

Cyclin Dependent Kinase 19 Upregulation Correlates With Unfavorable Prognosis in Hepatocellular Carcinoma

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Research Article

Keywords: Cyclin dependent kinase 19, hepatocellular carcinoma, prognosis, knocking down, proliferation, migration, invasion

Posted Date: April 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-445045/v1>

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Abstract

Objectives: Cyclin dependent kinase 19 (CDK19) is a component of the Mediator co-activator complex, which is required for transcriptional activation. In this study, we will utilize the public data and combine it with wet-bench experiments in hepatic cell lines to elucidate the potential roles of CDK19 in hepatocellular cancer (HCC).

Materials and Methods: We studied the relationships between CDK19 expression and several clinical features related with HCC by consulting Oncomine and UALCAN. The prognostic value of CDK19 was tested using the Kaplan-Meier Plotter database. We presented the mutations of CDK19 and addressed its relations with immune cells with the use of cBioPortal, and COSMIC and TIMER database. Hub genes were obtained and further analysed using the STRING database. To test the *in silico* findings, we knocked down CDK19 with short hairpin RNA (shRNA) technology in two hepatic cell lines, and then several functional characterization experiments were conducted.

Results: A remarkably higher level of CDK19 expression was found in HCC tissues than normal liver tissues, and CDK19 mRNA expression has high diagnostic value in HCC patients. Subgroup analysis showed that CDK19 overexpression were associated with gender, tumor stage and TP53 mutant. Prognostic values of CDK19 upregulation for overall survival (OS) were significant in patients with stage 2-3, stage 3-4, grade 2 and etc. 1% of the patients have mutations at CDK19, and we did not observe a potential relationship between CDK19 mutation and prognosis. CDK19 showed positive correlations with the abundances of CD4+ T cells, macrophages and dendritic cells. We identified 10 genes that correlated with CDK19, 8 of which presented excellent prognostic value in HCC. Besides, these hub genes were directly involved in cell division and regulation of G2/M transition of mitotic cell cycle. PPI and pathway predictions indicated that CDK19 should have a high possibility to be involved with several cellular functions, such as proliferation, migration, and invasion. These functions were strongly interfered in two independent hepatic cell lines, after knocking down CDK19.

Conclusions: CDK19 could serve as a prognostic marker in HCC and it deserves further work to test its therapeutic potential to HCC.

Background

Hepatocellular carcinoma (HCC), accounting for 75%~85% of primary liver cancer, ranked 6th most commonly diagnosed and ranked 3th in cancer-related death globally [1]. In recent years, the incidence of HCC increased dramatically and will continue to rise over the next 10–20 years [2]. The major challenges are metastasis and recurrence after resection, which contributes to the dismal prognosis of HCC. Nowadays, several novel therapeutic options for HCC are emerging and have shown to improve the survival rates, but the overall prognosis is still unsatisfactory [3]. Thus, to identify promising prognostic biomarkers is still urgent and necessary.

Cyclin dependent kinase 19 (CDK19) is a cyclin-dependent transcription-regulating kinase. CDK19 and its homolog CDK8, being termed as 'Mediator Kinase', have been shown to have crucial roles in cellular homeostasis and developmental programming. In a mutually exclusive manner, CDK19 or CDK8 can form a Mediator co-activator complex with another three proteins, CCNC, MED12 and MED13[4]. CDK19 or CDK8 reversibly regulate RNA polymerase II to control transcriptional activity. CDK8 has been reported to involve in the development of malignancies, including cancers of colon, breast and pancreas [5–7]. In contrast, the role of CDK19 in carcinogenesis is rarely studied and only sporadically reported in prostatic cancer, colorectal cancer, breast cancer and etc [8–10]. Meanwhile, some small-molecule CDK8/19 inhibitors have been found to possess beneficial effects on tumor treatment, and a clinical trial (ClinicalTrials.gov Identifier: NCT03065010) with estrogen receptor-positive breast cancer has been on its way [11]. Studies have shown that Sorafenib is a molecular inhibitor of CDK19 [12]. Here, we analyzed the HCC patients treated with Sorafenib by bioinformatics, and found that they had a better prognosis (Fig. 3I).

In this study, we first investigated the expression of CDK19 and the prognostic value of CDK19 in HCC patients. Next, we evaluated the relationship between CDK19 and immune infiltrates, identified a 10-gene hub correlated with CDK19 strongly, and explored the underlying roles of CDK19 by PPI and pathway analysis. Then, we conducted CDK19-knockdown in two HCC cell lines and conformed its relevant functions from *in vitro* assays.

Materials And Methods

mRNA/protein expression and survival analysis

We search 'CDK19' as the gene symbol in the Oncomine database (<http://www.oncomine.org> ; accessed from 2 February, 2021). CDK19 expression values from four GEO datasets (including Roessler liver, Wurmbach liver, Roessler liver2 and Chen liver) were obtained and the dot plots were generated using GraphPad Prism 7.0 software. Then, we collected and collated the results about CDK19 expression levels in different subgroups using UALCAN database (<http://ualcan.path.uab.edu> ; accessed from 2 February, 2021) [13]. Moreover, we investigated the protein expression of CDK19 in the Human Protein Atlas database (www.proteinatlas.org; accessed from 2 February, 2021) [14, 15]. Next, CDK19 expression and overall survival in HCC patients were evaluated using Kaplan-Meier Plotter database (<http://kmplot.com>; accessed from 2 February, 2021) [16].

Mutant and immune infiltrates analysis

We evaluated the mutant frequenc of CDK19 in HCC patients using the cBioPortal database (<http://www.cbioportal.org/>; accessed from 2 February, 2021) [17, 18]. We selected all listed 1000 HCC samples of 5 studies as study object. Then, we validated the mutation of CDK19 in HCC in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (<http://cancer.sanger.ac.uk>; accessed from 2 February, 2021) [19, 20]. Next, we further explored the associations between CDK19 and immune cells

using the Tumor Immune Estimation Resource (TIMER) database (<https://cistrome.shinyapps.io/timer/>; accessed from 2 February, 2021) [21].

Differential expression genes and hub genes analysis

We investigated the differential expression genes (DEGs) using LinkedOmics (<http://www.linkedomics.org>; accessed from 2 February, 2021) database, where contains 32 cancer types' multi-omics data[22]. 371 TCGA-HCC RNAseq samples were analysed by Spearman test. Then the hub genes were determined using cytoscape software (<https://cytoscape.org/>; accessed from 2 February, 2021) based on the top 200 significantly correlated DEGs. The prognostic significance of hub genes and the association between CDK19 and hub genes were investigated using the Kaplan-Meier Plotter database (<http://kmplot.com>; accessed from 2 February, 2021) [16] and GEPIA (<http://gepia.cancer-pku.cn/>; accessed from 2 February, 2021)[23].

Protein-Protein Interaction network and GO/KEGG analysis

Protein-Protein Interaction (PPI) analysis for CDK19 were performed using STRING database (<https://string-db.org/>; accessed from 2 February, 2021) [24]. We picked out the top 200 significantly related genes to establish the cluster of the network with default criteria. Next, we conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) biological process enrichment analysis, and the results were visualized with the bioinformatics online tool (<http://www.bioinformatics.com.cn>; accessed from 2 February, 2021).

