Clinical significance of cyclin-dependent kinase inhibitor 3 in hepatocellular carcinoma

XING LIU[1], KAIYUAN CHANG[2] and JIAN LIU[3]

[1]Department of Emergency, The Second People's Hospital of Futian District Shenzhen, Shenzhen, Guangdong 518049, P.R. China;
[2]Department of Laboratory Medicine, Longhua District Central Hospital, Shenzhen, Guangdong 518100, P.R. China;
[3]Department of Emergency, The First Hospital of Hunan University of Chinese Medicine, Changsha, Hunan 410007, P.R. China

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Abstract. The aim of the present study was to determine the expression of cyclin-dependent kinase inhibitor 3 (CDKN3) as well as its clinical value in hepatocellular carcinoma (HCC) using bioinformatic analysis. The expression of CDKN3 in HCC and its correlation with HCC prognosis were analyzed using the UALCAN and SangerBox databases. The findings obtained from the UALCAN database were validated using immunohistochemistry. The Assistant for Clinical Bioinformatics database was employed for the unifactorial and multifactorial analyses regarding the correlation between CDKN3 and pathways, and the STRING database served for the correlation analysis between CDKN3 and proteins. The results revealed that CDKN3 expression increases in tumor tissues and corresponds with an increase in the tumor stage. CDKN3 crucially affects prognosis of patients with HCC and is an essential factor for the diagnosis and prognosis of this condition. CDKN3 expression in HCC is mainly related to pathways for DNA repair, fatty acid degradation, G2M checkpoints, MYC targets, selenium compound metabolism and tumor proliferation. The role of CDKN3 in HCC was found to be related to genes including *PTTG2*, *BIRC5*, *CKS2*, *CCNB2* and *CCNA2*. In conclusion, CDKN3 exhibits a high expression in HCC tumor tissues, and such expression substantially affects tumor patients' prognosis. The role of CDKN3 role in HCC is closely related to cell cycle-related pathways, showing its potential as a therapeutic target for HCC and as an indicator for the determination of prognosis.

Correspondence to: Dr Jian Liu, Department of Emergency, The First Hospital of Hunan University of Chinese Medicine, 95 Shaoshan Middle Road, Yuhua, Changsha, Hunan 410007, P.R. China
E-mail: ljian_0731@163.com

Abbreviations: CDKN3, cyclin-dependent kinase inhibitor 3; LIHC, liver hepatocellular carcinoma; GEO, Gene Expression Omnibus; ROC, receiver operating characteristic; OS, overall survival; PFI, progression-free interval; DFI, disease-free interval; DSS, disease-specific survival; HR, hazard ratio; CI, confidence interval; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes

Key words: hepatocellular carcinoma, CDKN3, prognosis, immunohistochemistry, bioinformatics, diagnostic value

Introduction

Liver cancer is a prevalent form of malignant tumor and the deadliest malignant tumor of the digestive system. In total, ~85% of these cases are of hepatocellular carcinoma (HCC). China accounts for nearly 50% of all newly diagnosed HCC cases and 51% of all deaths worldwide (1). Surgery is considered the first choice of treatment, which, however, is accompanied by high rates of recurrence and metastasis, very low 5-year survival rate, and extremely poor prognosis (2). Despite advancements in immunotherapy and targeted therapies over the past decade, patients suffering from liver cancer still present a 5-year survival rate <20% (3). Consequently, the mechanisms underlying the prognosis of HCC shall be well elucidated and biomarkers with strong sensitivity and specificity shall be identified to contributed to a valuable regime for HCC diagnosis and treatment.

Dysfunction of cell cycle regulators is a significant event in carcinogenesis and progression. Cyclin-dependent kinase inhibitor 3 (CDKN3) is part of a family of bispecific protein phosphatases located on human chromosome 14 and is a cell cycle protein-dependent kinase inhibitor that functions well in the cell cycle regulation (4). In previous studies, CDKN3 was identified to remarkably affect the progression of several types of cancers, including rectal and cervical cancer (5,6). CDKN3 has been found to be expressed in HCC tissues, and its overexpression drives HCC cell proliferation. Therefore, CDKN3 can be regarded as an oncogene in HCC (7). Existing research have not well elucidated the specific mechanisms underlying the role of CDKN3 in HCC development. On these accounts, the present study holds the primary objective of delving deeper into the mechanism of CDKN3 in HCC.

Materials and methods

Materials. Among the HCC specimens and normal paraneoplastic tissues from the Department of Pathology of Longhua Central Hospital from January 2022 to December 2023,

16 cases with complete medical records and comprehensive clinical information were selected. The cases were not treated with radiotherapy or chemotherapy, and all pathological indices were reevaluated by two pathologists who reached a unanimous opinion. The present study adhered to the declaration of Helsinki, and approval (approval no. (2024-097-01) was obtained of the Ethics Committee of Longhua Central Hospital (Shenzhen, China). Written informed consent was acquired by all participants.

Inclusion criteria were as follows: i) Patients with liver cancer who underwent surgery at Longhua Central Hospital; ii) age ≥18 years old; iii) the specimen contains both cancerous and control adjacent or normal liver tissue; iv) complete clinical information; and v) the patient did not receive any adjuvant therapy such as chemotherapy or radiotherapy before surgery. Exclusion criteria were as follows: i) age <18 years old; ii) the specimen does not contain corresponding adjacent cancerous tissue or normal liver tissue; iii) received adjuvant therapy such as chemotherapy or radiotherapy before surgery; and iv) clinical information is incomplete.

The cancer genome atlas (TCGA) database. General information of patients with liver HCC (LIHC) was downloaded from the TCGA database (https://www.cancer.gov/ccg/research/genome-sequencing/tcga) and data on 371 patients with LIHC were obtained, including 245 male patients and 117 female patients. There were 27 patients aged 21-40, 140 patients aged 41-60, 181 patients aged 61-80, and 10 patients aged >81. There were 54 patients with stage I tumors, 173 patients with stage II tumors, 118 patients with stage III tumors, and 12 patients with stage IV tumors.

The Assistant for Clinical Bioinformatics database. The Assistant for Clinical Bioinformatics database (https://www.aclbi.com/static/index.html) is a platform that integrates information from multiple databases (8). Currently, it contains sample information for all 33 tumors from TCGA database, seven pediatric/hematologic tumors from the target database, tumor samples from the Gene Expression Omnibus (GEO) database, non-tumor sample information, cell line data from the cancer cell line encyclopedia database, and sample information of 24 tumors from the international cancer genome consortium database. The analysis steps were as follows: i) pan-cancer analysis; ii) select sample: HCC; iii) select gene: CDKN3; and iv) expression.

SangerBox database. The SangerBox database (http://sangerbox.com/home.html) is a web-based platform (9) that offers interactive graphical analysis tools, integrates multiple databases and conducts fast batch processing of these data, remarkably weakening the difficulty for users to obtain data and improving the efficiency of data processing in the analysis of raw information. The steps used were as follows: i) pan-cancer analysis; ii) prognostic cancer gene expression analysis; iii) input gene: CDKN3; iv) sample source selection: all samples; v) data source: TCGA + GTEx; vi) survival data: overall survival (OS), progression-free interval (PFI), disease-free interval (DFI) and disease-specific survival (DSS).

