Research Article

Elevated CDK5R1 predicts worse prognosis in hepatocellular carcinoma based on TCGA data

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Background: Hepatocellular carcinoma (HCC) is a malignant tumor with rapid progression, high recurrence rate and poor prognosis. The objective of our investigation was to explore the prognostic value of CDK5R1 in HCC.

Methods: The raw data of HCC raw data were downloaded from The Cancer Genome Atlas (TCGA) database. The Wilcoxon signed-rank test, Kruskal–Wallis test and logistic regression were applied to investigate the relevance between the CDK5R1 expression and clinicopathologic characteristics in HCC. Kaplan–Meier and Cox regression analysis were employed to examine the association between clinicopathologic features and survival. Gene set enrichment analysis (GSEA) was applied to annotate the biological function of CDK5R1.

Results: CDK5R1 was highly expressed in HCC tissues. The high expression of CDK5R1 in HCC tissues was significantly associated with tumor status ($P=0.00$), new tumor event ($P=0.00$), clinical stage ($P=0.00$) and topography ($P=0.00$). Elevated CDK5R1 had significant correlation with worse overall survival (OS; $P=7.414e-04$), disease-specific survival (DSS; $P=5.642e-04$), disease-free interval (DFI; $P=1.785e-05$) and progression-free interval (PFI; $P=2.512e-06$). Besides, univariate and multivariate Cox regression analysis uncovered that increased CDK5R1 can independently predict adverse OS ($P=0.037$, hazard ratio [HR] = 1.7 (95% CI [1.0–2.7])), DFI ($P=0.007$, hazard ratio [HR]= 3.0 (95% CI [1.4–6.7])), PFI ($P=0.007$, hazard ratio [HR]= 2.8 (95% CI [1.3–5.9])). GSEA disclosed that notch signaling pathway and non-small cell lung cancer were prominently enriched in CDK5R1 high expression phenotype.

Conclusions: Increased CDK5R1 may act as a promising independent prognostic factor of poor survival in HCC.

Introduction

Primary liver cancer ranks as the fourth most common malignant tumor and the sixth leading cause of cancer incidence in the world, with a 5-year survival rate of 18% [1]. Hepatocellular carcinoma (HCC) constitutes 85–90% of primary liver cancer [2], we mainly focus on HCC in the present study. Although local hepatectomy makes it possible to cure HCC, the overall survival outcome of HCC remains poor. The 5-year local recurrence rate after radical resection is much more than 70% [3]. When HCC related symptoms occur, the average survival time of patients is just approximately 3–4 weeks [4]. Take into account this situation, early prediction of the prognosis before and after treatment is of great significance to improve the 5-year survival rate. On the one hand, it is the key step for the doctor to formulate the correct treatment plan [5]; on the other hand, it is helpful to encourage patients to actively strengthen the monitoring of abnormal indicators, detect abnormalities in time, and treat as early as possible. However, a robust prognostic biomarker of HCC remains limited.
Cyclin-dependent kinase 5 (CDK5) is a unique member of the cyclin-dependent kinases (Cdks) family of serine/threonine kinases [6]. CDK5 not only plays an important regulatory role in the physiological and pathological processes of the nervous system, but also regulates cell apoptosis and senescence, and works in a variety of tumors [7–9]. Recent studies have found that CDK5 has the effect of driving G1-S and RB phosphorylation in medullary thyroid carcinoma models [10]. It must bind to the activator to exert its activity. P35 is one of the two activators of CDK5, which is encoded by Cyclin-dependent kinase 5 regulatory subunit 1 (CDK5R1), and thus CDK5R1 plays a crucial role in the proper activity of CDK5 [8]. Previous studies have reported that overexpressed CDK5 and CKD5R1 (P35) could promote the progression and metastasis of lung cancer [11], similar results can be seen in melanoma [12], pancreatic cancer [13], large B-cell lymphoma [14] and head and neck squamous cell carcinoma [15]. However, the role and clinical significance of CDK5 and CKD5R1 (P35) in hepatocellular carcinoma have not been reported so far. This article seeks to explore the role of CDK5R1 in HCC and its potential prognostic value.

Materials and methods
Patient information
The RNA-sequencing data and corresponding patient clinical information were collected from the TCGA data repository (https://portal.gdc.cancer.gov/repository), involving 374 HCC samples and 50 normal samples, and workflow type was HTSeq-FPKM. The clinical features of HCC patients including age, serum AFP value, BMI, family history, clinical stage, topography (T), lymph node (N), metastasis (M), residual tumor, tumor status, gender, vascular invasion, histologic grade, Child-Pugh, new tumor event, virus, tumor weight, risk factor (alcohol consumption and/or viral hepatitis), postoperative ablation embolization and radiation were recorded. Some unavailable or unclear clinical information was removed. Moreover, in order to verify the expression of CDK5R1 in HCC tissues, gene expression profiles of GSE121248 and GSE62232 were downloaded from the Gene Expression Omnibus (GEO) database. The selection criteria for the data set were: (1) primary hepatocellular carcinoma; (2) complete microarray data; (3) containing cancerous and matched paracancerous tissues (4) the cause of HCC has a wide coverage, including viral infections such as HCV and HBV, heavy drinking, non-alcoholic steatohepatitis and so on.

Enrichment analysis of GSEA
GSEA is a method that can be used for analysis and calculations so as to ascertain whether the apriori defined group of genes has a consistent and statistically significant difference between two biologic status [16]. In the present study, an ordered list of all genes was firstly produced based on the basis of their association with CDK5R1 expression by GSEA. The expression level of CDK5R1 was served as a phenotype label. The number of gene set permutations were 1000 times for each analysis. The statistical significance of pathways is dependent on normal $P$-value $<0.05$ and false discovery rate (FDR) $q$-val $<0.05$.