Reagents and cell culture

HCC cell lines (Hep.G2 and SK-Hep-1) were purchased from Chinese Academy of Sciences. Both cell lines have been cell line certified and are free of mycoplasma infection. These cells were cultured in DMEM medium with 10% fetal bovine serum and maintained in an environment suitable for cell growth. For CDK19 knockdown, we used the short hairpin RNA (shRNA) delivered by lentivirus (purchased from Genomeditech, shanghai, China), which targeting the sequence 5'-GCATGACTTGTGGCATATTAT-3' (Gene ID: 23097).

qPCR analysis

After 48h of lentiviral transduction, the mRNA expression of CDK19 were evaluated. Total cellular RNA were extracted and relative mRNA expression was determined. The primers for CDK19 were: forward primer 5'-GTTTCACCGTGCATCAAAGC-3'; reverse primer 5'-ACCCAATTTGCATGGAGGTAATG-3'. GAPDH was set as an internal reference gene.

Cell proliferation assay

After 48h of viral transduction, the cells of sh-CDK19-NC and sh-CDK19 were seeded evenly into the 96-well plates with 10000 cells/well. Then, we detected cell viability using Cell Counting Kit-8 at 0h, 24h, 48h,

72h. These cells were incubated with 10 ul CCK-8 for 2h and 450 nm absorption value was recorded using VICTOR Nivo Multimode Plate Reader (PerkinElmer Inc., Massachusetts, USA).

Migration and invasion assay

Wound healing assay and transwell assay were proceeded to evaluate the migration and invasion ability of HCC cells. 48h post transfection, cells were trypsinized and seeded into culture insert (80209, IBIDI Co., Germany) and migration transwell chambers (3422, Corning Co., USA). After 48 hours, the migration and invasion ability were detected as previously detailed protocols[25].

Statistical analysis

We used GraphPad Prism 7.0 software to analyse partial results. Data were summarized as the means \pm SEM. Differences between 2 groups were evaluated using Student's t-test.

Results

High expression of CDK19 in HCC

CDK19 is a cyclin-dependent transcription-regulating kinase. Both CDK19 and its homolog CDK8, being termed as 'Mediator Kinase', have been shown to play crucial roles in cellular homeostasis and to be related with several diseases. Based on Oncomine, we evaluated the expression of CDK19 in HCC from 4 GEO datasets (Roessler liver, Roessler liver 2, Chen liver, and Wurmbach liver) [26-28]. As a result, the expression of CDK19 in HCC tissues was significantly upregulated. For example, CDK19 showed 1.71-fold increases in the Roessler liver datasets (Figure 1A). The difference of CDK19 expression across these four studies was significant, indicating that there may be to some extent inter-patient variations existed. ($P \leq 0.05$) (Figure 1B). We next investigated the protein expression of CDK19 in HCC by using HPA database. As shown in Figure 1C, CDK19 was hardly detected in a normal liver tissue (Patient ID 2556), but from a liver tumor tissue (Patient ID 2177) it showed quite strong signal (Figure 1C).

To further explore the inter-patient variations of CDK19, we studied the expression patterns of CDK19 in TCGA-LIHC by using UALCAN, according to several different clinical features. As shown in Figure 2A, CDK19 had much higher expression in LIHC patients (n=371) than in normal control group (n=50) (Figure 2A). While clustering the patients into different subgroups based on their age, gender, race and weight, we can observe differential expression profiles (Figure 2B-2E). For example, the expression difference would not be significant between the control and patients at the age of 81-100 Yrs. Besides, there was seemingly a correlation between CDK19 expression and different tumor severity (Figure 2F-2H). Among it, the difference of patients between stage 1 and stage 3 was quite obvious ($P=0.0021$). Considering that TP53 is one of the most commonly mutated genes in HCC, we found that there may be a correlation link between TP53 and CDK19. Intriguingly, CDK19 was accumulated much more in patients with TP53 mutation than others, $p=0.00012$ (Figure 2I).

Survival results and multivariate analysis in HCC

We evaluated the prognostic significance of CDK19 in 364 patients using the Kaplan-Meier Plotter database. We found that the higher the expression of CDK19, the worse the overall survival (OS, HR = 1.55, log-rank $P = 1.8E-2$) in HCC patients (Figure 3A). The prognostic value of CDK19 in different clinical subgroups of HCC was investigated. The results indicated that OS was relatively poor in patients with high CDK19 mRNA expression, under the conditions of stage 2-3, stage 3-4, grade 2, male sex, Asian race, alcohol consumed and non-hepatitis virus infected (Figure 3B-3H). Collectively, CDK19 expression level can serve as a valuable prognostic biomarker in HCC patients and the prognostic significance varies depending on different clinical subgroups, which can guide our clinical practice in a personalized pattern.

Mutations of CDK19 in HCC

Next, we investigated mutation landscape of CDK19 in a large number of HCC patients by using cBioPortal software. Overall, 1000 samples from 998 patients allocated in five studies (AMC, INSERM, RIKEN, MSK and TCGA-PanCancer Atlas) were selected for analysis [29-33]. From our datamining, 8 alterations of CDK19 were found, with one missense mutation which appeared in early HCC (Figure 4A). And the somatic mutant frequency of CDK19 was around 1% (Figure 4B). However, CDK19 somatic status can not be used to distinguish the OS in HCC patients (Figure 4C). In addition, we used COSMIC database to verify the mutation of CDK19 in HCC. There were only 9 mutations from 951 tissues (somatic mutation frequency: 0.95%) and the only type of mutations was missense substitution (Figure 4D). The substitution mutations solely occurred at C>A (100%) (Figure 4E).

The relationship between CDK19 and immune infiltrates in HCC

To investigate the correlation between CDK19 and immune infiltrates, we used TIMER online tool. The relationships between 6 immune cell types (B cell, CD8+T cells, CD4+ T cells, macrophage, neutrophil and myeloid dendritic cell) and CDK19 expression were determined by Spearman tests (tumor purity adjusted, Figure 5A). It revealed that CDK19 was significantly in positive correlation with these 6 immune infiltrates, especially macrophage ($R=0.48$) and myeloid dendritic cell ($R=0.458$) (Figure 5B-5G). Then it inspired us to further research if there may be a potential association between CDK19 and immune cell gene markers. Similar to the findings above, CDK19 had positive correlations with the respective gene markers of those 6 immune cells (Table 1). Among the listed gene markers, QRSL1 ($R=0.700$), IRF5 ($R= 0.483$), STAT1 ($R= 0.469$), NRP1 ($R=0.469$) and PTGS2 ($R=0.467$) are most relevant ones (Table 1).