STRING database. STRING (https://string-db.org/) is a search tool used for analyzing biological gene or protein interactions. The interaction data hosted on the database were sourced from high-throughput experimental data, automated text mining, computer genome prediction, and data from other databases, which has the largest coverage of species and the largest amount of information on interactions among numerous protein interaction databases currently available, including a biological database containing proven and predictable proteins and protein interactions (10). In the present study, the search conditions were set as follows: i) select the protein by its name; ii) enter CDKN3 in the protein names; and iii) select *Homo sapiens* as the organism.

Gene expression profiling interactive analysis (GEPIA) database. The GEPIA database (http://gepia.cancer-pku.cn/) comprises both the TCGA cancer database and the GTEx normal tissue database (11). The screening criteria for the differential expression analysis were as follows: i) expression DIY, expression on box plots; ii) gene, CDKN3; iii) dataset selection, HCC; iv) matched normal data, TCGA normal and GETx data. The settings of the conditions for survival analysis were as follows: i) dataset selection: HCC; ii) methods: OS; iii) group cut-off: median.

Kaplan-Meier plotter database. Microarray and RNA-seq data derived from GEO, EGA and TCGA databases were utilized for the construction of the database. A total of 54,675 genes were subjected to meta-analysis by integrating gene expression information with clinical prognostic information, and survival-related molecular markers were identified and validated (12,13). The steps used were as follows: i) start KM Plotter for liver cancer; ii) input the target gene: CDKN3; and iii) draw a Kaplan-Meier plot.

Genomic data commons (GDC) database. GDC database (https://gdc.cancer.gov/) was developed by the National Cancer Institute, consolidating data from multiple cancer databases, offering unified storage, management, and visualization while facilitating global sharing with cancer genomics researchers.

Immunohistochemistry. For 16 samples of HCC tissues and normal tissues adjacent to the cancer, paraffin-embedded 4-μm thick tissue sections were received from each sample and stained (100˚C, 15 min) with the Roche Ventana BenchMark XT immunohistochemistry system using multimer technology. The samples were heat-repaired using EDTA (pH 9.0). The slides then underwent 30 min of incubation with rabbit anti-human CDKN3 antibodies (1:200; cat. no. abs115945; Absin) at 37˚C, and another 32 min of incubation with horseradish peroxidase-labeled secondary antibodies [1:100, cat. no. K20716; Roche Diagnostics (Shanghai) Co., Ltd.] at 37˚C in succession. Tissue slices then underwent color development using diaminobenzidine and hematoxylin re-staining. Positive CDKN3 was localized in the perinuclear region and cytoplasm, with yellowish to tan coloration observed under a light microscope. Based on this staining, the percentage of positive cells for CDKN3 protein expression in tumor cells was assessed.

The proportion of positive cells to the total number of cells is ≤10%, 11-25%, 26-50% and >50%, respectively, rated as 0, 1, 2 and 3 points. At the same time, the degree of staining of positive cells was observed and no staining, light yellow, brownish yellow, and yellow brown were rated as 0, 1, 2, and 3 points, respectively. The final staining score for each slice is obtained by multiplying the positive cell percentage score with the positive cell staining degree score. A final score of 0-2 indicates no expression, while a score of 3-9 indicates positive expression.

Statistical analysis. Data analysis and processing relied on R software (version 4.2.2; https://cran.r-project.org/). The mRNA expression levels were converted to expression $\log_2$(TPM+1). Measurements with normal distribution presented in the format of the mean ± standard deviation (SD), and an independent sample t-test served for the between-group variance comparison. Measurements failing to obey a normal distribution presented as M (P25, P75), and the Mann-Whitney U test assisted in the relevant comparison. Counting data presented as the number of samples or percentages, comparison between groups relied on the χ^2 test, and grading information comparison relied on the rank-sum test. A receiver operating characteristic (ROC) curve was plotted for analyzing the diagnostic value of CDKN3 in patients. The survival of high- and low-CDKN3 expression groups were analyzed using the Kaplan-Meier survival curves followed by the log-rank test. Univariate and multivariate Cox regression analyses together assisted in ascertaining risk factors affecting the poor prognosis of HCC. P<0.05 was considered to indicate a statistically significant difference.

Results

CDKN3 gene expression analysis. According to the analysis results of the CDKN3 expression in HCC via the SangerBox, UALCAN and GEPIA databases, tumor tissue presented significantly higher CDKN3 expression versus normal tissues, and the difference exhibited a statistical significance (P<0.05) (Fig. 1A-C).

Immunohistochemistry. Immunohistochemical analysis suggested that CDKN3 was localized in the perinuclear region and cytoplasm of the cells and presented a high expression in HCC tissues versus normal tissues (P<0.05) (Fig. 2). Immunohistochemical scoring was performed on the expression of CDKN3 in 16 cases of liver cancer tissues, and the expression of CDKN3 in corresponding normal tissues was also evaluated. After statistical analysis, the positive expression rate of CDKN3 in LIHC was 93.75%, while the positive expression rate of CDKN3 in normal liver tissues was 18.75%, and the difference was statistically significant (P<0.05) (Table I).

CDKN3 at different expression levels. According to the analysis results of how CDKN3 expression affected the OS of patients with HCC by the UALCAN database, patients in the low-expression group presented considerably extended survival time versus the high-expression group and P<0.05 (Fig. 3).

Table I. Comparison of immunohistochemical positivity rates between tumor tissue and normal tissue (P<0.05).

	Positive (%)	Negative (%)
Normal	18.75 (3/16)	81.25 (13/16)
Tumor	93.75 (15/16)	6.25 (1/16)

The tumor prognosis included OS, PFI, DFI and DSS, and their correlation with CDKN3 was measured by utilizing the SangerBox database. The specific method was as follows: the optimal cut-off values for CDKN3 were calculated by virtue of the 'maxstat' package in R (maximally selected rank statistics with some P-value approximations version: 0.7-25), with the minimum grouping of samples >25% and the maximum grouping <75%. The optimal cut-off values were calculated as -9.9658 (OS), -5.0116 (PFI), -5.0116 (DFI) and -9.9658 (DSS). Based on these values, patients fell into high- and low-expression groups, and the log-rank test method was employed for a deeper interpretation of their difference in prognosis by virtue of the survfit function of the 'survival' package in R. The low-expression group showed more favorable prognosis versus the high-expression group (P<0.05) (Fig. 4).

Moreover, the prognosis of the two expression groups in patients with HCC was compared using the GEPIA database. The high-expression group exhibited considerably better DFS and OS versus the low-expression group (P<0.05) (Fig. 5).

According to the analysis on the linkage between CDKN3 expression and HCC tumor stage grading using the GEPIA database, the expression level of CDKN3 underwent an obvious elevation in higher tumor stage (P<0.05). However, in stage IV, because patients with tumors at this stage were in the advanced stage, fewer specimens could be obtained by surgical resection. Because this led to an insufficient number of specimens, the statistical data may have been skewed. However, based on the samples obtained, the levels of CDKN3 in grade I, II and III patients increased progressively with grading (Fig. 6).