Statistical analysis
All statistical analyses were performed with R (version 3.6.1, 2019-07-05, R Foundation, Vienna, Austria), the expression of CDK5R1 between HCC and normal groups was compared by Wilcoxon rank sum tests, and adjacent normal tissues by Wilcoxon signed-rank tests. The relationship between CDK5R1 expression and clinicopathologic characteristics were conducted on the Wilcoxon signed-rank test or Kruskal–Wallis test and logistic regression. The association between the expression of CDK5R1 and survival outcome along with other clinicopathological characteristics was carried out using Cox regression analysis and Kaplan–Meier. In the Cox regression analysis, $P<0.05$ means statistically significant. The median expression value of CDK5R1 was considered to be the cut-off value.

Construction of PPI network
To investigate the interaction between CDK5R1 and other genes, we established a CDK5R1-related PPI network via the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https://string-db.org/) [17] with a minimum required interaction score $>0.4$, and Cytoscape 3.7.1 [18] was applied to visualize these interactions after hiding the disconnected nodes.

Results
Clinicopathologic features of patients with HCC
Clinicopathologic features of patients with HCC including age, serum AFP value, BMI, family history, stage, topography (T), lymph node (N), metastasis (M), residual tumor, tumor status, gender, vascular invasion, histologic grade,
CDK5R1 was overexpressed in HCC

In our research, Wilcoxon rank sum test was used to compare the CDK5R1 expression in 374 HCC tissues and 50 normal tissues. CDK5R1 was significantly elevated in HCC (P=1.565e−17) (Figure 1A). In addition, compared with 50 adjacent normal tissues, the expression of CDK5R1 was prominently increased in HCC (P=3.536e−09) based on Wilcoxon signed-rank tests (Figure 1B). Further, to verify CDK5R1 expression in other datasets, we downloaded GSE 121248 and GSE 62232 datasets from GEO database. The results also indicated that the expression of CDK5R1 was high in HCC compared with normal tissues (Figure 1C,D).

The effects of overexpressed CDK5R1 on clinicopathological characteristics

As shown in (Figure 2A–F), increased CDK5R1 had a significant correlation with histologic grade ((G1-2 vs. G3-4, P=0.004), clinical stage (Stage I–II vs. Stage III–IV, P=2.185e−04), topography (T1-2 vs. T3-4, P=5.232e−04), tumor status (P=0.002), AFP (AFP<20 vs. 20≤AFP<400 vs. AFP≥400, P=0.023) and new tumor event (P=0.016).

Logistic regression was applied to analyze the relationship between CDK5R1 expression and clinicopathologic features (Table 2). We found that overexpressed CDK5R1 was significantly associated with tumor status (OR = 2.28 for with tumor vs. tumor free, P=0.00), new tumor event (OR = 1.95 for yes vs. no, P=0.00), clinical stage (OR = 2.10 for III-IV vs. I-II, P=0.00) and topography (OR = 2.08 for T3-4 vs. T1-2, P=0.00). Taken together, high expression of CDK5R1 (based on median expression value) was closely related to worse clinicopathologic characteristics and prone to have a poor prognosis.

Correlation between clinicopathologic features and survival

Kaplan–Meier unclosed that elevated CDK5R1 had a significant correlation with worse overall survival (OS; P=7.414e−04), disease-specific survival (DSS; P=5.642e−04), disease-free interval (DFI; P=1.785e−05) and progression-free interval (PFI; P=2.512e−06), which suggested that HCC patients with high CDK5R1 had a tendency to have shorter survival time than that with low CDK5R1 (Figure 3A–D).

Univariate analysis for OS with Cox regression model showed that poor OS had prominently correlation with CDK5R1 expression (high vs. low; P=0.033, HR = 2.0 (95% CI [1.1–3.9])), new tumor event (yes vs. no; P=0.012, HR = 3.0 (95% CI [1.3–7.0])), tumor status (with tumor vs. tumor free; P=0.001, HR = 4.0 (95% CI [1.8–9.2])), CDK5 expression (high vs. low; P=0.032, HR = 2.4 (95% CI [1.1–5.2])), CDC25B expression (high vs. low; P=0.005, HR = 3.1 (95% CI [1.4–6.8])) (Table 3). However, at multivariate Cox regression analysis, CDK5R1 expression (high vs. low; P=0.037, HR = 1.7 (95% CI [1.0–2.7])), tumor status (with tumor vs. tumor free; P=0.004, HR = 3.3 (95% CI [1.5–7.6])), the expression of CDC25B (high vs. low; P=0.011, HR = 1.9 (95% CI [1.2–3.1])) could independently predict adverse OS (Table 3, Figure 4A). Besides, this revealed that patients with elevated CDK5R1 have a 1.7 times higher risk of adverse OS than patients with low CDK5R1 expression.