The genes correlated with CDK19 in HCC

We investigated the genes correlated with CDK19 using LinkedOmics software. As the volcano map showed (Figure 6A), the negatively and positively related genes were located to the left and right areas, respectively. The top 50 positively and negatively related genes were identified based on Spearman test, and were shown in heatmap separately (Figure 6B-6C). To address whether there is some hub genes existed, we input the top 200 positively related genes with CDK19 into the STRING online database and cytoscape software. Based on gene degree, the 10 most relevant hub genes were obtained, including CEP135, CEP162, CEP192, CEP290, CNTRL, HAUS6, IQCB1, NEDD1, TCTN2 and WDHD1 (Figure 6D). We

were surprised to find that almost all Hub genes are directly involved in cell division and regulation of G2/M transition of mitotic cell cycle. The correlations between CDK19 and the 10 hub genes were validated by using GEPIA web-tool (Supplementary Figure 1). Lastly, we found that 8 of the 10 top hub genes presented excellent prognostic value in HCC (Figure 6E), especially IQCB1 (HR=2.05) and NEDD1 (HR=1.93) (Figure 6E).

PPI and KEGG/GO enrichment of CDK19 in HCC

By utilizing the STRING software, we constructed a protein-protein interaction (PPI) network based on the top 200 significantly related genes (Figure 7A). As shown in the network, CDK19 can directly interact with MED23 and CNOT2, through which further interact with other proteins. As we all know, MED23 and CNOT2 are both involved in regulation of gene expression and transcription. Meanwhile, we also found a lot of proteins in the network took part in tumor development, including PHIP driving glioblastoma motility and invasion[34], HDAC2 regulating breast cancer progression and proliferation[35] and ZFN292 participating hepatoma proliferation and vasculogenic mimicry[36].

Then, we investigated the GO/KEGG enrichment signaling pathways, which CDK19 may be involved in. GO biological process analysis of CDK19 showed that binding to transcription cofactors, regulations of transcription factors and mRNA were significantly affected and enriched (Figure 7B). KEGG pathway enrichment showed that CDK19 is mainly involved in mitosis through different ways (Figure 7C).

CDK19 involved in several cellular functions of hepatic tumor cell lines

To validate the findings related with CDK19 from bioinformatics analysis, we chose two independent hepatic cell lines, Hep.G2 and SK-Hep-1, as our *in-vitro* models. Here, small hairpin RNA (shRNA) based method was utilized to knock down CDK19, and the lentivirus containing sh-CDK19 was employed as the transgene delivery tool. Firstly, after 48h of lentivirus infection, we isolated mRNA from the cells, and performed qPCR to check the CDK19 level. As shown in Figure 8A, CDK19 was knocked down successfully in both cell lines, comparing with non-targeting control (NC). To address if CDK19 is involved in cell growth, we next conducted cell viability assay. In comparison to the control, knocking-down of CDK19 clearly inhibited the proliferation of both SK-Hep-1 and Hep.G2 cells (Figure 8B).

As suggested from the bioinformatics analysis, CDK19 may have relations with migration and invasion abilities, directly or indirectly through interaction with other invasion-relevant proteins. Aiming to validate its involvement in migration, wound healing assay was performed in the two cell lines. As seen from Figure 8C, much less tumor cells can migrate while comparing with the control. In order to conform CDK19 has contributions to invasion ability, we did transwell invasion assay to the cell lines. Similar as above, knocking-down of CDK19 can significantly decrease the amount of invaded tumor cells (Figure 8D).

Taken together, knocking-down of CDK19 can decrease the proliferation, migration and invasion abilities of hepatic tumor cells, indicating that CDK19 may serve as a promising therapeutic target in HCC.

Discussion

HCC is one of the most aggressive cancers with poor survival [37]. Globally, about 750,000 new cases and 780,000 death cases of HCC are recorded every year [38]. Because of late diagnosis, metastasis and quick progression, HCC has become a major killer in cancers. Currently, effective therapeutic strategies for HCC patients is still in a hard dilemma. However, with the development of next-generation sequencing and multi-omics, the cellular and molecular mechanisms responsible for HCC tumorigenesis becomes gradually clear[39]. Meanwhile, many new biomarkers for HCC have been discovered, which provide valuable information for diagnosis and/or prognosis of HCC.

The human Mediator complex (MED) is a transcriptional regulator consisting of 30 subunits. It is designated to 4 distinct MED modules including head, middle, tail and kinase modules. By interacting with RNA-polymerase-II and transcription factors, MED impact almost all cellular processes, such as elongation, initiation, and chromatin architecture[40]. It is not surprising that MED contributes to pathogenesis of diseases, and that the genetic variations or changes of Mediator subunits can lead to a number of pathologies including cancers. For example, CDK8 kinase module and MED29 promoted the expression of β -catenin in colorectal cancer, and they were shown to have oncogenic and tumour-suppressive functions in pancreatic cancer cells, respectively[6, 41]. Moreover, a few studies found that MED1/17 and MED19 were extremely highly expressed in prostate cancer and breast cancer. By reducing the expression of MED1/17 and MED19, the growth and proliferation of cancer cells were inhibited[42, 43]. Therefore, particular Mediator subunit was proposed as hallmarks or therapeutic targets in cancers. CDK19 and its gene paralog CDK8 are part of the Mediator's kinase module, which reversibly associates with the Mediator core structure, and leads to gene transcription activity as signaling pathway coactivators and corepressors[44]. As previously mentioned, multiple studies found CDK19 and CDK8 involved in diverse cancer entities [5–10]. However, to our knowledge, the detailed characteristics of CDK19 in HCC are not well understood yet. Here, we tried to answer the following questions with great enthusiasm. First, what is the potential mechanism of CDK19 in HCC? Second, what mutations of CDK19 occur in HCC? Does these mutations have a relationship with the prognosis? Third, is CDK19 involved in the pathological process of HCC through immune infiltration? To answer such questions, we conducted a series informatics analysis and experiments in this study.

Based on our work, we found that CDK19 is upregulated in HCC patients. High expression of CDK19 showed tight correlations with the clinical features of HCC patients, such as OS. These results indicated that CDK19 can be used as a novel prognostic marker in HCC. As for mutational alterations of CDK19 in HCC, we found the mutation frequency was only 0.1%, mainly missense substitutions. Nowadays, immunotherapy is a most promising treatment for cancers. To explore whether there is a possibility to combine CDK19-targeting and canonical immunotherapy, we researched if there was a potential relationship between CDK19 expression and immune infiltration in HCC. Our work showed that the expression of CDK19 was in positive correlation with immune infiltrates. These results indicated that CDK19 may play an important role in HCC immune microenvironment. Hofmann et al. recently found that CDK8/19 inhibitors could enhance the killer function of NK cells and promote the lysis of primary

leukemia cells[45]. Their works inspired us that CDK8/19 inhibitors may be used in treatment of HCC in the future. With datamining on PPI network and GO/KEGG analysis, we found that CDK19 took part in regulations of some critical transcription factors and it may be involved in mitosis, proliferation, invasion and migration from the mRNA level. Hence, we further explored whether CDK19 could affect the malignancies of HCC cell lines. A serial of phenotypic functional experiments demonstrated that CDK19 may participate in HCC development, by promoting the proliferation, migration and invasion. These phenotypic characteristics were similar with the ones found in prostate cancer, gastric cancer, and head and neck squamous cell carcinoma where CDK19 participated[46–48].