ROC curves for the diagnosis of HCC by CDKN3. The Kaplan-Meier analysis was conducted on specific genes for the examination of their prognostic values. The different quartiles of gene expression were taken as the criteria to classify patients into two cohorts, with the relevant comparison results illustrated in the Kaplan-Meier survival plot, from which we obtained the hazard ratio (HR), 95% confidence interval (CI) and log-rank P-values. Collectively, CDKN3 had a favorable diagnostic value for HCC, with an area under curve (AUC) of 0.98 and 95% CI of 0.99-0.97. CDKN3 could also well predict the poor prognosis of patients with HCC (P<0.05), proving its statistical significance (Fig. 7).

Univariate and multivariate analysis. One-way multifactorial analysis was conducted using Assistant for Clinical Bioinformatics. Univariate and multifactorial analyses revealed the association between prognosis and CDKN3 expression and tumor-node-metastasis staging for patients with HCC (P<0.05) (Fig. 8).

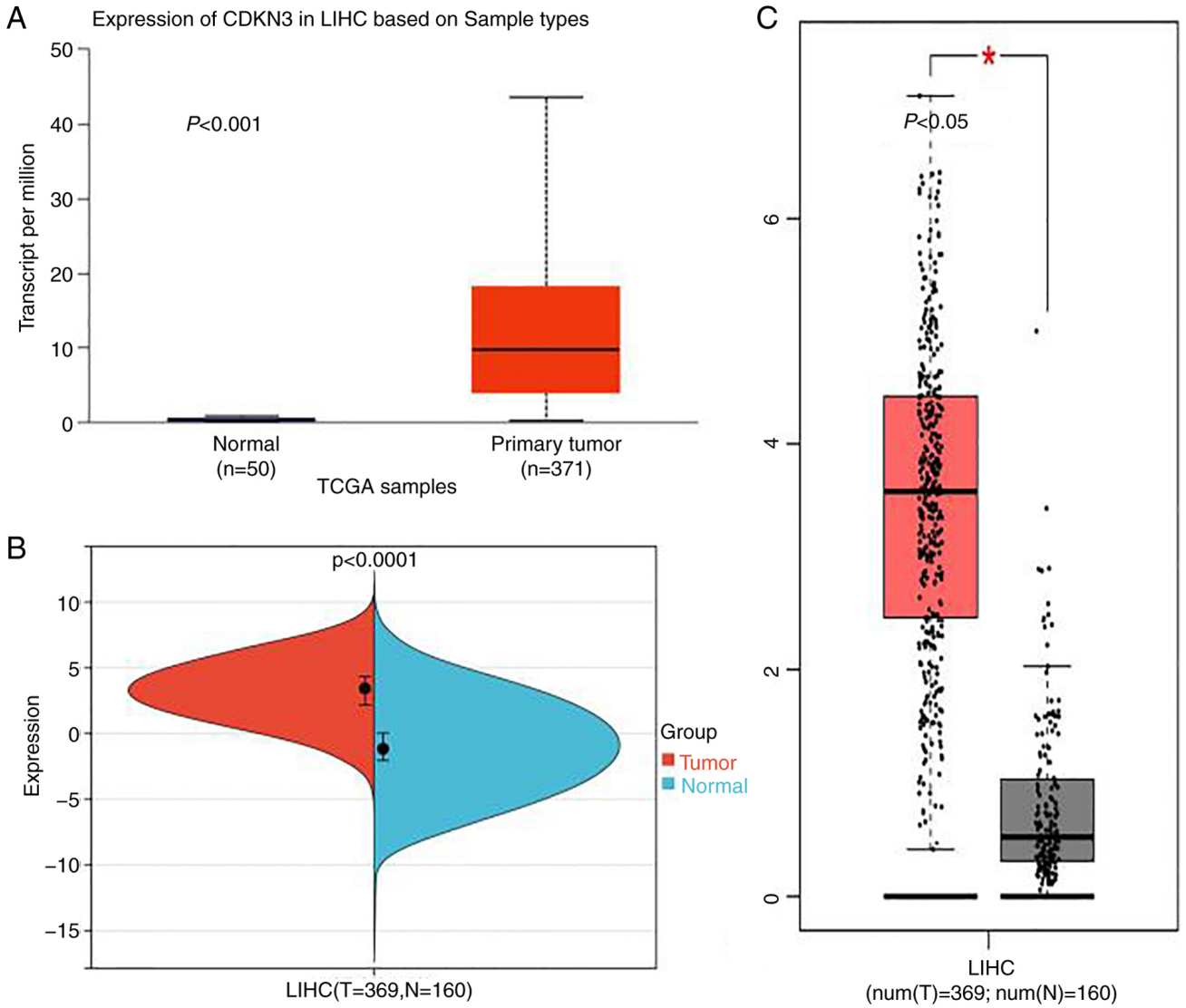

Figure 1. Expression of CDKN3. (A) Differential expression of CDKN3 between normal and tumor tissues through UALCAN database. (B) Differential expression of CDKN3 between normal and tumor tissues through SangerBox database. (C) Differential expression of CDKN3 between normal and tumor tissues through GEPIA database. *P<0.05.

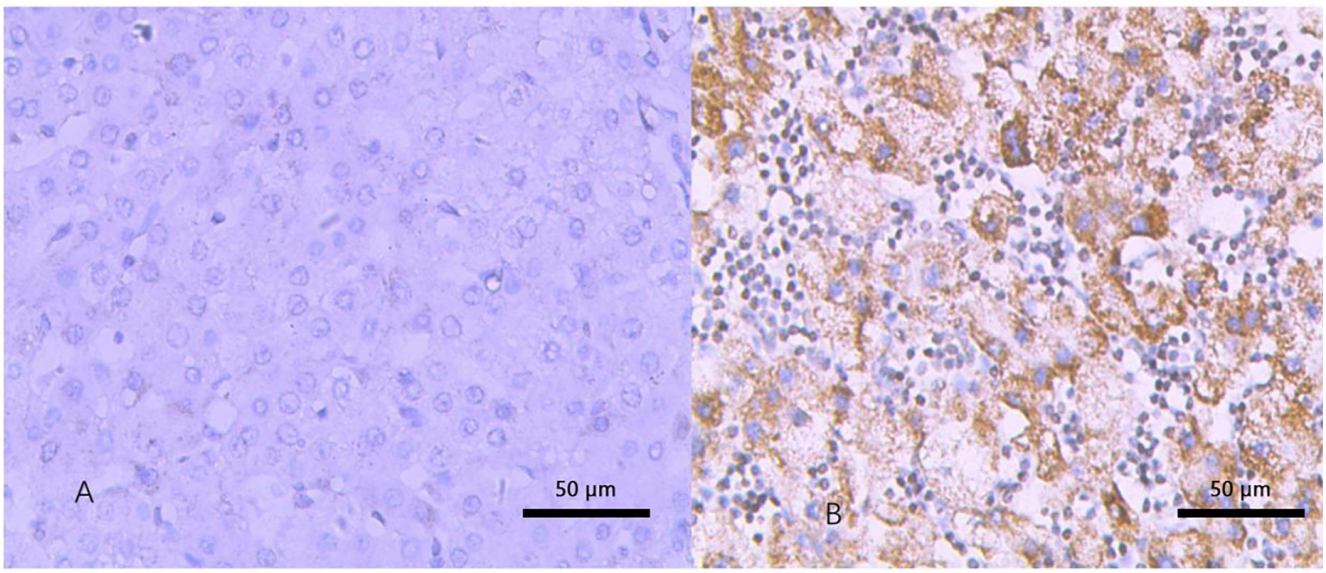

Figure 2. Immunohistochemical analysis. (A) Low-expression in tumor-adjacent normal tissues. (B) High-expression in tumor tissue.

