Identification of CDC25C as a Potential Biomarker in Hepatocellular Carcinoma Using Bioinformatics Analysis

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Abstract

Hepatocellular carcinoma (HCC) is the most aggressive type of gastrointestinal tumor, with a high rate of mortality. However, identifying biomarkers for the treatment of HCC remains to be developed. We aimed to determine whether cell division cycle 25C (CDC25C) could be used as a novel diagnostic and therapeutic biomarker in HCC. Expression of CDC25C in HCC was analyzed by using GEPIA (Gene Expression Profiling Interactive Analysis) and UALCAN databases. GEPIA and CBioPortal databases were applied to analyze patients'survival and CDC25C mutations, respectively. PPI (Protein-Protein Interaction) network was further built by STRING (Search Tool for the Retrieval of Interacting Genes) and Metascape Web portals. To the best of our knowledge, the novel observations identified in the present study reveal that the expression of CDC25C in HCC was significantly enhanced when compare to that in normal liver tissues (P < 0.001). A higher CDC25C expression resulted in a remarkably shorter disease free survival as well as overall survival. Moreover, the expression of CDC25C in HCC was related to HCC patients'grade and race, but not gender. The expression levels of CDC25C elevated gradually from stage 1 to 3 but decreased in stage 4. The specific gene mutations V41A, L87 H, N222 K and X309-splice of CDC25C occurred in HCC samples and these unique mutations were not detected in any other tumor tissues. Finally, PPI networks and GO enrichment analysis suggested that CDC25C might be associated with cell cycle and p53 signaling pathway. Taken together, bioinformatics analysis revealed that CDC25C might be a potential diagnostic predictor for HCC.

Introduction

Although hepatocellular carcinoma (HCC) is the sixth common cancer, it is the second cause of cancer deaths in Asian/Pacific islander.[1,2] During the past 2 decades, the great advancement of HCC treatment has been achieved, including surgical resection, ablation, liver transplantation and targeted therapy.[3] However, the 5-year survival rate for HCC has remained unsatisfied. Due to toxicity to the liver, radiotherapy is not used for the treatment of HCC.[4] Although liver transplantation is a potential approach for HCC, its application is limited by a number of factors.The reason for liver transplantation is not only liver donor shortage but liver transplantation have low success rate beyond Milan criteria, and etiology is also important because of high relapse rate in HCV infection. Recurrence of hepatitis C virus (HCV) infection following liver transplantation is a major source of morbidity and mortality. Targeted therapy can block the action of key molecules in the formation and progression of

liver cancer. However, only a few drugs are currently available for patients with advanced liver cancer. Therefore, it is significant to investigate the key genes and major signal pathways involving in the development for better treatment of HCC. To date, serum alpha-feto protein (AFP) and PIVKA-II (protein induced by vitamin K absence or antagonist-II) are 2 best used

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biomarker for HCC in clinical screening, diagnosis and monitoring.[5-10] AFP is not only important for diagnosis but also is important as a prognostic and predictive factor for anti-VEGF treatment and outcome after transplantation and surgery.The combination use of both biomarkers has significantly improved HCC sensitivity detection despite that sensitivity and specificity are far from satisfactory.[11-14] In order to improve the clinical diagnosis and treatment of HCC, it is necessary to find new genes that are useful for screening, diagnosis and monitoring of HCC.

Dual specificity phosphatase CDC25 originally discovered in yeast can dephosphorylate phospho-threonine and phospho-tyrosine.[15,16] In humans, CDC25 family is composed of CDC25A, B and C.[17] Several studies have shown the association between CDC25C and human cancers. Ozen et al[18] found that CDC25C was closely related to the occurrence, development and prognosis of prostate cancer. Wang et al[19] further confirmed that overexpression of CDC25C played an important role in the occurrence and development of squamous cell carcinoma of female vulva. Furthermore, Xia Z.et al[20] found that CDC25C predicts poor prognosis in Lung Adenocarcinoma(LUAD)and may function in cell cycle regulation and FAS-mediated apoptosis. Al-Matouq et al[21] also found that CDC25B and CDC25C were increased in mouse and human skin cancers.

In this study, to verify the value of CDC25C for the diagnosis and treatment of HCC, the GEPIA and UALCAN databases analysis were performed. Analogously, we observed here that the expression level of CDC25C in HCC tissues was significantly higher than that in normal liver tissues.[22] The expression level of CDC25C in HCC is closely related to tumor size, differentiation, stages and grades.[23] Furthermore, CBioPortal databases were applied to unveil the specific mutations of CDC25C in HCC patients. PPI network and GO enrichment analysis mined some promising CDC25C relevant genes, further suggested that these genes are significant enriched in cell cycle, signal transduction involved in DNA integrity checkpoint, cell cycle G2/M phase transition and P53 signaling pathway.