Conclusion

In conclusion, we firstly demonstrated that upregulated CDK19 expression was correlated with a poor prognosis in HCC from several clinic-related features. CDK19 is in a positive correlation with immune infiltrates in. We addressed that CDK19 shall be involved with several cellular functions, such as proliferation, migration, and invasion. These findings highlighted the potential value of CDK19 expression as a prognostic marker, and meanwhile targeting CDK19 deserved further work to test its therapeutic use for HCC.

Abbreviations

CDK19	Cyclin dependent kinase 19
HCC	hepatocellular cancer
DEGs	differential expression genes
GEPIA	Gene Expression Profiling Interactive Analysis
MED	Mediator complex
COSMIC	Catalogue of Somatic Mutations in Cancer
TIMER	Tumor Immune Estimation Resource
KEGG	Kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
shRNA	short hairpin RNA

Declarations

Acknowledgements

Not applicable

Authors' contributions

XPC, JWD conceived this idea and performed cells' trials. JMZ conducted partial analysis. HQC and ZC revised the manuscript. All authors contributed to this manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by National Science and Technology Major Project of China (2018ZX10302206 and 2017ZX10202203).

Availability of data and materials

The datasets generated and analyzed were obtained from online databases including Oncomine (<http://www.oncomine.org>), UALCAN (<http://ualcan.path.uab.edu>), Human Protein Atlas database (www.proteinatlas.org), Kaplan-Meier Plotter (<http://kmplot.com>), Catalogue of Somatic Mutations in Cancer (COSMIC) (<http://cancer.sanger.ac.uk>), cBioPortal (<http://www.cbioportal.org>), Tumor Immune Estimation Resource (TIMER) (<https://cistrome.shinyapps.io/timer>), LinkedOmics (<http://www.linkedomics.org>), cytoscope software (<https://cytoscape.org>), GEPIA (<http://gepia.cancer-pku.cn>), STRING (<https://string-db.org>), bioinformatics online tool (<http://www.bioinformatics.com.cn>).

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Table

Table 1. Correlations between CDK19 and immune cells' gene markers in HCC

Cells Subtypes	Markers	Non-purity Adjusted		Purity Adjusted	
		$\rho(\rho)$	P	$\rho(\rho)$	P
B cells	CD19	0.213	4.90E-04	0.245	8.60E-05
	CD79A	0.189	2.60E-03	0.26	1.90E-05
T cells (general)	CD3D	0.173	4.70E-03	0.237	1.80E-04
	CD2	0.183	2.30E-03	0.272	5.80E-06
	CD3E	0.182	2.10E-03	0.278	3.20E-06
CD8+ T cells	CD8B	0.126	5.00E-02	0.186	2.60E-03
	CD8A	0.195	1.20E-03	0.265	7.70E-06
CD4+ T cells	QRSL1	0.698	2.60E-54	0.7	5.10E-51
	STAT1	0.443	4.20E-18	0.469	5.30E-19
	STAT4	0.312	1.60E-08	0.348	3.70E-10
	STAT5A	0.404	7.60E-15	0.435	3.10E-16
	STAT6	0.339	1.10E-10	0.335	1.00E-09
	CD4	0.303	1.70E-08	0.362	5.20E-11
	TBX21	0.096	1.60E-01	0.16	9.40E-03
Tumor Associated Macrophages	CCL2	0.217	2.10E-04	0.289	4.50E-07
	CD68	0.193	1.30E-03	0.239	6.90E-05
	IL10	0.264	1.00E-05	0.34	1.80E-09
Type I Macrophages	IRF5	0.483	1.90E-21	0.483	6.40E-20
	NOS2	0.156	6.10E-03	0.172	3.30E-03
	PTGS2	0.364	8.50E-12	0.467	1.60E-18
Type II Macrophages	CD163	0.15	1.00E-02	0.216	1.70E-04
	MS4A4A	0.156	8.60E-03	0.235	5.40E-05
	VSIG4	0.173	3.40E-03	0.245	2.90E-05
Neutrophil	CCR7	0.181	2.30E-03	0.26	9.90E-06
	ITGAM	0.315	5.20E-09	0.365	2.60E-11
Dendritic Cells	CD1C	0.252	6.00E-06	0.3	1.30E-07
	HLA-DPB1	0.175	3.70E-03	0.234	7.30E-05

	HLA-DQB1	0.086	2.80E-01	0.133	4.80E-02
	HLA-DRA	0.171	4.60E-03	0.23	7.90E-05
	ITGAX	0.337	1.00E-09	0.425	6.20E-15
	NRP1	0.459	1.00E-19	0.469	2.70E-19