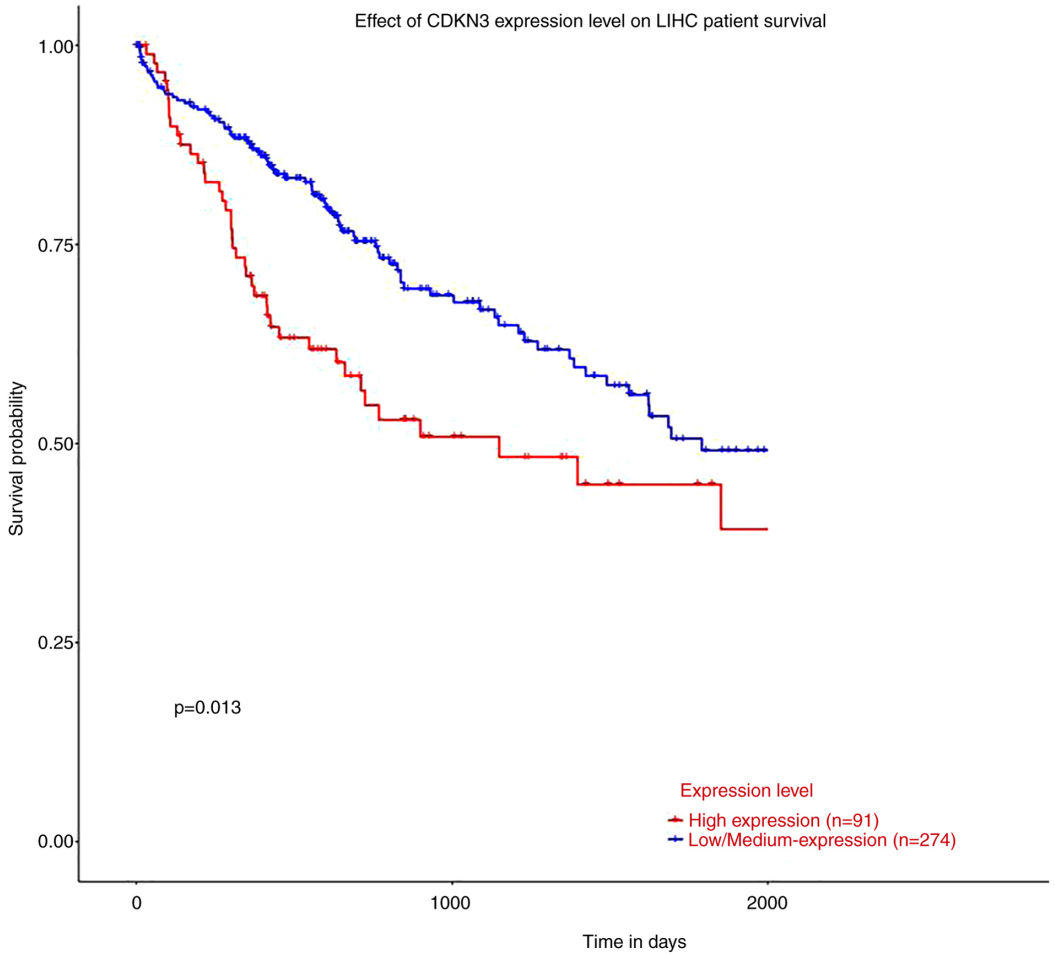

Figure 3. UALCAN database analysis of the effect of different expression levels of CDKN3 gene on survival of patients with hepatocellular carcinoma. LIHC, liver hepatocellular carcinoma.

CDKN3 and pathway correlation. RNA-seq data and the respective clinical information for HCC were obtained from the Genomic Data Commons data portal (https://portal.gdc.com). The gene set variation analysis package in R was utilized for analyzing the genes contained in relevant pathways. Lastly, the gene collection analysis was conducted based on pathway scores using Spearman correlation. All of the analysis relied on R (version 4.0.3). The present study demonstrated the participation of CDKN3 in several biological processes, including DNA repair (0.581), fatty acid degradation (0.521), G2/M checkpoint regulation (0.881), MYC targets (0.655), seleno-compound metabolism (0.484), tumor proliferation signature (0.878) and other pathways ($P<0.05$) (Fig. 9A-F).

Gene enrichment analysis. Numerous functional nodes that exhibit a conceptual overlapping phenomenon will be generated upon the direct annotation of a set of genes; hence the redundant analysis dose does not benefit the further examination. Therefore, the obtained functional nodes must be filtered and screened, thereby obtaining a larger number of meaningful functional information. The most commonly used methods are based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. According to KEGG analysis, CDKN3 is associated with the cell cycle, p53 signaling pathway, cellular senescence, human T-cell leukemia virus 1 infection, oocyte meiosis, small-cell lung cancer, progesterone-mediated oocyte maturation, cancer pathway, viral oncogenesis, FOXO signaling pathway, hepatitis B, EB virus infection, human papillomavirus infection, and other pathways; the cell cycle pathway was the most dominant pathway. GO analysis indicated that mitotic cell cycle phase transition, cell cycle phase transition, cell cycle protein-dependent protein kinase holoenzyme complex, mitotic cell cycle process, cell division, cell cycle process, mitotic cell cycle, serine/threonine protein kinase complex, protein kinase complex, cell cycle regulation, cell cycle, cell cycle protein-dependent protein kinase activity, transferase complexes, transfer of phosphorus-containing groups, protein phosphorylation, cell cycle G1/S phase transition, microtubule organizing centers, microtubule cytoskeleton, centrosome, and other pathways were related, wherein the mitotic cell cycle phase transition and cell cycle pathways were the dominant pathways (Fig. 10).

Protein-protein interaction network of CDKN3 in HCC. Proteins usually perform their function by interacting with other proteins. By studying the interaction networks between proteins, the functional regulation and signaling processes