Univariate Cox analysis of DFI disclosed that highly expressed CDK5R1 had a prominent effect on DFI (P=0.000, hazard ratio [HR]= 2.7 (95% CI [1.6–4.5])), other clinical factor, for instance TNM (T (T3-4 vs. T1-2): P=0.043, HR = 2.0 (95% CI [1.0–3.9])), clinical stage (III-IV vs. I-II) (P=0.022, hazard ratio [HR]= 2.1 (95% CI [1.1–4.1])) was also associated with shorter DFI. At multivariate analysis, CDK5R1 (high vs. low; P=0.007, hazard ratio [HR]= 3.0 (95% CI [1.4–6.7])) were the clinicopathologic characteristics that remained significantly correlated with DFI (Table
Table 1 HCC patient characteristics based on TCGA

| Clinical characteristics | Total | %  |
|--------------------------|-------|----|
| Age (years)              |       |    |
| >40                      | 336   | 90.8|
| ≤40                      | 34    | 9.2 |
| Gender                   |       |    |
| male                     | 250   | 67.4|
| female                   | 121   | 32.6|
| BMI                      |       |    |
| ≥25                      | 158   | 47.2|
| <25                      | 177   | 52.8|
| Family history           |       |    |
| Yes                      | 112   | 35.0|
| No                       | 208   | 65.0|
| Histologic grade         |       |    |
| G1-G2                    | 232   | 63.4|
| G3-G4                    | 134   | 36.6|
| Clinical stage           |       |    |
| I-II                     | 257   | 74.1|
| III-IV                   | 90    | 25.9|
| T                        |       |    |
| T1-T2                    | 275   | 74.7|
| T3-T4                    | 93    | 25.3|
| N                        |       |    |
| N0                       | 252   | 98.4|
| N1                       | 4     | 1.6 |
| M                        |       |    |
| M0                       | 266   | 98.5|
| M1                       | 4     | 1.5 |
| Residual tumor           |       |    |
| R0                       | 324   | 94.7|
| R1                       | 18    | 5.3 |
| Tumor status             |       |    |
| tumor free               | 201   | 57.1|
| with tumor               | 151   | 42.9|
| Vascular invasion        |       |    |
| Yes                      | 109   | 34.6|
| No                       | 206   | 65.4|
| Child-Pugh               |       |    |
| A                        | 217   | 90.8|
| B-C                      | 22    | 9.2 |
| AFP                      |       |    |
| AFP<20                   | 147   | 52.9|
| 20<AFP<400               | 66    | 23.7|
| AFP≥400                  | 65    | 23.4|
| New tumor event          |       |    |
| Yes                      | 169   | 48.3|
| No                       | 181   | 51.7|
| Risk factor              |       |    |
| Alcohol consumption and viral hepatitis | 39 | 11.0 |
| Alcohol consumption      | 79    | 22.1|
| Viral hepatitis           | 117   | 32.7|
| Neither                  | 123   | 34.4|
| Postoperative ablation embolization |   |     |
| Yes                      | 28    | 8.1 |
| No                       | 319   | 91.9|

Continued over
Table 1 HCC patient characteristics based on TCGA (Continued)

| Clinical characteristics | Total | %     |
|--------------------------|-------|-------|
| Radiation therapy        |       |       |
| Yes                      | 8     | 2.3   |
| No                       | 338   | 97.7  |

AFP = alpha fetal protein; BMI = Body Mass Index; M = distant metastasis; N = lymph node metastasis; T = topography distribution.

Figure 1. CDK5R1 is elevated in HCC

(A) CDK5R1 showed prominently high expression in HCC samples than in normal samples via Wilcoxon rank sum test. (B) The expression of CDK5R1 was significantly increased in HCC tissues compared with adjacent non-cancerous tissues via Wilcoxon signed-rank test. (C and D) showed CDK5R1 was prominently elevated in HCC samples from GSE 121248 and GSE 62232; CDK5R1, Cyclin-dependent kinase 5 regulatory subunit 1.
Figure 2. Association between CDK5R1 expression and clinicopathologic characteristics

As we can see from panels (A–F), elevated CDK5R1 was significantly correlated with (A) histologic grade, (B) clinical stage, (C) topography, (D) tumor status, (E) AFP, (F) New tumor event; AFP, alpha fetoprotein; M, distant metastasis; N, lymph node metastasis; T, topography distribution.

4, Figure 4B). This showed that patients with increased CDK5R1 have a 3.0 times higher risk of poor DFI than patients with low CDK5R1 expression.

Univariate Cox regression analysis of progression free interval (PFI) revealed that worse PFI was significantly associated with advanced TNM (T (T3-4 vs. T1-2): \( p=0.047 \), hazard ratio [HR]= 1.9 (95% CI [1.0–3.6])), clinical stage (III-IV vs. I-II) (\( p=0.026 \), HR = 2.0 (95% CI [1.1–3.8])), postoperative ablation embolization (yes vs. no) (\( p=0.003 \), HR = 2.7 (95% CI [1.4–5.1])), tumor status (with tumor vs. tumor free) (\( p=0.000 \), HR = 37.1 (95% CI [14.2–97.4])), residual tumor (R1-2 vs. R0) (\( p=0.001 \), HR = 6.4 (95% CI [2.2–18.3])), elevated CDK5R1 (\( p=0.000 \), HR = 2.5 (95% CI [1.5–4.2])), as shown in Table 4. What's more, multivariate analysis with Cox regression model uncovered that high expression of CDK5R1 was an independent prognostic factor for PFI, with an HR of 2.8 (\( p=0.007 \), 95% CI [1.3–5.9]), while other clinical factor, for instance, the expression of CDC25B (high vs. low) (\( p=0.044 \), HR = 1.8 (95% CI [1.0–3.2])) was also independently associated with poor PFI (Table 5, Figure 4C). This uncovered that patients with highly expressed CDK5R1 have a 2.8 times higher risk of poor PFI than patients with low CDK5R1 expression.
Table 2 Relationship between CDK5R1 expression and clinicopathologic figures by logistic regression