Figures

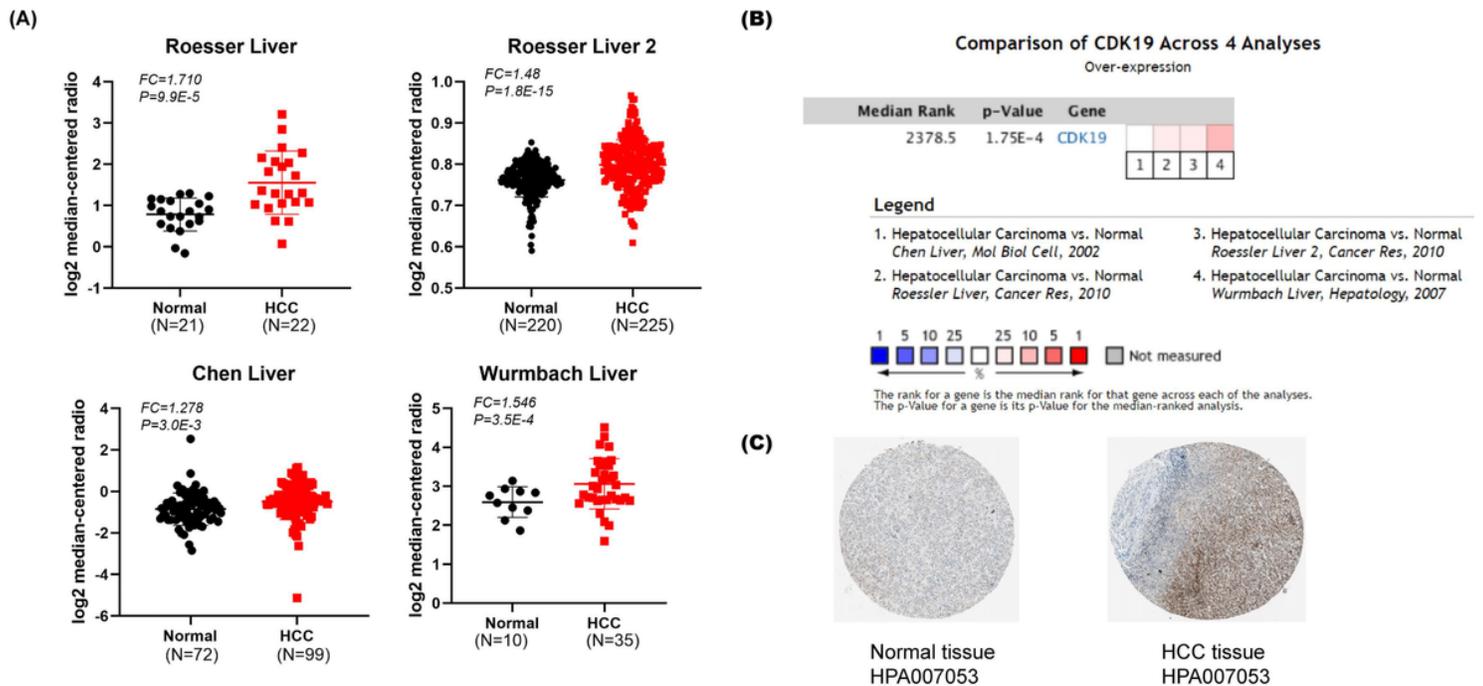


Figure 1

The expressed profiles of CDK19 in hepatocellular carcinoma (HCC). A, The profiles in four different studies of HCC. B, Compared analysis of CDK19 across the four studies. C, Protein expression of CDK19 in HCC tissues.

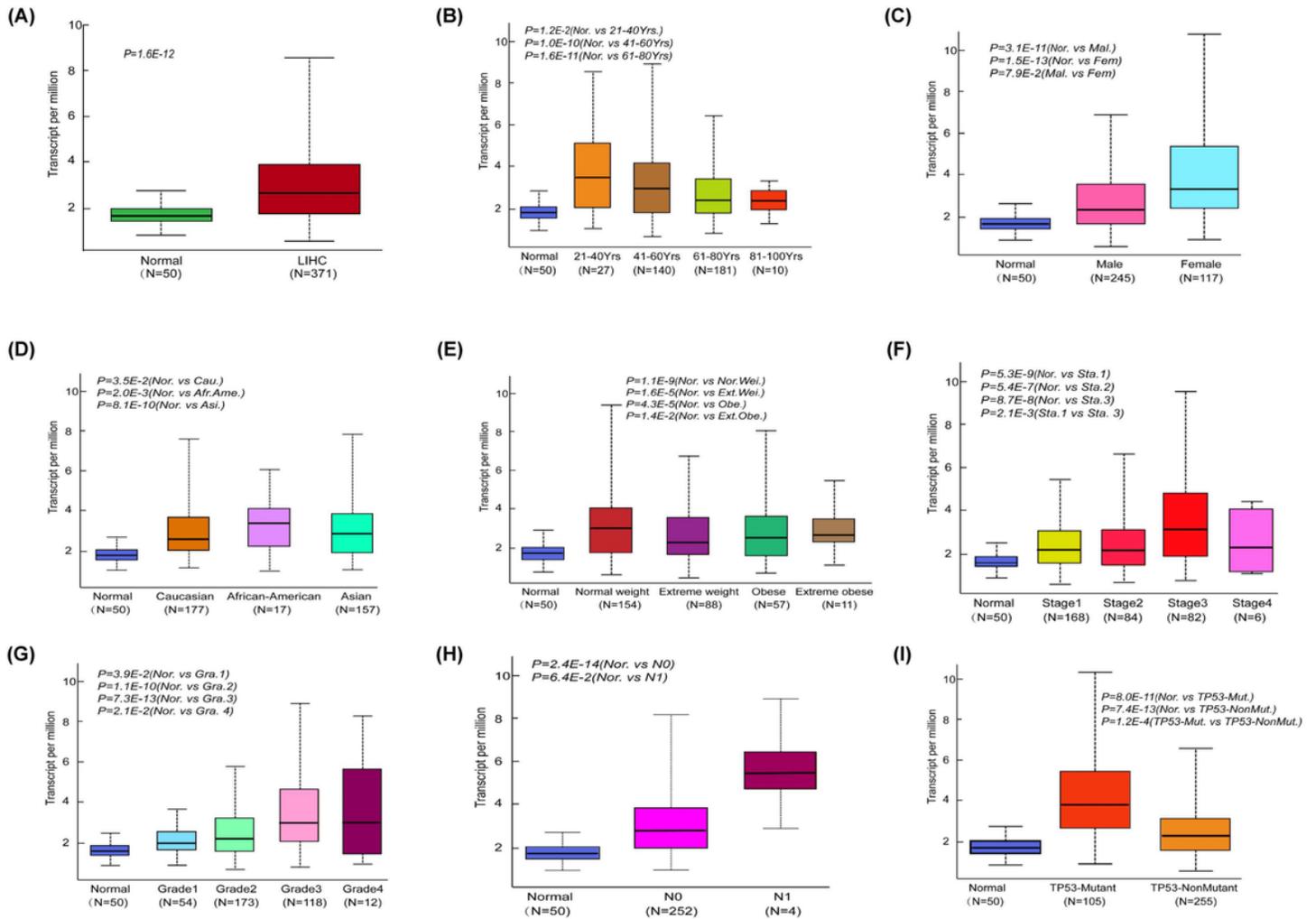


Figure 2

Analysis of CDK19 mRNA expression in subgroups of HCC using UALCAN software. A, mRNA expression of CDK19 was compared in normal tissue and LIHC tissues from TCGA. B-E, CDK19 mRNA expression levels of HCC patients were analyzed in subgroups with different ages, genders, races and weights. F-H, CDK19 mRNA expression levels of HCC patients with different tumour stages, tumour grades and metastasis status. I, CDK19 mRNA expression levels of HCC patients with TP-53 mutant.