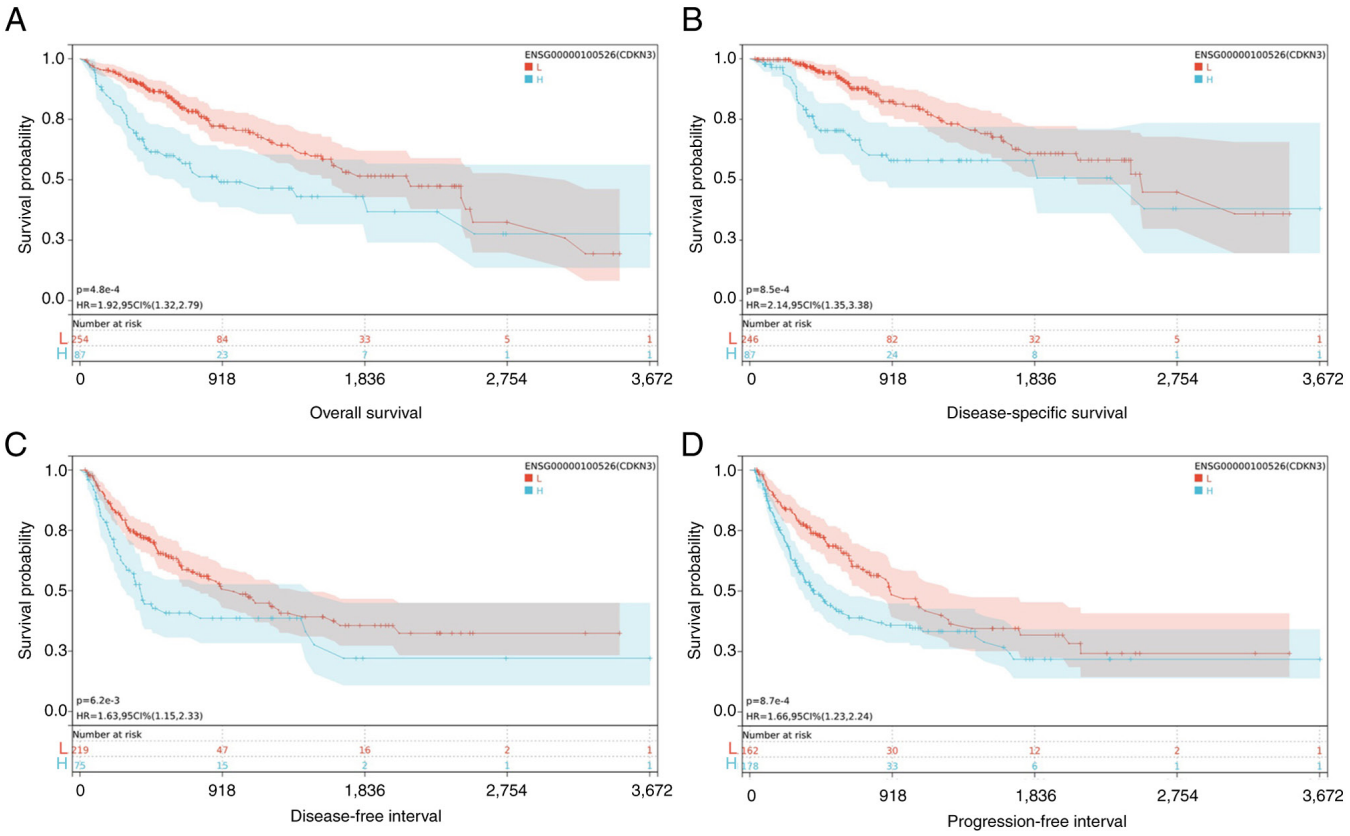

Figure 4. Association between different expression levels of CDKN3 and (A) overall survival; (B) disease-specific survival; (C) disease-free interval; (D) progression-free interval. HR, hazard ratio.

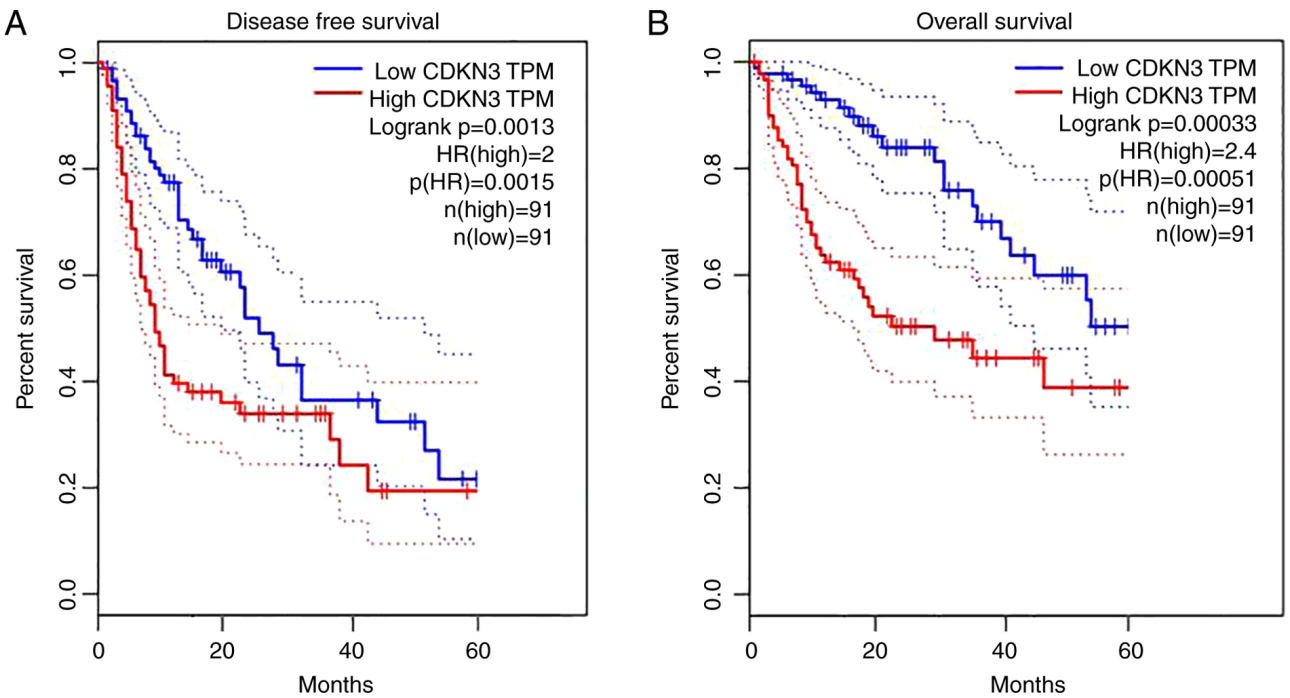

Figure 5. (A) Association through Gene Expression Profiling Interactive Analysis database between different expression levels of CDKN3 and (A) disease-free survival; (B) overall survival. CDKN3, cyclin-dependent kinase inhibitor 3; HR, hazard ratio.

of proteins within the cell can be better understood. The interactions of CDKN3 with other proteins were analyzed using the STRING database, in which multiple interacting proteins were found to be centered on CDKN3, such as PTTG2, BIRC5, CKS2, CCNB2 and CCNA2, as shown in Fig. 11.

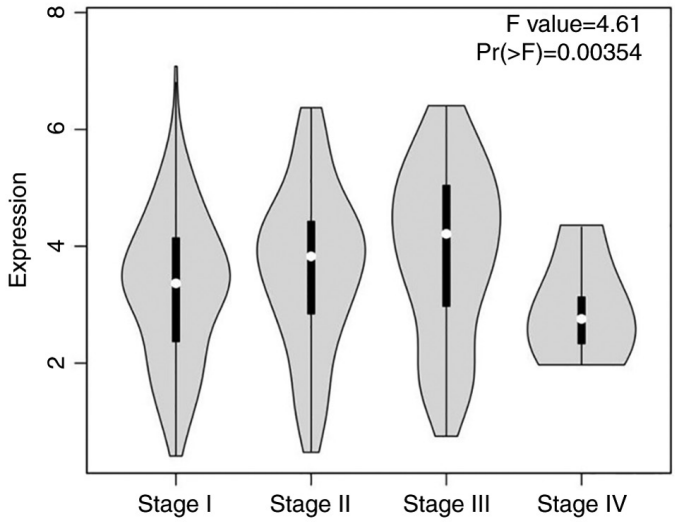

Figure 6. Relationship between tumor staging and cyclin-dependent kinase inhibitor 3 expression level.