Materials and Methods

UALCAN Analysis

CDC25C expression data and clinical data of HCC patients in TCGA were downloaded from UALCAN(http://ualcan.path.uab.edu/analysis.html).[5] In this study, we utilized this online tool to analyze the expression levels of CDC25C between HCC specimen and normal tissues.

Survival Analysis

GEPIA(http://gepia.cancer-pku.cn/) is an interactive web resource and database for analyzing cancer transcriptome and patients' survival.In this study, we utilized this online tool to analyze patients' survival.Using GEPIA, overall survival(OS) and disease free survival(DFS) were presented and the hazards ratio was calculated based on Cox PH Model.95% confidence interval was added as dotted line.The thresholds for high and low expression level cohorts are 50% respectively.

Correlation Genes Analysis

Protein-protein interaction of CDC25C was conducted in STRING online service.The STRING database (http://string-db.org/) was used to analyze PPI networks.The non-interacting genes were excluded.The top 10 genes with the highest degree of connection to the others were presented.

Pathway and Process Enrichment Analysis

The Metascape database (http://metascape.org/) is an online tool for high-throughput functional analysis of genes.All the genes interacted with CDC25C in STRING were included in the enrichment analysis in Metascape database.Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway, Gene ontology (GO) biological process, Reactome Gene Sets and Canonical Pathways were enriched.

Mutations Analysis

CBioPortal (https://www.cbioportal. org/) is a database with integrated genetic data, including DNA mutations, methylations, gene amplifications and proteinalterations.In our study, the cBioPortal database was used to analyze the association between genetic mutations and the development of HCC.The top 10 geng which are related to CDC25C were analyzed by using cBioPortal database.We performed CDC25C gene mutations analysis across all tumor samples (n = 14) from the TCGA-HCC database.