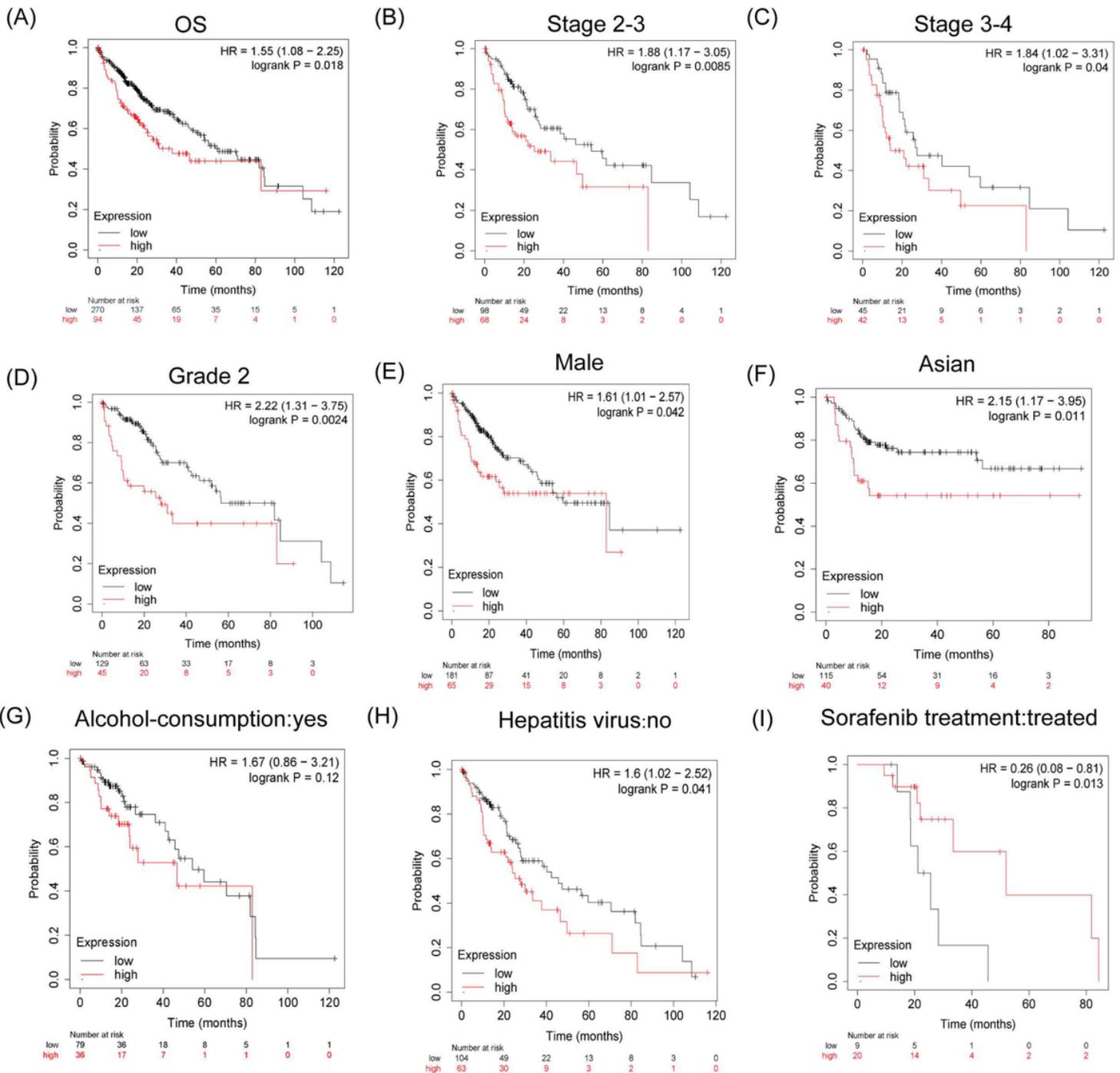


Figure 3

The overall survival (OS) values were analyzed in regards to the mRNA expression level of CDK19 in all tumors and subgroups of HCC patients. OS analysis of (A) All tumors, (B) Tumor stage II-III, (C) Tumor stage III-IV, (D) Tumor grade III, (E) Male, (F) Asian race, (G) Alcohol consumption, (H) Non-hepatitis virus infected, and (I) sorafenib treated. Graphs were generated from the Kaplan-Meier Plotter database.

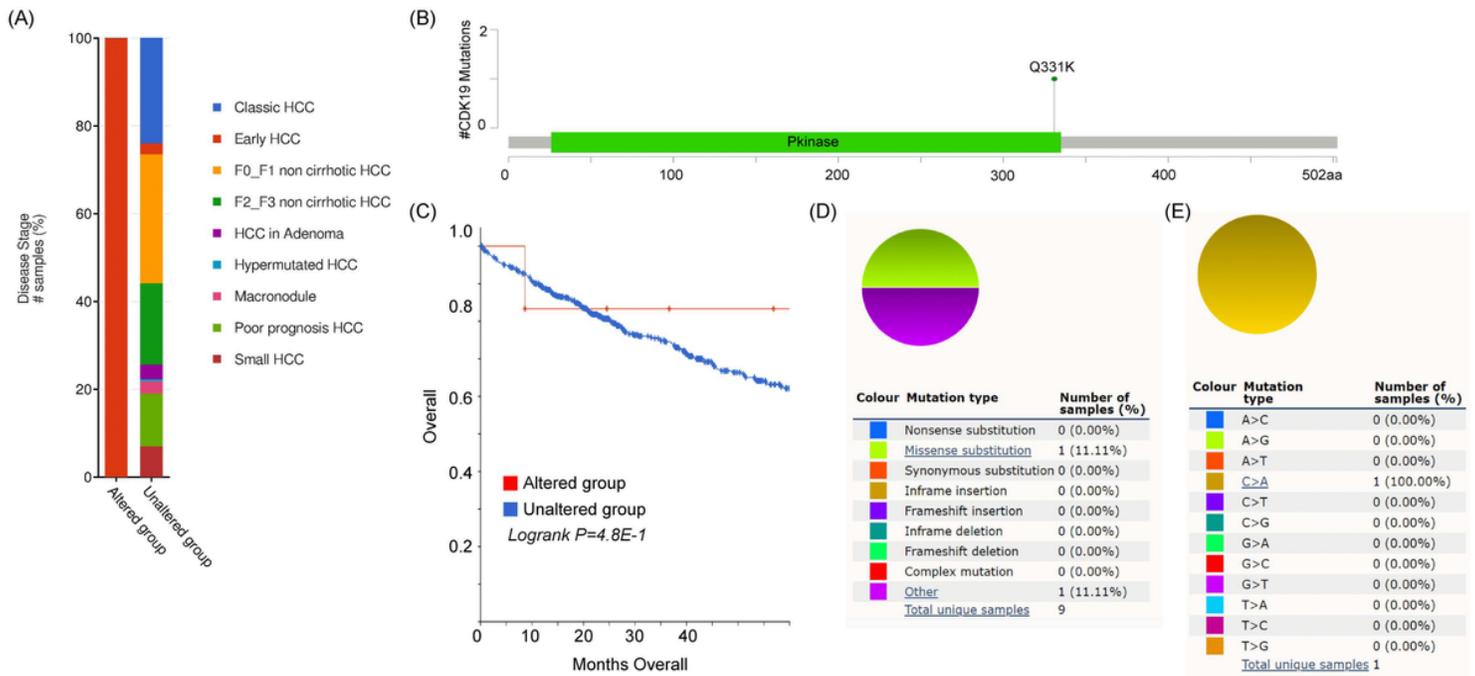


Figure 4

CDK19 mutations in hepatocellular carcinoma (HCC). A. The alterations of CDK19 gene in HCC. B. The schematic representation of CDK19 mutations in HCC. C. The relationship between CDK19 alterations and the prognosis of HCC patients. D-E, The mutation types of CDK19 (%) in HCC.