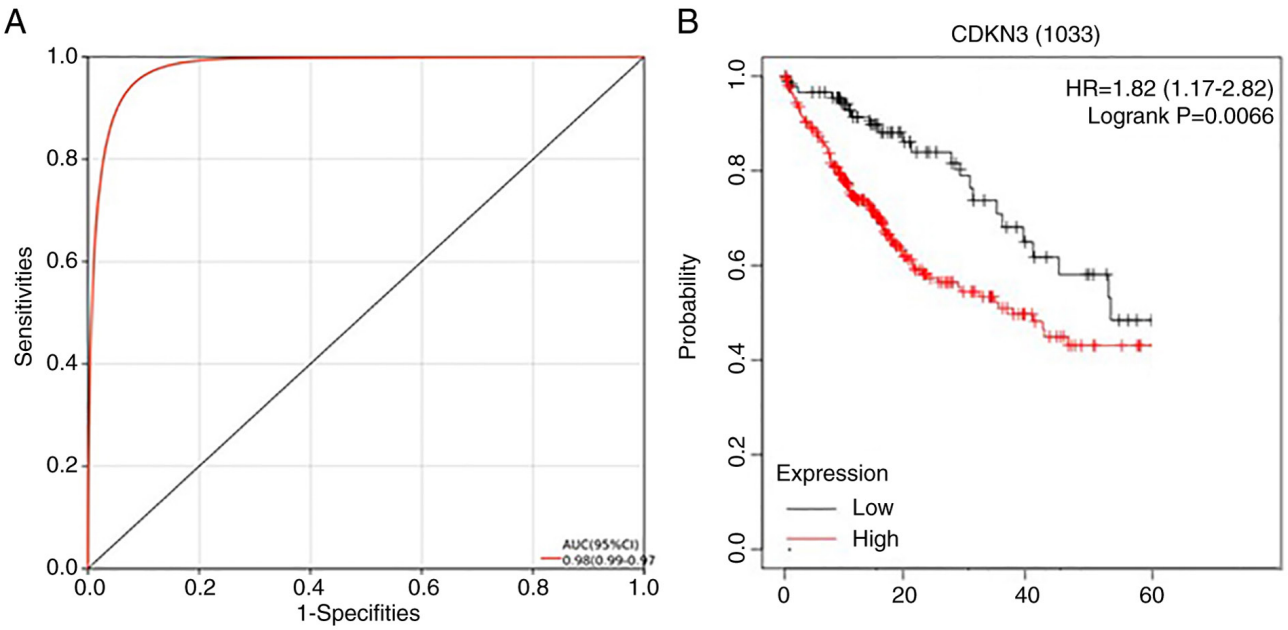

Figure 7. (A) Receiver operating characteristic curve for diagnosis of HCC by CDKN3. (B) Kaplan-Meier survival curve of CDKN3 predicting poor prognosis in patients with HCC. HCC, hepatocellular carcinoma; CDKN3, cyclin-dependent kinase inhibitor 3; AUC, area under the curve; HR, hazard ratio.

Discussion

HCC is the second most common cause of cancer-related deaths and ranks 6th in incidence, with rates currently increasing in China (14). Advances in surgical procedures, chemotherapy and targeted immunotherapy obviously prolong the life span of patients with HCC. Additionally, the widespread adoption of multidisciplinary comprehensive treatment protocols has improved patient outcomes. Despite these developments, the prevention and control of HCC remain challenging (15). On these accounts, efforts shall be made to identify new HCC markers and potential therapeutic targets, and to investigate their molecular mechanisms in the pathogenesis of HCC to prevent and control the disease.

In the present study, CDKN3 presented a high expression in HCC tumor tissues, which was further validated by immunohistochemistry, conforming to previous findings (16-18). The value of CDKN3 in diagnosing HCC and determining its prognosis was well recognized here. According to Fig. 9, CDKN3 expression in HCC is mainly related to DNA repair, fatty acid degradation, G2M checkpoint, MYC target, selenium compound metabolism, tumor proliferation markers and other pathways. According to unifactorial and multifactorial analyses, CDKN3 significantly marks worse prognosis of patients with HCC. As demonstrated in Fig. 10, the function of CDKN3 in HCC was closely correlated with cell cycle-related pathways, as demonstrated previously in other studies (7). The CDKN3 gene interacts with multiple pathways related to the cell cycle, such as DNA repair and

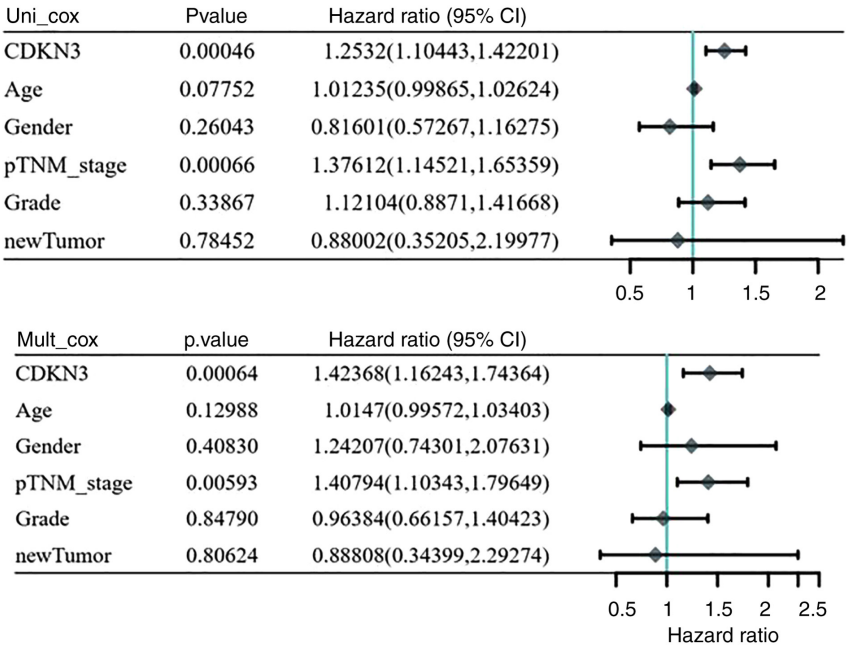

Figure 8. Single and multiple factor analysis. CDKN3, cyclin-dependent kinase inhibitor 3; CI, confidence interval.

