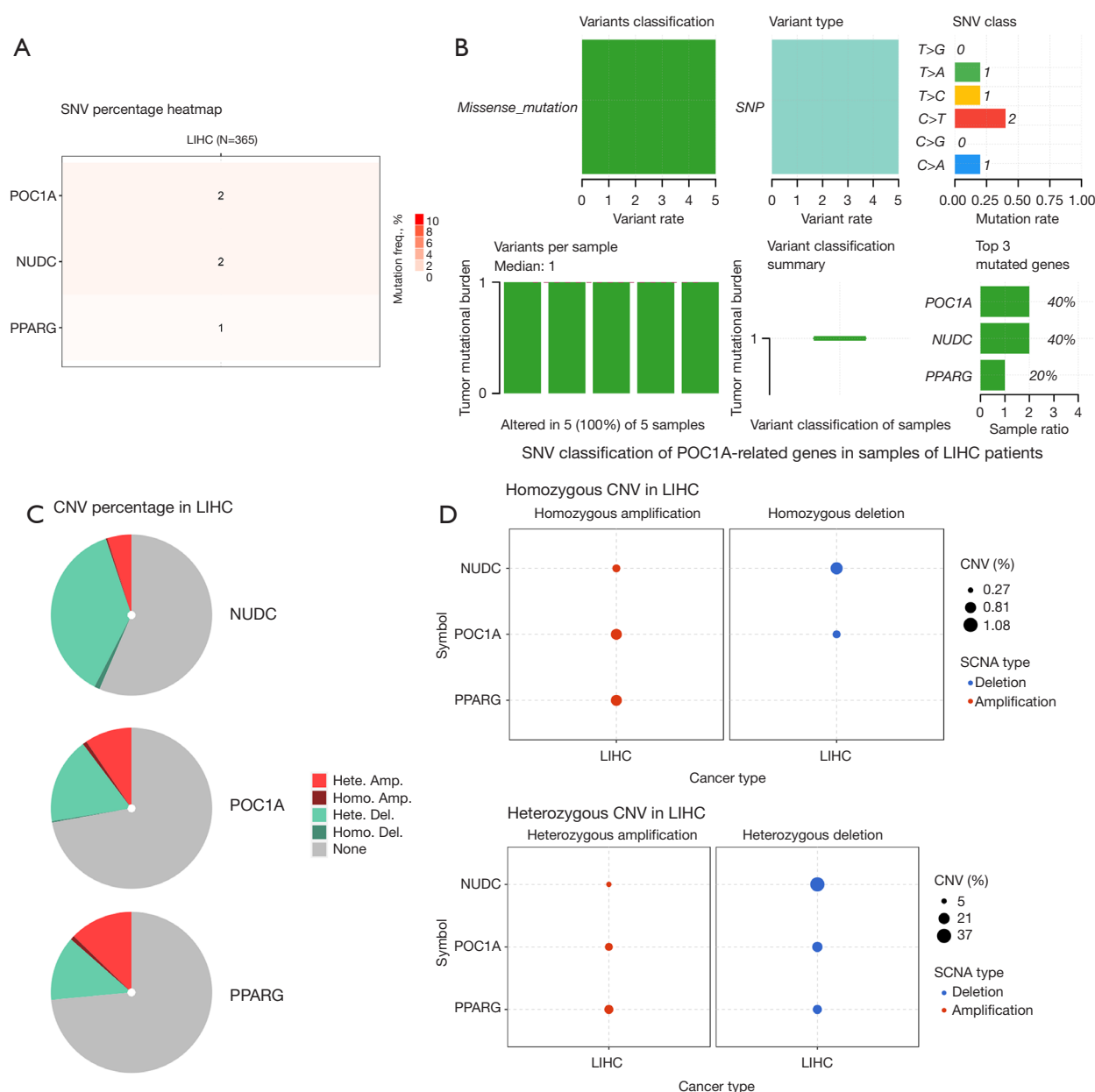
Figure 7 Analysis of the level of SNV and CNV changes in *POC1A* and related genes in LIHC samples. (A) SNV characterization of related genes in LIHC. (B) SNV categories of related genes in LIHC, A (adenine), T (thymine), G (guanine) and C (cytosine) represent the four bases that make up DNA. (C) Percentage change of the CNVs of *POC1A*, *NUDC*, and *PPARG* in LIHC. (D) Homozygous and heterozygous CNV mapping of *POC1A*, *NUDC*, and *PPARG*. SNP, single nucleotide polymorphism; SCNA, somatic copy number alterations; SNV, single nucleotide variation; CNV, copy number variation; LIHC, liver hepatocellular carcinoma; *POC1A*, POC1 centriolar protein A.

and KEGG enrichment analyses were then conducted. The genes associated with *POC1A* were presented in volcanic maps, and the top 50 genes positively or negatively correlated with *POC1A* were presented in heat maps. The GO enrichment analysis revealed that *POC1A* is involved

in the biological process of chromosome separation, which may result in aneuploidy and micronuclei, leading to chromosomal instability (CIN).