Results

The Expression of CDC25C in HCC Patients

To verify CDC25C expression levels in HCC tissues and the value to the diagnosis and surveillance of HCC, GEPIA database was applied. As shown in Figure 1A, the expression level of CDC25C was significantly higher in HCC (n = 371) compared with that in normal tissues (n = 50;P < 0.001). Moreover, the relationship between CDC25C expression levels and HCC patients'clinicopathological parameters were further analyzed by UALCAN databases. The result demonstrated that expression of CDC25C was higher in Asian HCC patients than that in Caucasian patients(P < 0.05, Figure 1B). The expression levels of CDC25C increased from grade 1 to grade 4, suggesting CDC25C was remarkably correlated with HCC patients'grade (Figure 1C) (grade1 vs grad e3, P < 0.001;grade 1 vs grade 4, P < 0.001;grade 2 vs grade 3, P < 0.001;grade 2 vs grade 3, P < 0.05). As shown in Figure 1D, there are gradually increased expression levels of CDC25C from stage 1 to stage 3 but obviously declined in stage 4 (stage 1 vs stage 2, P < 0.05;stage 1 vs stage 3, P < 0.001).
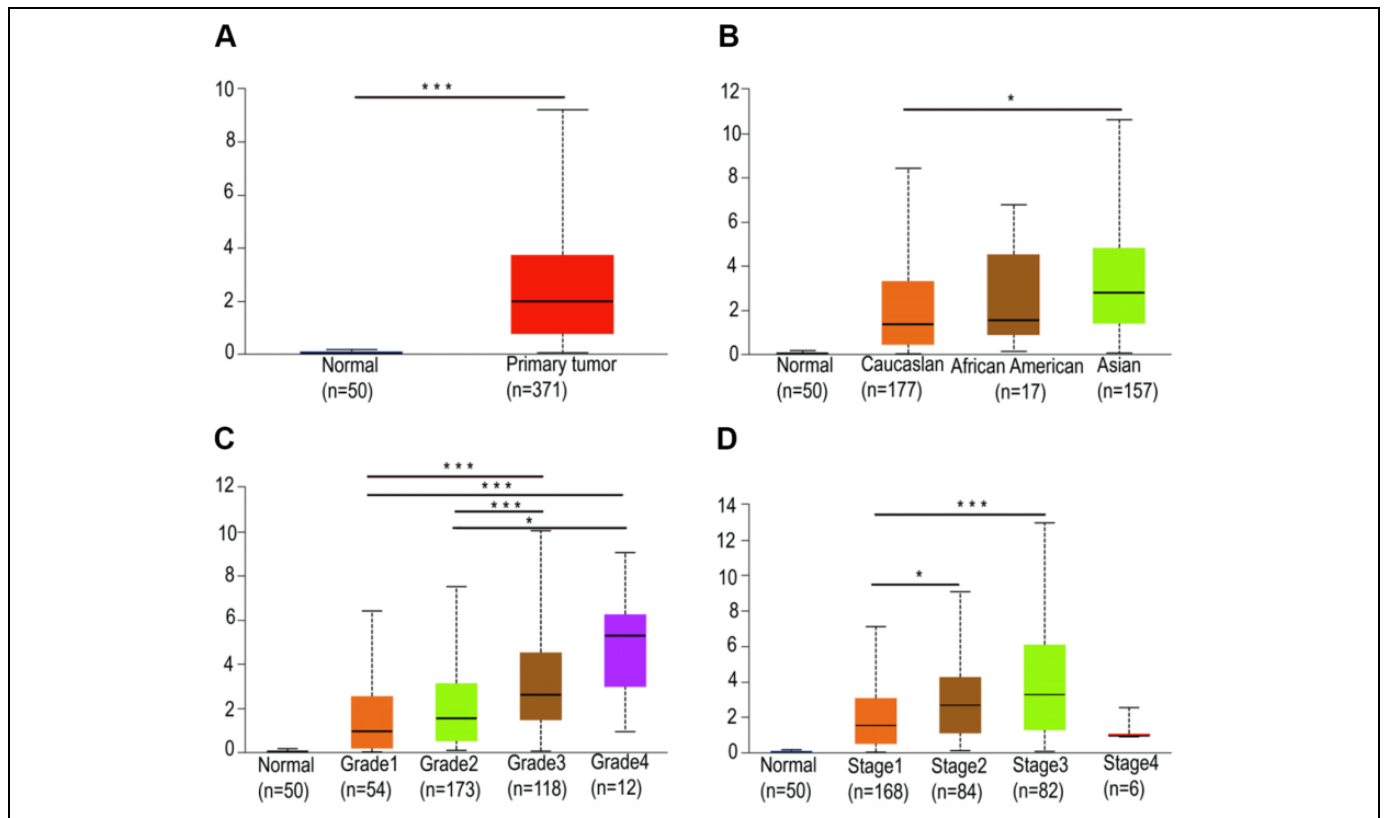
Figure 1. Expression of CDC25C in patients with HCC. (A) The expression of CDC25C in HCC obtained from GEPIA online tool (P < 0.001; tumor vs. normal). (B) Expression of CDC25C in HCC based on race (P < 0.05; Caucasian vs. Asian), and (C) individual cancer grade (P < 0.001; grade 1 vs. grade 3; grade 2 vs. grade 3;grade 1 vs. grade 4; grade 2 vs. grade 4) and (D) individual cancer stage (P < 0.001; stage 1 vs. stage 2; stage 1 vs. stage 3).*, P < 0.05;**, P < 0.01;***, P < 0.001.
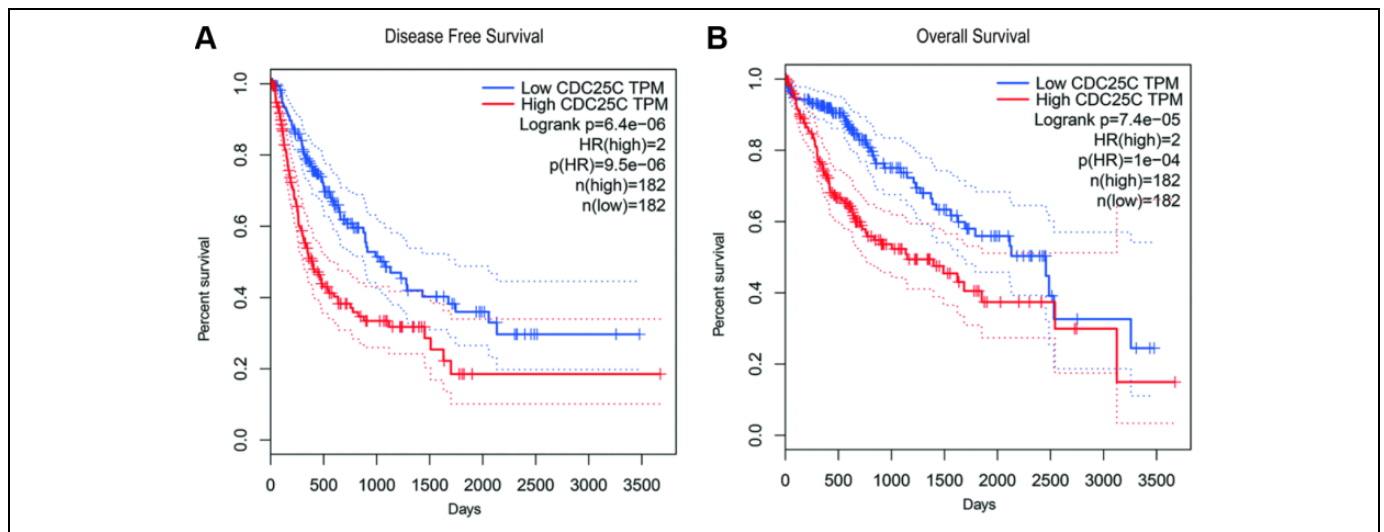
Figure 2. Higher expressions of CDC25C was associated with poorer DFS (A) and OS (B) in HCC patients. (P < 0.0001).