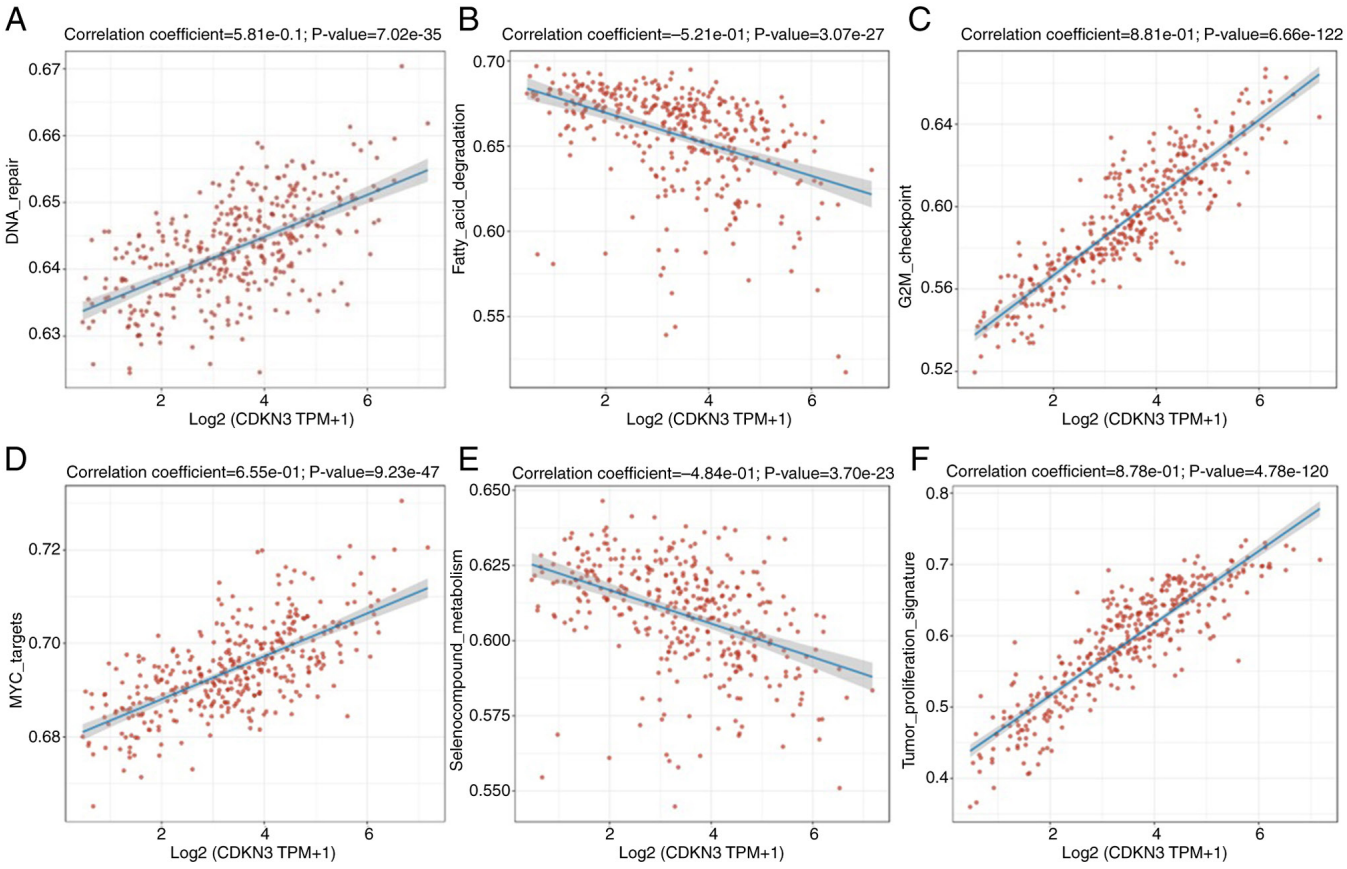

Figure 9. Correlation in hepatocellular carcinoma between CDKN3 and (A) DNA repair pathway; (B) Fatty_acid_degradation pathway; (C) G2M_checkpoint pathway; (D) MYC_targets pathway; (E) Selenocompound_metabolism pathway; (F) tumor_proliferation_signature pathway. CDKN3, cyclin-dependent kinase inhibitor 3.

G2/M checkpoint, in liver cancer to regulate tumor growth, metastasis and drug resistance. Research has shown that the KAP protein encoded by CDKN3 can inhibit the activity of CDK2, thereby affecting the normal progression of the cell cycle. In terms of DNA repair, CDKN3 may interfere with the function of CDK2, weaken the cell's ability to repair DNA

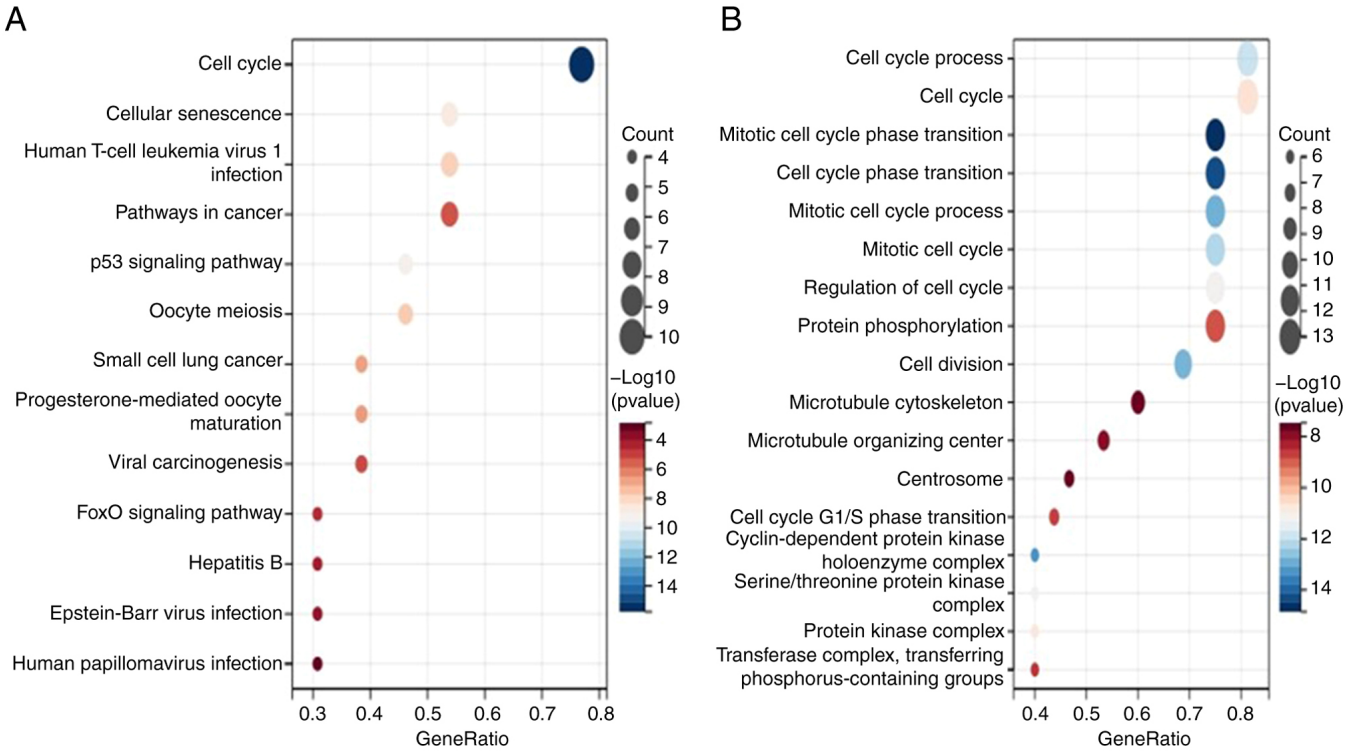

Figure 10. Gene enrichment analysis by (A) Kyoto Encyclopedia of Genes and Genomes; and (B) Gene Ontology.