| Clinical characteristics                              | Total (N) | Odds ratio in CDK5R1 expression | P-value |
|-------------------------------------------------------|-----------|---------------------------------|---------|
| Age (>40 vs. ≤40)                                     | 370       | 1.00 (0.49–2.04)                | 1.000   |
| Gender (male vs. female)                              | 371       | 0.88 (0.57–1.35)                | 0.560   |
| BMI (≥25 vs. <25)                                     | 335       | 0.76 (0.49–1.17)                | 0.210   |
| Family history (yes vs. no)                           | 320       | 0.9 (0.57–1.42)                 | 0.640   |
| Child-Pugh (B-C vs. A)                                | 239       | 1.23 (0.51–3.04)                | 0.640   |
| AF (AFP≥400 vs. AFP<20)                               | 212       | 1.68 (0.93–3.05)                | 0.080   |
| 20≤AFP<400 vs. AFP<20                                  | 213       | 1.27 (0.71–2.28)                | 0.420   |
| AF≥400 vs. 20AFP<400                                   | 131       | 1.32 (0.67–2.65)                | 0.420   |
| Tumor status (with tumor vs. tumor free)              | 352       | 2.28 (1.48–3.52)                | 0.000   |
| Tumor weight                                          |           |                                 |         |
| 1000 °W > 1000 vs. W ≤ 500                            | 268       | 1.05 (0.42–2.64)                | 0.920   |
| 1000 ≤ W ≥ 500 vs. W < 500                            | 278       | 1.57 (0.73–3.49)                | 0.250   |
| 1000 > W > 1000 vs. W ≤ 500                           | 50        | 0.67 (0.21–2.09)                | 0.490   |
| Vascular invasion (yes vs. no)                         | 315       | 1.23 (0.77–2.00)                | 0.380   |
| New tumor event (yes vs. no)                           | 350       | 1.95 (1.28–3.00)                | 0.000   |
| Residual tumor (R1-2 vs. R0)                          | 342       | 1.00 (0.38–2.62)                | 1.000   |
| Histologic grade (≥3 vs. 1-2)                          | 366       | 1.53 (1.00–2.35)                | 0.050   |
| Clinical stage (III vs. II)                            | 347       | 2.10 (1.29–3.47)                | 0.000   |
| T (T3-4 vs. T1-2)                                     | 368       | 2.08 (1.29–3.40)                | 0.000   |
| N (N1 vs. N0)                                         | 256       | 1.00 (0.12–8.44)                | 1.000   |
| M (M1 vs. M0)                                         | 270       | 0.33 (0.02–6.93)                | 0.340   |
| Radiation therapy (yes vs. no)                         | 352       | 1.00 (0.23–4.29)                | 1.000   |
| Postoperative ablation embolization (yes vs. no)       | 353       | 1.01 (0.46–2.23)                | 0.987   |
| Risk factor                                           |           |                                 |         |
| Viral hepatitis versus neither                         | 240       | 1.21 (0.72–2.02)                | 0.467   |
| Alcohol consumption versus neither                    | 202       | 1.16 (0.66–2.06)                | 0.600   |
| Alcohol consumption and viral hepatitis versus neither | 162       | 0.66 (0.31–1.38)                | 0.264   |
| Alcohol consumption versus viral hepatitis             | 196       | 0.96 (0.54–1.72)                | 0.897   |
| Alcohol consumption and viral hepatitis versus alcohol | 156       | 0.54 (0.25–1.13)                | 0.107   |
| consumption                                          | 118       | 0.56 (0.25–1.22)                | 0.152   |

Abbreviations: AFP, alpha fetal protein; BMI, body mass index; CDK5R1, cyclin-dependent kinase 5 regulatory subunit 1; M, distant metastasis; N, lymph node metastasis; T, topography distribution.

CDK5R1-related signaling pathway performed on GSEA

We employed Gene Set Enrichment Analysis (GSEA) to screen significantly activated signaling pathways between high and low CDK5R1 expression phenotype group, FDR <0.05 and NOM P-val <0.05 indicated significant differences in enrichment of MSigDB collection (c2.cp.kegg.v7.0.symbols). In our analysis, 2 signaling pathways that were prominently enriched in high CDK5R1 expression phenotype were filtered out, including notch signaling pathway and non-small cell lung cancer. (Figure 5A, Table 6).

CDK5R1-associated PPI network

The CDK5R1-associated PPI network was established with 11 points, 27 edges and an average point degree of 4.91(Figure 5B). The PPI network showed that some genes had close relationship with CDK5R1, for instance, CDK5, CAPN2, CAPN1, MAPT, CDC25B, PKA1, PPP1R1B, NTRK2, NDEL1 and YWHAE.

Discussion

Despite considerable progress has been achieved in recent years, the morbidity and mortality of HCC are still increasing. Effective prediction of prognosis is of great significance for improving the survival of patients with HCC. However, so far, the prognostic biomarker has been limited. Although accumulative studies have demonstrated the
Figure 3. Survival outcomes based on Kaplan–Meier analysis
Kaplan–Meier survival analysis showed that increased CDK5R1 was prominently associated with poor (A) OS, (B) DSS, (C) DFI, (D) PFI; DFI, disease-free interval; DSS, disease-specific disease; OS, overall survival; PFI, progression-free interval.

clinical significance of CDK5R1 in various cancer types, up to date, the effect of CDK5R1 on HCC has not been reported. Therefore, a better understanding of the role of CDK5R1 in HCC and its potential prognostic value, as well as molecular mechanisms underlying its effects are required.