CIN is a significant marker of cancer. It is caused by persistent errors in chromosome separation during mitosis,
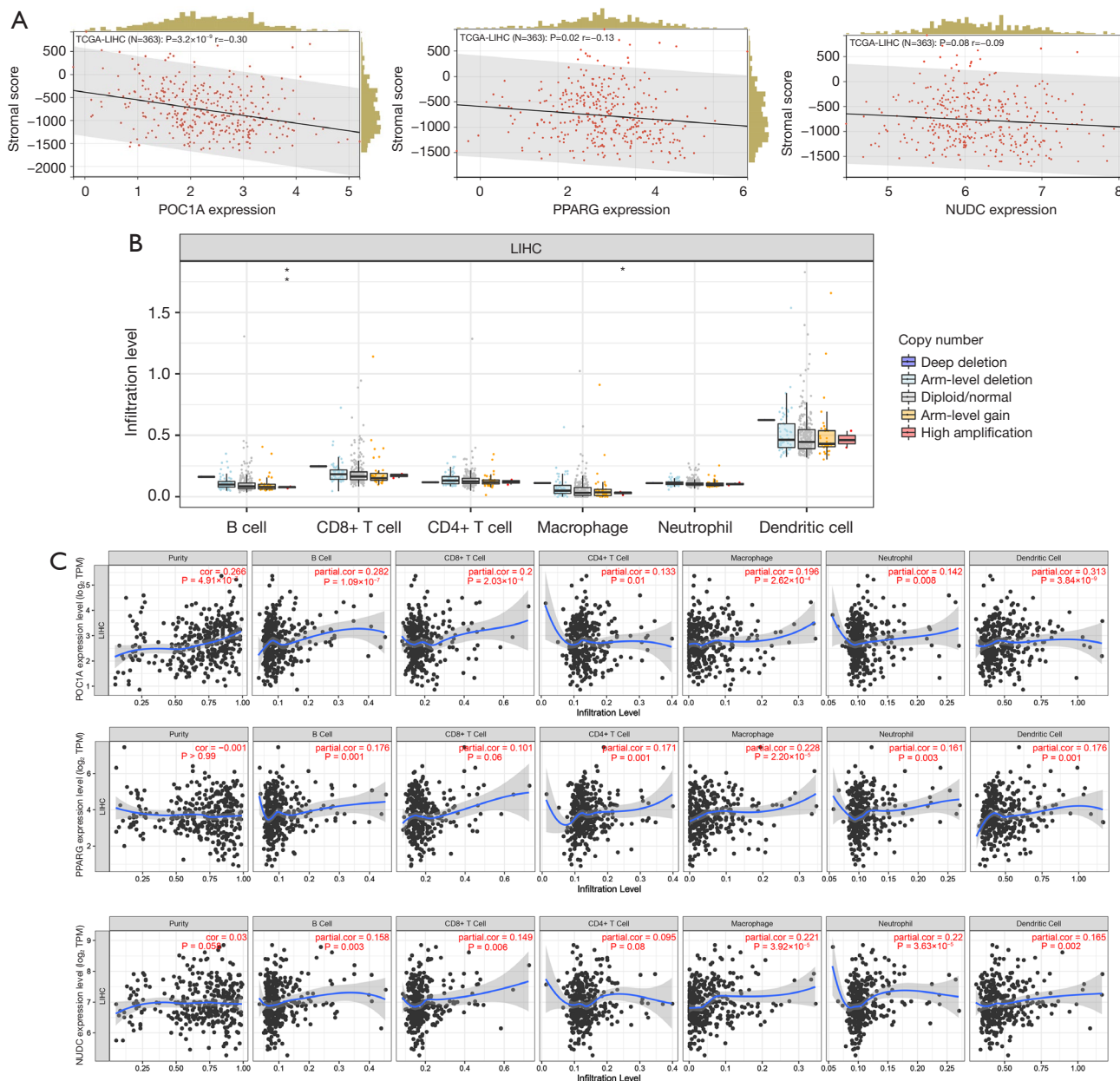
Figure 8 Analysis of the infiltration and expression of *POC1A* and related genes in LIHC immune cells. (A) The level of immune infiltration of *POC1A*, *NUDC*, and *PPARG* in patients with LIHC (B) The putative copy number alterations of *POC1A*. (C) The expression levels of *POC1A*-related genes in immune cells. *, P<0.05; **, P<0.01. cor, correlation; TPM, transcripts per million; LIHC, liver hepatocellular carcinoma; *POC1A*, POC1 centriolar protein A.