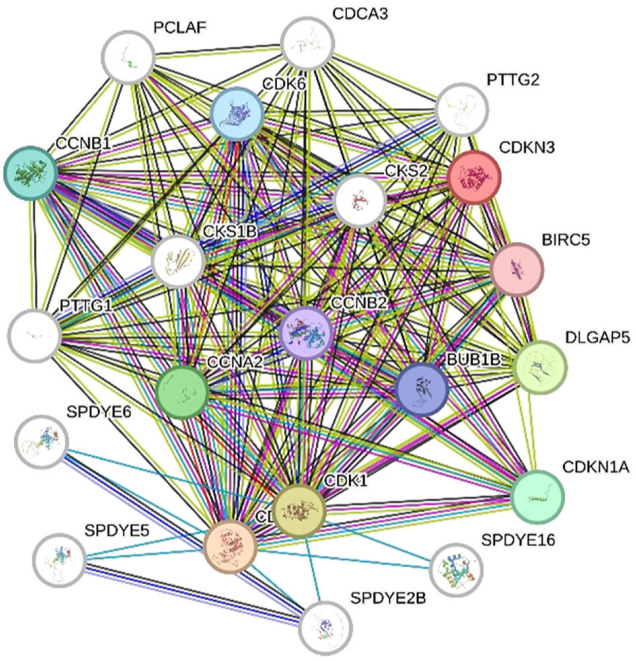

Figure 11. Protein interaction network of CDKN3. CDKN3, cyclin-dependent kinase inhibitor 3.

damage, increase genomic instability, and promote tumor occurrence and progression. In G2/M checkpoint regulation, CDKN3 may hinder the transition of cells from G2 phase to M phase by inhibiting the activity of CDK1, leading to cell cycle arrest or abnormal division, which may be related to the invasiveness and metastatic ability of tumor cells. In addition, overexpression of CDKN3 may lead to tumor cell resistance to chemotherapy drugs by affecting the expression of cell cycle related proteins. However, the specific molecular mechanisms by which CDKN3 regulates tumor growth, metastasis, and drug resistance through these pathways have not been fully elucidated, and further research is needed to reveal its detailed functional network. Thus, CDKN3 affects HCC development by controlling the cell cycle.

Cell cycle dysregulation leads to uncontrolled and abnormal cell proliferation, during which cell cycle-related proteins associated with tumorigenesis undergo aberrant changes (19). CDKN3 can well control the cell cycle by acting as a dual-specificity protein phosphatase dephosphorylating cell cycle protein dependent kinase (20). Hence, CDKN3 can negatively regulate cell cycle progression, thus crucially impacting the development of various tumors (4,21-22).

In summary, the role of CDKN3 gene in LIHC has received widespread attention in recent years. Research has shown that CDKN3 plays an important role in the occurrence and development of liver cancer by regulating the cell cycle progression. The protein KAP (CDK2 Associated Protein) encoded by CDKN3 can inhibit the activity of cell cycle dependent kinase, thereby affecting cell proliferation. In liver cancer, CDKN3 often exhibits abnormal expression, and its overexpression is closely related to the invasiveness, metastatic ability, and poor prognosis of the tumor. Therefore, CDKN3 is considered a potential therapeutic target. By targeting and inhibiting the expression or function of CDKN3, it may effectively suppress the proliferation of liver cancer cells and induce their apoptosis, providing a new approach for precise treatment of liver cancer.

From the perspective of clinical translational value, the study of CDKN3 has important practical significance. Firstly, the expression level of CDKN3 may serve as a biomarker for the diagnosis and prognosis of liver cancer, helping clinicians identify high-risk patients earlier and develop personalized treatment plans. Secondly, the development of small molecule inhibitors or gene editing technologies targeting CDKN3 may provide new drug options for the treatment of liver cancer. In addition, the study of the interaction between CDKN3 and other signaling pathways (such as PI3K/AKT, p53) may reveal multi-target treatment strategies for liver cancer, thereby improving treatment efficacy and reducing the occurrence of drug resistance.

However, there are still some limitations in current research on CDKN3. Firstly, the present study is retrospective, and information bias may be caused by incomplete records (such as missing clinical parameters, interrupted follow-up) or improper sample preservation (such as degradation of immunohistochemical specimens). It is also possible that differences in immunohistochemistry between different batches (such as antibody clone numbers, staining processes) may lead to measurement errors in CDKN3 expression. Secondly, the specific mechanism of action of CDKN3 in liver cancer has not been fully elucidated, especially the differential expression and functional heterogeneity in different subtypes of liver cancer still need further exploration. Thirdly, existing research is mostly based on cell experiments and animal models, lacking support from large-scale clinical data, which limits the clinical translation process of CDKN3 as a therapeutic target. In addition, the safety and efficacy of CDKN3 inhibitors still need to be validated through rigorous clinical trials. Although immunohistochemistry was used to validate the expression of CDKN3 in tumor samples, the present study mainly relied on bioinformatics tools for data analysis; In the future, the authors will further validate the role of CDKN3 in LIHC through western blotting and PCR methods.

Although the present study has numerous limitations, it still has some innovation: combining clinical sample analysis, public database mining, and experimental verification to confirm the role of CDKN3 in LIHC; Multi-level research from expression characteristics, clinical significance to molecular mechanisms; A new standard for CDKN3 as a prognostic biomarker was proposed, and the optimal critical value was determined. CDKN3 was also proposed as a potential biomarker for immunotherapy, demonstrating the association between CDKN3 and tumor microenvironment.

Future research directions should focus on the following aspects: firstly, in-depth analysis of the molecular mechanism of CDKN3 in liver cancer, especially its interaction with other key signaling pathways; Secondly, conducting multi-center and large-scale clinical studies to verify the reliability of CDKN3 as a biomarker and therapeutic target; The third is to develop efficient and low toxicity CDKN3 targeted drugs, and explore their combined effects with other treatment methods such as immunotherapy and radiotherapy. Through interdisciplinary collaboration and the application of cutting-edge technologies, CDKN3 is expected to become an important breakthrough point in the field of liver cancer treatment.

In summary, the present study has laid a theoretical foundation for the pathogenesis and immunotherapy research of LIHC. However, there are also some shortcomings: differences in sample sources, sequencing platforms, and batches in public databases may lead to biased results and affect the comparability of CDKN3 expression. Lack of functional validation: Bioinformatics only provides relevant conclusions and lacks experimental validation of the protein level and specific molecular mechanism of CDKN3; The molecular characteristics of subtypes of liver cancer (such as HBV/HCV related and non-alcoholic fatty liver cancer) are different, but some datasets are not clearly stratified, which limits the generalization of conclusions Although the co-expression network or pathway enrichment of CDKN3 can be predicted, its upstream regulation (such as methylation, miRNA) and downstream effects in liver cancer still require experimental exploration.

In addition, the present study also has certain limitations, such as not being able to fully control for all potential confounding factors, such as the patient's genetic background, environmental exposure, comorbidities and treatment history. These factors may affect the association between CDKN3 expression and liver cancer progression, leading to biased results. In future research, it will be attempted to collect treatment response data and information on co-existing diseases in patients, conduct supplementary analysis on existing molecular marker data and concurrently improve the analysis method to control as numerous known confounding factors as possible.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XL conducted experiments, wrote and edited the manuscript. KC wrote the original draft and collected data. JL designed the study, performed data curation and project administration. XL and KC confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study adhered to the declaration of Helsinki, and approval (approval no. (2024-097-01) was obtained of the Ethics Committee of Longhua Central Hospital (Shenzhen, China). Written informed consent was acquired by all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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