Up to date, there have been no reports on the role of CDK5R1 in HCC, but to our knowledge, CDK5R1 encodes the activator p35 of CDK5, which must be combined with the activator to work and thus CDK5R1 plays a pivotal role in regulating the appropriate activity of CDK5 [8]. That is to say, CDK5R1 has a close relationship with CDK5, which is consistent with our results in PPI network. Many studies have demonstrated that elevated CDK5R1 (p35) promotes the overexpression and activation of CDK5, which in turn promotes the initiation, progression, and metastasis of various tumors [11–15]. Accumulative studies have been proved that CDK5 is overexpressed and activated in HCC, and its excessive activation promotes the initiation and progression of HCC. Inhibition of CDK5 can increase the sensitivity of HCC cells to DNA-damaging agents and improve the responsiveness of patients with advanced HCC to sorafenib [19,20]; CDK5 knockout can inhibit the proliferation and promote apoptosis of HCC cells [21]. Taken
Figure 4. Association between clinicopathologic characteristics and survival outcome of HCC patient through univariate and multivariate Cox regression analysis

Panel (A) showed CDK5R1 can independently predict adverse OS. Panel (B) indicated CDK5R1 can independently predict poor DFI. Panel (C) suggested that CDK5R1 can independently predict worse PFI; DFI, disease-free interval; OS, overall survival; PFI, progression-free interval.
Figure 5. Enrichment plots from gene set enrichment analysis (GSEA) and PPI network of CDK5R1
(A) Results of GSEA showed notch signaling pathway were differentially enriched in high CDK5R1 expression phenotype. (B) PPI network of CDK5R1 suggested that CDK5R1 had close relationship with CDK5; ES, enrichment score; NES, normalized ES; FDR, false discovery rate; NOM P-val, normalized P-value; CDK5, cyclin-dependent kinase 5; CDK5R1, cyclin-dependent kinase 5 regulatory subunit 1; PPI, protein–protein interaction.
### Table 3

Association between clinicopathologic characteristics and HCC patient OS through univariate and multivariate analysis with Cox regression survival model

| Characteristics                                    | Univariate analysis | Multivariate analysis |
|-----------------------------------------------------|---------------------|-----------------------|
|                                                     | HR 95%CI  | P-value   | HR 95%CI  | P-value   |
| Child-Pugh (B-C vs. A)                              | 1.3 0.4-4.2 | 0.715     | 0.8 0.6-1.0 | 0.084     |
| Risk factor (Alcohol consumption and/or viral hepatitis vs. neither) | 0.6 0.4-1.0 | 0.064     |           |           |
| AFP (AFP≥400/20<AFP<400 vs. AFP<20)                 | 1.0 0.7-1.6 | 0.961     |           |           |
| New tumor event (yes vs. no)                        | 3.0 1.3-7.0 | 0.012     | 0.8 0.4-1.9 | 0.669     |
| Age (>40 vs. ≤40)                                   | 4.7 0.6-34.8 | 0.133     |           |           |
| Gender (male vs. female)                            | 0.5 0.2-1.1 | 0.086     | 1.4 0.8-2.5 | 0.281     |
| Histologic grade (G3-4 vs. G1-2)                   | 1.6 0.7-3.3 | 0.247     |           |           |
| M (M1 vs. M0)                                       | 5.4 0.7-40.7 | 0.099     | 2.8 0.6-12.4 | 0.177     |
| N (N1 vs. N0)                                       | 4.3 0.6-31.7 | 0.157     |           |           |
| T (T3-4 vs. T1-2)                                   | 1.2 0.5-3.0 | 0.691     |           |           |
| Clinical stage (III-IV vs. I-II)                    | 1.4 0.6-3.3 | 0.454     |           |           |
| Postoperative ablation embolization (yes vs. no)    | 1.1 0.4-3.2 | 0.870     |           |           |
| Radiation therapy (yes vs. no)                      | 0.8 0.5-1.3 | 0.997     |           |           |
| Vascular invasion (yes vs. no)                      | 1.3 0.6-2.9 | 0.560     |           |           |
| Tumor status (with tumor vs. tumor free)            | 4.0 1.8-9.2 | 0.001     | 3.3 1.5-7.6 | 0.004     |
| Family history (yes vs. no)                         | 1.8 0.9-3.7 | 0.116     |           |           |
| Residual tumor (R1-2 vs. R0)                        | 1.4 0.2-10.2 | 0.761     |           |           |
| CDK5 (high vs. low)                                 | 2.4 1.1-5.2 | 0.032     | 1.6 1.0-2.6 | 0.077     |
| CAPN2 (high vs. low)                                | 1.4 0.6-2.9 | 0.426     |           |           |
| CAPN1 (high vs. low)                                | 1.6 0.7-3.3 | 0.242     |           |           |
| MAPT (high vs. low)                                 | 1.3 0.6-2.8 | 0.447     |           |           |
| CDC25B (high vs. low)                               | 3.1 1.4-6.8 | 0.005     | 1.9 1.2-3.1 | 0.011     |
| PAK1 (high vs. low)                                 | 1.5 0.7-3.0 | 0.310     |           |           |
| PPP1R1B (high vs. low)                              | 1.0 0.5-2.2 | 0.947     |           |           |
| NTRK2 (high vs. low)                                | 0.7 0.3-1.5 | 0.404     |           |           |
| NDEL1 (high vs. low)                                | 0.9 0.4-2.0 | 0.829     |           |           |
| YWHAE (high vs. low)                                | 0.9 0.4-1.9 | 0.840     |           |           |
| CDK5R1 (high vs. low)                               | 2.0 1.1-3.9 | 0.033     | 1.7 1.0-2.7 | 0.037     |

Abbreviations: CI, confidence interval; HR, hazard ratio; M, distant metastasis; N, lymph node metastasis; OS, overall survival; T, topography distribution.