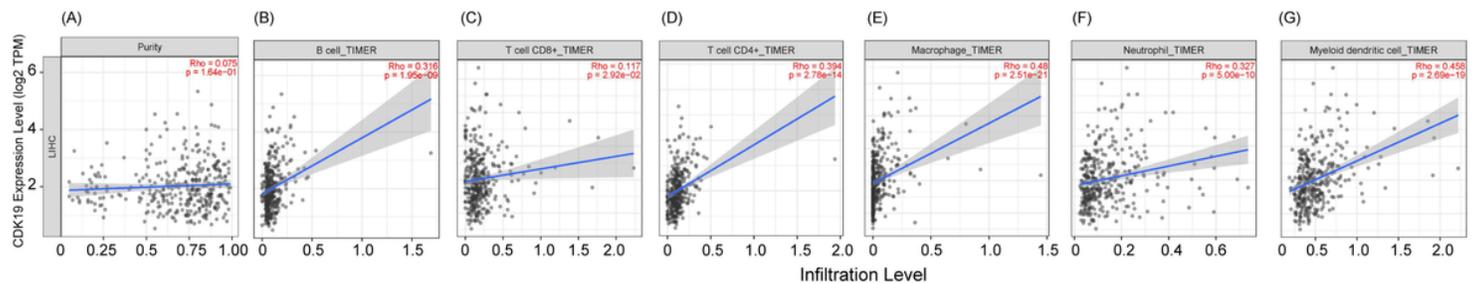


Figure 5

CDK19 was associated with immune infiltration in hepatocellular carcinoma (HCC). A. The correlation between CDK19 and tumor purity. B-G Graphs showed the correlations between CDK19 and (B) B cell, (C) T cells CD8+, (D) T cells CD4+, (E) macrophage, (F) neutrophil and (G) myeloid dendritic cell.

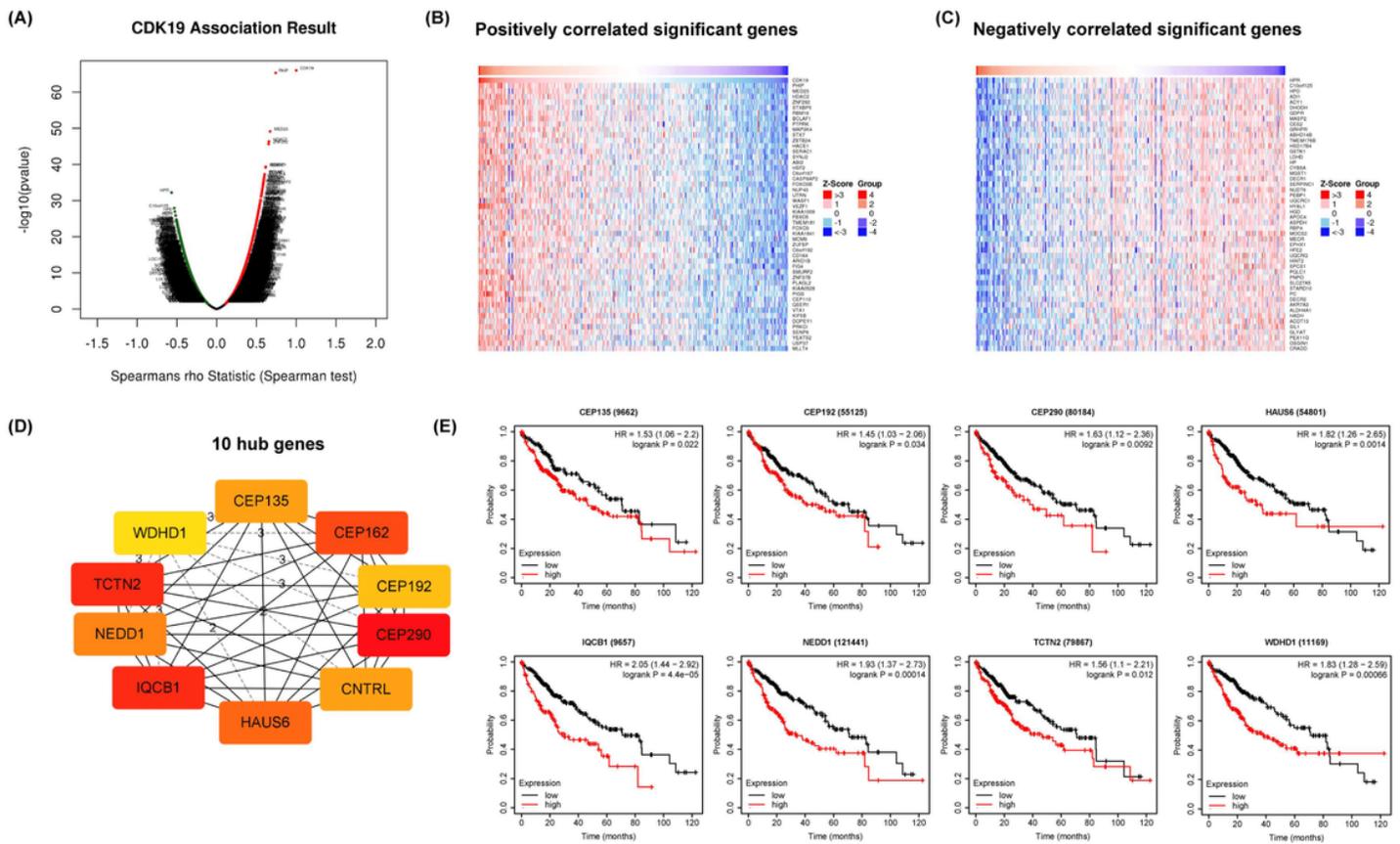


Figure 6

Genes correlated with CDK19 in hepatocellular carcinoma (HCC). A. Correlations between CDK19 and differently expressed genes (DEGs). B-C, The positively or negatively correlated genes with CDK19 (Top 50 genes). D. The 10 hub genes of CDK19 in HCC. E. The prognostic significance of the top 10 hub genes.