and is the main factor promoting tumor development. Micronuclei have been found in the regenerative and developmental nodules of the liver, which suggests that CIN can occur in the early stages of liver cancer (18). The number of abnormal chromosomes is a prominent feature of cancer cells, and polyploidy, where the DNA content increases to an integer multiple of the entire chromosome set, is also considered a possible mechanism leading to the occurrence of liver cancer. Chromosome aberrations can endow cancer cells with drug resistance, ultimately leading to treatment failure. The degree of drug resistance is related to the degree of CIN (19,20).

POC1A is also enriched in the process of non-coding RNA, which has been continuously studied and was previously considered useless in mediating the regulatory factors involved in many functions but has since been identified as a carcinogenic driving factor and tumor suppressor (21). The KEGG enrichment analysis showed that *POC1A* is involved in ribosome related pathways, and genetic mutations in ribosomal biogenesis are associated with an increased risk of cancer. Dysregulated ribosomal biogenesis has a broader role in the development and progression of most spontaneous cancers (22). The protein encoded by the *POC1A* gene is an important protein that regulates the assembly of centrioles. As a protein located on the centrosome, it regulates tumor cells by participating in the cell cycle and DNA replication.

The use of PPIs and small molecule antagonisms in therapeutic approaches has become increasingly feasible. POC1A-encoded proteins contain WD40 repeat (WDR) domains, one of the most abundant PPI domains in human proteomics, consisting of WDR5 and EED proteins. The propeller domain has been successfully targeted by effective, specific, and cellular active drug-like chemical probes (23). Thus, *POC1A* is likely to be a new target for the early diagnosis and treatment of HCC. In this study, we focused on the pathways by which POC1A interacts to affect cell-cycle progression. We found that POC1A interacts with the NUDC protein, and genes such as *FOXC1*, *YY1*, and *NFIC* may be the TFs of *POC1A*.

Moreover, the high expression of *PPARG* and *NUDC* significantly affected the OS rate of the LIHC patients. Research has shown that *NUDC* may serve as a new natural killer cell-related marker to predict the prognosis of and efficacy of immunotherapy in liver cancer patients (24). *PPARG* may play an important role in cell proliferation, the complement system, and immune cell invasion of HCC, and may be independently associated with susceptibility to progressive liver fibrosis (25,26). In addition, many studies have found that the occurrence and development of tumors are closely related to the tumor microenvironment. Tumor tissue does not simply comprise tumor cells; rather, various cells form the tumor microenvironment. The difference in the infiltration degree of different immune cells is highly correlated with tumor progression and prognosis. The high expression of *POC1A* and its interaction genes could play a significant role in various immune cell types. These genes and their interaction with *POC1A* are worthy of further research.

There have been some advances in science and technology in recent years, and there have been improvements in the treatment of HCC, of which liver transplantation is still one of the most effective methods. However, due to many limiting factors such as a shortage of donor organs and long waiting times, patients may not be able to undergo transplantation due to tumor progression and may ultimately die. In addition to studying effective treatment methods after diagnosis, more accurate early screening and diagnosis methods are needed to develop a more targeted and effective method to improve the survival rate, especially in patients in the middle and late stages. Further validation studies are needed to assess the specific molecular mechanisms by which the *POC1A* gene affects the prognosis of HCC patients.

Conclusions

This study investigated the mode and mechanism of *POC1A* in HCC patients by integrating and analyzing a large number of HCC data from multiple databases. We found that *POC1A* was significantly highly expressed in HCC patients, and the OS and DFS rates of these patients were significantly reduced. The overexpression of *POC1A* may have an important effect on the development of HCC via the modulation of the cell-cycle pathway. This study provides a theoretical basis for the development of HCC, and suggests that *POC1A* could be used as a new potential biomarker for the treatment and prevention of HCC, which is of great significance in the study of liver cancer.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the REMARK reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2398/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2398/dss

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-23-2398/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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