Together, we speculated that CDK5R1 may also play a critical role in the initiation, progression and metastasis of HCC.

In the present study, high-throughput RNA-seq data provided evidence that CDK5R1 was overexpressed in HCC tissues and an elevated expression of CDK5R1 had a close relationship with worse histologic grade, advanced clinical stage, poorer TNM, new tumor event, higher serum AFP value as well as shorter survival time. These suggested that there may be a high probability of HCC recurrence, invasion and metastasis in patients with elevated CDK5R1, and highly expressed CDK5R1 may herald poor prognosis. Further, univariate and multivariate Cox regression analysis disclosed that under the influence of excluding other clinicopathological factors such as genes closely related to CDK5R1, CDK5R1 was still the factor that can independently predict poor OS, DFI and PFI. Although the gene CDC25B, which is strongly associated with CDK5R1, may also independently predict poor OS and PFI.

We further investigated the function of CDK5R1 and the probable mechanism underlying the effects of CDK5R1 on the progression and metastasis HCC based on GSEA. GSEA has wide applicability and is one of the most commonly used approaches for path enrichment analysis. Compared with traditional pathway enrichment analysis such as gene ontology (GO) and Kyoto Gene and Genome Encyclopedia (KEGG), GSEA can detect the expression changes of gene sets rather than individual genes, and GSEA can detect subtle enrichment signals, which makes the results more reliable and flexible [16]; However, GSEA’s functional class scoring (FCS) approach has some limitations. When FCS analyses each pathway, it is likely to treat genes with different fold changes equally, although some genes with larger fold changes should receive greater weight, which may overlook the biological significance of certain genes and their
Table 4 Association between clinicopathologic characteristics and HCC patient DFI through univariate and multivariate analysis with Cox regression survival model

| Characteristics                                      | Univariate analysis | Multivariate analysis |
|-------------------------------------------------------|---------------------|-----------------------|
|                                                       | HR      | 95%CI    | P-value | HR      | 95%CI    | P-value |
| Child-Pugh (B-C vs. A)                                | 2.1     | 0.9–4.9  | 0.096   | 5.2     | 1.9–14.2 | 0.001   |
| Risk factor (Alcohol consumption and/or viral hepatitis vs. neither) | 0.9     | 0.6–1.2  | 0.341   |         |          |         |
| AFP (AFP ≥ 400/20 ≤ AFP < 400 vs. AFP < 20)            | 0.9     | 0.7–1.3  | 0.632   |         |          |         |
| New tumor event (yes vs. no)                          | 2.4     | 1.4–4.0  | 0.996   |         |          |         |
| Age (>40 vs. ≤40)                                     | 1.0     | 0.4–2.3  | 0.926   |         |          |         |
| Gender (male vs. female)                              | 0.9     | 0.5–1.6  | 0.599   |         |          |         |
| Histologic grade (G3-4 vs. G1-2)                     | 1.2     | 0.7–2.1  | 0.486   |         |          |         |
| M (M1 vs. M0)                                         | 0.8     | 0.6–1.1  | 1.000   |         |          |         |
| N (N1 vs. N0)                                         | 4.2     | 0.6–31.4 | 0.159   |         |          |         |
| T (T3-4 vs. T1-2)                                     | 2.0     | 1.0–3.9  | 0.043   | 5.2     | 0.5–51.1 | 0.159   |
| Clinical stage (III-IV vs. I-II)                      | 2.1     | 1.1–4.1  | 0.022   | 0.4     | 0.0–3.8  | 0.430   |
| Postoperative ablation embolization (yes vs. no)      | 2.7     | 1.4–5.5  | 0.005   | 1.1     | 0.5–2.5  | 0.752   |
| Radiation therapy (yes vs. no)                        | 1.5     | 0.2–11.1 | 0.683   |         |          |         |
| Vascular invasion (yes vs. no)                        | 1.0     | 0.5–2.0  | 0.910   |         |          |         |
| Tumor status (with tumor vs. tumor free)              | 35.0    | 13.4–93.5| 0.000   | 36.7    | 13.2–101.6| 0.000   |
| Family history (yes vs. no)                           | 1.1     | 0.6–2.1  | 0.639   |         |          |         |
| residual tumor (R1-2 vs. R0)                          | 1.7     | 1.0–2.6  | 0.393   |         |          |         |
| CDK5 (high vs. low)                                   | 1.6     | 0.9–2.9  | 0.082   | 1.1     | 0.6–2.1  | 0.751   |
| CAPN2 (high vs. low)                                  | 0.8     | 0.5–1.4  | 0.506   |         |          |         |
| CAPN1 (high vs. low)                                  | 1.5     | 0.8–2.5  | 0.178   |         |          |         |
| MAPT (high vs. low)                                   | 1.2     | 0.7–2.1  | 0.546   |         |          |         |
| CDC25B (high vs. low)                                 | 2.1     | 1.2–3.7  | 0.007   | 1.8     | 1.0–3.4  | 0.056   |
| PAK1 (high vs. low)                                   | 1.1     | 0.6–2.0  | 0.676   |         |          |         |
| PPP1R1B (high vs. low)                                | 0.8     | 0.5–1.5  | 0.537   |         |          |         |
| NTRK2 (high vs. low)                                  | 0.9     | 0.5–1.5  | 0.567   |         |          |         |
| NDE1 (high vs. low)                                   | 0.9     | 0.5–1.6  | 0.682   |         |          |         |
| YWHAE (high vs. low)                                  | 1.0     | 0.6–1.7  | 0.987   |         |          |         |
| CDK5R1 (high vs. low)                                 | 2.7     | 1.6–4.5  | 0.000   | 3.0     | 1.4–6.7  | 0.007   |

Abbreviations: CI, confidence interval; DFI, disease-free interval; HR, hazard ratio; M, distant metastasis; N, lymph node metastasis; T, topography distribution.

complex interconnections. In addition, some pathway annotation information is insufficient, which makes it difficult to set the appropriate threshold to determine the gene set. Some genes also have insufficient annotation information, which reduces the sensitivity of GSEA detection [22]. As there is little literature on CDK5R1, with the performance of GSEA, we only found that notch signaling pathway and non-small cell lung cancer were significantly enriched in the CDK5R1 high expression phenotype.