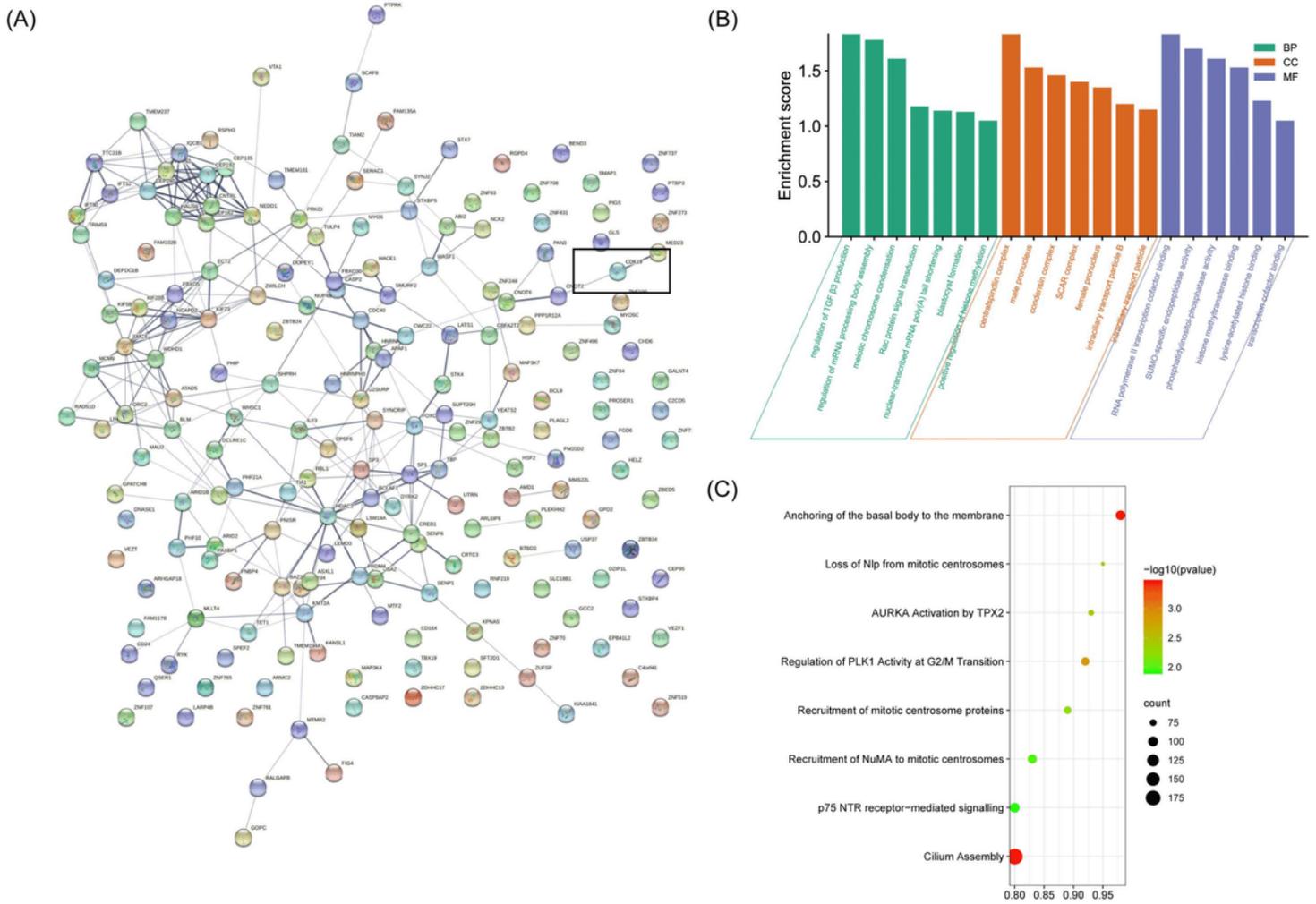


Figure 7

The Protein-Protein Interaction (PPI) and KEGG/GO biological process analysis of CDK19 in hepatocellular carcinoma (HCC). A. The interaction network of the top 200 genes. B. The GO biological process analysis of CDK19 in HCC. C. The KEGG pathway enrichment analysis of CDK19 in HCC.

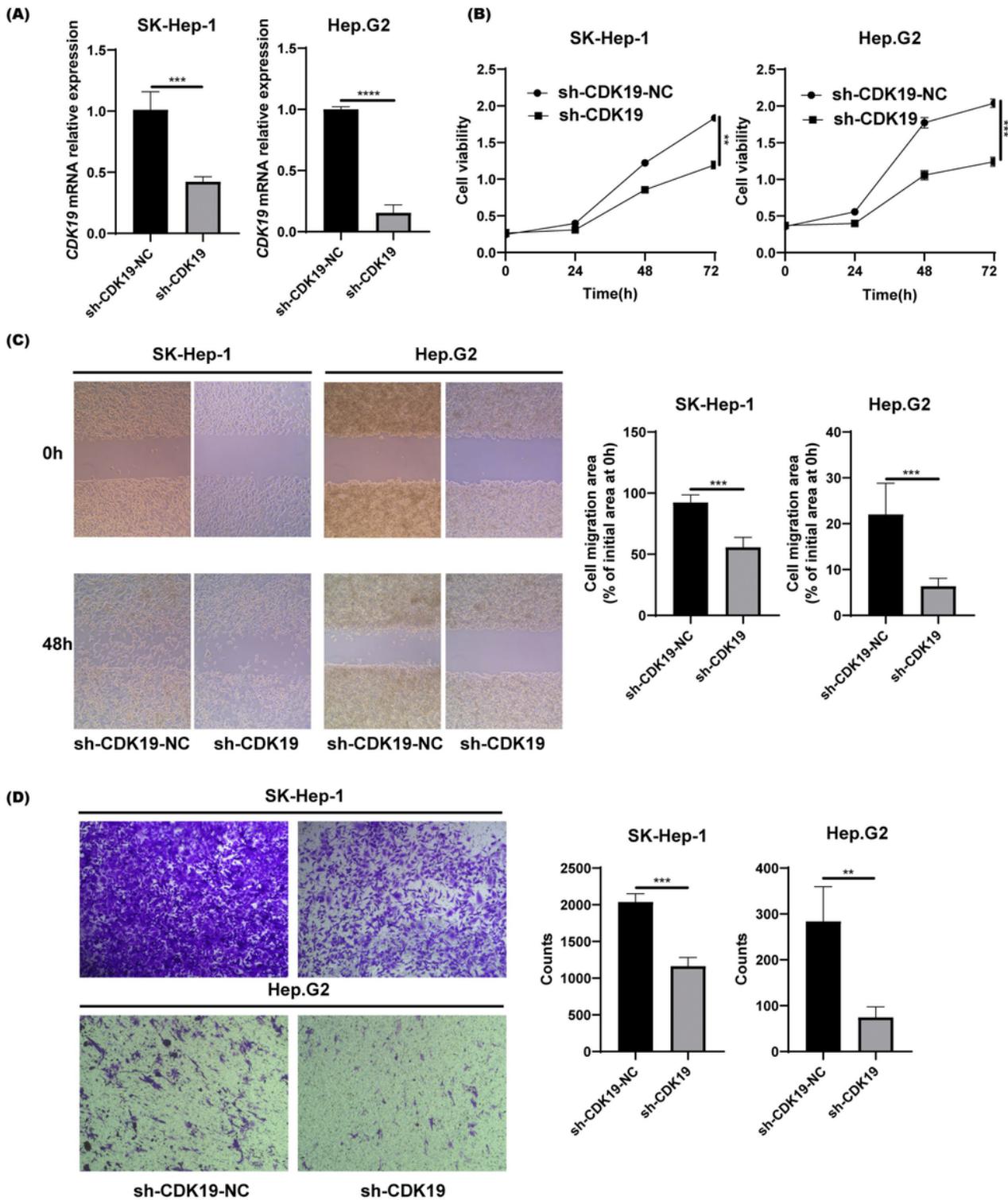


Figure 8

Functional analysis of CDK19 in hepatocellular carcinoma (HCC) cells. A. mRNA expression of CDK19 after lentiviral vector-mediated sh-CDK19-NC or sh-CDK19 infection. B. Cell viability measured by cell counting kit-8 at 0h, 24h, 48h, 72h after the infection. C. Wound healing experiment and D. Transwell experiment showed the migration and invasion of HCC cells after CDK19 knocking-down. * $P < .05$; ** $P < .01$; *** $P < .001$.

Supplementary Files

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