Effect of CDC25C Expression Level on Patient Survival

In survival analysis, CDC25C expression of HCC patients were divided into low-expression group and high-expression group (cutoff-high is 50%, cutoff-low is 50%). As shown in Figure 2A and Figure 2B, a lower CDC25C expression level resulted in a significantly higher survival probability (P < 0.001). Survival curves analysis showed that CDC25C was suitable for predicting HCC patients' prognosis.

PPI Networks and Process Enrichment Analysis of CDC25C

As shown in Figure 3, PPI network analysis indicated that CDC25C interacted with 10 functional genes, including CDK1, CCNB1, CHEK2, CHEK1, PLK1, CCNA2, YWHAZ, TP53, CCNB2 and WEE1. To further determine the potential function CDC25C in HCC, GO enrichment analysis and KEGG pathway analysis of CDC25C were performed using Metascape. The result revealed that those proteins were biologically closely associated with cell cycle and p53 signaling pathway (Figure 4 and Table 1).

Specific Mutations of CDC25C Genes in HCC Patients

In order to analyze the mutations of CDC25C gene in HCC patients' tissues, the CBioPortal database analysis was employed. As shown in Tables 2 and 3, we performed CDC25C gene mutations analysis across all tumor samples (n = 14) from the TCGA-HCC database (https://www.cbioportal. org/). These particular mutations of CDC25C in HCC patients might contribute greatly to HCC clinical diagnosis and monitoring. Moreover, the mutations of CDC25C and its interacted genes (CDK1, CCNB1, CHEK2, CHEK1, PLK1, CCNA2, YWHAZ, TP53, CCNB2 and WEE1) were analyzed by means of the

cBioPortal dataset. The alteration statuses of 10 key genes were analyzed. The frequency of alteration of 10 hub genes were shown in Figure 5A and Figure 5B. Intriguingly, there were 4 specific mutations V41A, L87 H, N222 K and X309-splice (Table 2, Figure 5C and Figure 5D) in the HCC samples that were not present in any other tumor samples. Furthermore, the OS (Figure 5E) and DFS (Figure 5F) were significant better in no alterations group than that in alterations group (P < 0.05). Survival curves analysis showed that CDC25C gene mutation may affect the prognosis of HCC patients.

## Prediction of Relevance Genes to CDC25C in HCC

The correlation between CDC25C and its 10 interacted genes were selected to further analyzed in HCC patients. We found that the 6 genes(CCNB1, CCNB2, CDK1, CHEK1, CHEK2, PLK1) were considered to be relevant genes and the scatter plots were shown in Figure 6.