Cancer stem cell is the origin of tumor, it promotes the growth and development of tumor cells and is an important cause of tumor recurrence [23–25]. In addition, cancer stem cells resist chemotherapy and radiation and are difficult to eradicate, which can lead to recurrence and metastasis for years after therapeutic treatment [26]. Studies have shown that tumor stem cells can make patients more susceptible to recurrence after HCC surgical resection [27]. The notch signaling pathway is one of the pivotal pathways that regulate the differentiation and development of cancer stem cells. It plays a key role in the self-renewal and angiogenesis of cancer stem cells. The abnormal notch signaling pathway as a carcinogen is closely linked to the occurrence, progression, and metastasis of a variety of cancers [28]. Notch signaling pathway blockers can delay the generation of tumors and effectively reduce the occurrence of tumors and self-renewal of cancer stem cells, which is expected to cure tumors by completely removing cancer stem cells [23].

Vitro experiments show that vascular endothelial CDK5 inhibitors can influence the migration and proliferation of vascular endothelial cells by inhibiting NOTCH-driven angiogenesis, thereby affecting tumor angiogenesis and ultimately inhibiting tumor growth [29]. Previous studies have also reported that DAPT, a Notch inhibitor in the nervous system, can down-regulate CDK5 activity [30]. In summary, CDK5R1 may participate in the progression
Table 5 Association between clinicopathologic characteristics and HCC patient PFI through univariate and multivariate analysis with Cox regression survival model

| Characteristics                                      | Univariate analysis |                      |                      | Multivariate analysis |                      |                      |
|------------------------------------------------------|---------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                                                      | HR                  | 95% CI               | P-value              | HR                  | 95% CI               | P-value              |
| Child-Pugh (B-C vs. A)                               | 1.8                 | 0.8–4.3              | 0.164                |                      |                      |                      |
| Risk factor (Alcohol consumption and/or viral hepatitis vs. neither) | 0.8                 | 0.6–1.1              | 0.244                |                      |                      |                      |
| AFP (AFP > 400/20 ≤ AFP < 400 vs. AFP < 20)           | 1.0                 | 0.7–1.3              | 0.914                |                      |                      |                      |
| New tumor event (yes vs. no)                         | 3.4                 | 2.1–5.7              | 0.995                |                      |                      |                      |
| Age (≥ 40 vs. < 40)                                  | 0.9                 | 0.4–2.1              | 0.887                |                      |                      |                      |
| Gender (male vs. female)                             | 0.8                 | 0.5–1.4              | 0.406                |                      |                      |                      |
| Histologic grade (G3-4 vs. G1-2)                     | 1.1                 | 0.7–1.9              | 0.845                |                      |                      |                      |
| M (M1 vs. M0)                                        | 5.0                 | 0.7–37.4             | 0.116                |                      |                      |                      |
| N (N1 vs. N0)                                        | 3.7                 | 0.5–27.5             | 0.197                |                      |                      |                      |
| T (T3-4 vs. T1-2)                                    | 1.9                 | 1.0–3.6              | 0.047                | 1.1                  | 0.1–8.9              | 0.925                |
| Clinical stage (III-IV vs. I-II)                     | 2.0                 | 1.1–3.8              | 0.026                | 2.0                  | 0.2–15.5             | 0.525                |
| Postoperative ablation embolization (yes vs. no)      | 2.7                 | 1.4–5.1              | 0.003                | 1.2                  | 0.6–2.4              | 0.646                |
| Radiation therapy (yes vs. no)                       | 1.3                 | 0.2–9.6              | 0.787                |                      |                      |                      |
| Vascular invasion (yes vs. no)                       | 1.2                 | 0.7–2.1              | 0.590                |                      |                      |                      |
| Tumor status (with tumor vs. tumor free)             | 37.1                | 14.2–97.4            | 0.000                | 33.2                 | 12.1–90.9            | 0.000                |
| Family history (yes vs. no)                          | 1.1                 | 0.6–1.9              | 0.785                |                      |                      |                      |
| residual tumor (R1-2 vs. R0)                         | 6.4                 | 2.2–18.3             | 0.001                | 3.8                  | 1.2–11.7             | 0.021                |
| CDK5 (high vs. low)                                  | 1.5                 | 0.9–2.5              | 0.131                |                      |                      |                      |
| CAPN2 (high vs. low)                                 | 1.0                 | 0.6–1.7              | 0.978                |                      |                      |                      |
| CAPN1 (high vs. low)                                 | 1.4                 | 0.8–2.4              | 0.186                |                      |                      |                      |
| MAPT (high vs. low)                                  | 1.1                 | 0.7–1.9              | 0.665                |                      |                      |                      |
| CDC25B (high vs. low)                                | 2.1                 | 1.2–3.5              | 0.007                | 1.8                  | 1.0–3.2              | 0.044                |
| PAK1 (high vs. low)                                  | 1.3                 | 0.8–2.2              | 0.350                |                      |                      |                      |
| PPP1R1B (high vs. low)                               | 1.0                 | 0.6–1.6              | 0.858                |                      |                      |                      |
| NTRK2 (high vs. low)                                 | 0.9                 | 0.6–1.6              | 0.796                |                      |                      |                      |
| NDEL1 (high vs. low)                                 | 1.0                 | 0.6–1.8              | 0.870                |                      |                      |                      |
| YWHAE (high vs. low)                                 | 1.1                 | 0.6–1.8              | 0.797                |                      |                      |                      |
| CDK5R1 (high vs. low)                                | 2.5                 | 1.5–4.2              | 0.000                | 2.8                  | 1.3–5.9              | 0.007                |

Abbreviations: CI, confidence interval; HR, hazard ratio; M, distant metastasis; N, lymph node metastasis; PFI, progression free interval; T, topography distribution.

Table 6 Gene sets enriched in phenotype high.

| MSigDB collection | Gene set name                                      | NES   | NOM P-val | FDR q-val |
|-------------------|----------------------------------------------------|-------|-----------|-----------|
| c2.cp.kegg.       | KEGG_NOTCH_SIGNALING_PATHWAY                       | 1.76  | 0.008     | 1.000     |
| v7.0.symbol.gmt [Curated] | KEGG_NON_SMALL_CELL_LUNG_CANCER                  | 1.60  | 0.040     | 1.000     |

Abbreviations: FDR, false discovery rate; NES, normalized enrichment score; NOM, nominal. Gene sets with NOM P-val < 0.05 and FDR q-val < 0.05 are considered as significant.

and migration of HCC by regulating the notch signaling pathway. The present study is the first to report the role of CDK5R1 in HCC and the regulatory effect of CDK5R1 on the notch signaling pathway in HCC.

Although our current study has improved our understanding of the role of CDK5R1 in HCC, there are still some limitations. First, the sample size of cancer patients in the TCGA database was significantly higher than that of the control patients. Second, the absence of clinical factors in the public database, such as specific details of the patient’s medication and/or surgical treatment, also affects the patient’s prognosis. Third, the protein level of CDK5R1 in HCC and its direct role in HCC progression and metastasis remain to be further validated in vitro. Fourth, due to the limitations of GSEA, and so far, too little research has been done on CDK5R1, other important signaling pathways...
regulated by CDK5R1 may be missed. Finally, the present study is a retrospective study, and prospective studies should be conducted in the future to make up for the limitations of the retrospective study. Although the present study has some limitations, it does provide clues for studying the function of CDK5R1 in HCC, and provides targets and potential prognostic markers for the treatment of HCC.

Conclusion

Patients with elevated CDK5R1 may have a poor prognosis, increased CDK5R1 may act as a promising independent prognostic marker of poor survival and therapeutic target in HCC. Besides, it may participate in the progression and migration of HCC through regulating the notch signaling pathway.

Data Availability

The datasets generated and/or analyzed during the current study are available in the TCGA repository, https://portal.gdc.cancer.gov/repository?facetTab=cases; and GEO repository https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE121248 and https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62232.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

All authors contributed to the paper and agreed to be responsible for all aspects of the work. Conceptualization: Z.Z and Z.C; Data curation: Z.Z, Z.C; Funding acquisition: E.Z; Methodology: Z.Z, Z.C, E.Z, Y.T and H.H; Visualization: Z.Z and Z.C; Writing—original draft: Z.Z; Writing—review and editing: Z.C, E.Z, H.H and YT; Language editing, Y.T and H.H.

Ethics Approval and Consent to Participate

No ethics approval was required for this work. All utilized public data sets were generated by others who obtained ethical approval.

Abbreviations

CDK5, cyclin-dependent kinase 5; CDK5R1, cyclin-dependent kinase 5 regulatory subunit 1; DFI, disease-free interval; DSS, disease-specific survival; GEO, Gene Expression Omnibus; GSEA, Gene Set Enrichment Analysis; OS, overall survival; PFI, progression-free interval.

References

1 Villanueva, A. (2019) Hepatocellular Carcinoma. N. Engl. J. Med. 380, 1450–1462, https://doi.org/10.1056/NEJMra1713263
2 Zhou, J. et al. (2018) Guidelines for Diagnosis and Treatment of Primary Liver Cancer in China (2017 Edition). Liver Cancer 7, 235–260, https://doi.org/10.1159/000488035
3 Erridge, S. et al. (2017) Meta-analysis of determinants of survival following treatment of recurrent hepatocellular carcinoma. Br. J. Surg. 104, 1433–1442, https://doi.org/10.1002/bjs.10597
4 Yuen, M.F. and Lai, C.L. (2003) Screening for hepatocellular carcinoma: survival benefit and cost-effectiveness. Ann. Oncol. 14, 1463–1467, https://doi.org/10.1093/annonc/mdg400
5 Petrizzo, A. et al. (2018) Cellular prognostic markers in hepatitis-related hepatocellular carcinoma. Infect. Agent Cancer 13, 10, https://doi.org/10.1186/s13027-018-0183-8
6 Arif, A. (2012) Extraneuronal activities and regulatory mechanisms of the atypical cyclin-dependent kinase Cdk5. Biochem. Pharmacol. 84, 985–993, https://doi.org/10.1016/j.bcp.2012.06.027
7 Lintas, C. et al. (2019) An Interstitial 17q11.2 de novo Deletion Involving the CDK5R1 Gene in a High-Functioning Autistic Patient. Mol. Syndromol. 9, 247–252, https://doi.org/10.1159/000491802

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