Discussion

Bioinformatics methods can provide us with gene expression levels and predict potential therapeutic targets.[24] A large number of clinical data showed that the death rate of liver cancer is very high.One of the best ways to reduce mortality is to detect accurately and treat successfully. Identifying novel key genes associated with the development and the progression of HCC is crucial for its diagnosis and treatment. In this study, our data showed that CDC25C expression was higher in HCC patients than that in control normal tissues, and correlated with HCC patients' grade. The results demonstrated that CDC25C expression enhanced gradually from stage 1 to stage 3 but decreased in stage 4. A higher CDC25C expression resulted in a significant shorter DFS and OS in HCC patients, suggesting that CDC25C may play an important role in the prognosis of HCC. The CBioPortal tool have unveiled 4 specific mutations V41A, L87 H, N222 K and X309-splice unique presented in the HCC. These characteristic mutations of CDC25C in HCC patients might facilitate to HCC clinical diagnosis and monitoring. To determine the probably pathogenic mechanism of CDC25C in HCC, PPI network and GO enrichment analysis were further
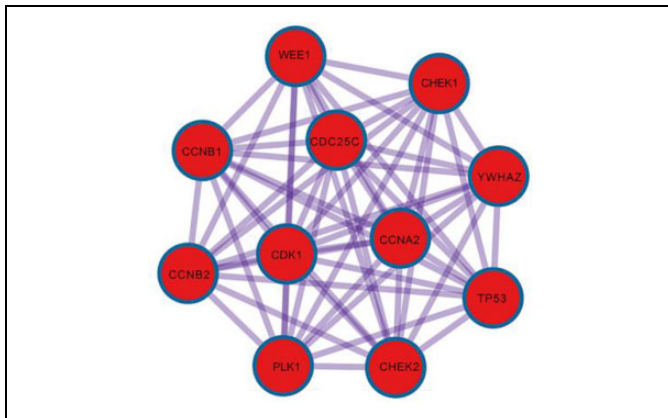
Figure 3. PPI network of the top 10 hub genes related to CDC25C.
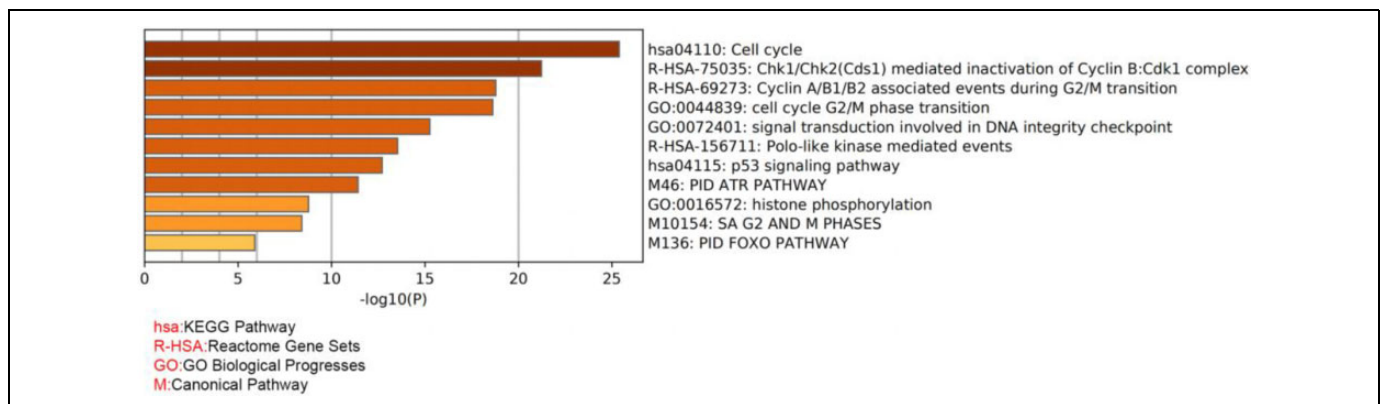
Figure 4. GO enrichment analysis of the top 10 hub genes.

Table 1. Significantly Enriched GO Terms and KEGG Pathways of CDC25C.

Category	Terms	Count	%	P
GO-TERM-BP-DIRECT	G2/M transition of mitotic cell cycle	7	70	2.2E-11
GO-TERM-BP-DIRECT	cell division	6	60	4.5E-7
GO-TERM-BP-DIRECT	mitotic nuclear envelope disassembly	4	40	1.4E-6
GO-TERM-BP-DIRECT	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	4	40	4.0E-6
GO-TERM-BP-DIRECT	mitotic nuclear division	5	50	5.5E-6
GO-TERM-BP-DIRECT	replicative senescence	3	30	1.7E-5
GO-TERM-BP-DIRECT	regulation of cell cycle	4	40	3.2E-5
GO-TERM-BP-DIRECT	regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	3	30	6.4E-5
GO-TERM-BP-DIRECT	positive regulation of ubiquitin-protein ligase activity involved in regulation of mitotic cell cycle transition	3	30	7.1E-4
GO-TERM-BP-DIRECT	anaphase-promoting complex-dependent catabolic process	3	30	7.7E-4
GO-TERM-BP-DIRECT	cellular response to hypoxia	3	30	1.1E-3
GO-TERM-BP-DIRECT	negative regulation of apoptotic process	4	40	1.5E-3
GO-TERM-BP-DIRECT	protein complex assembly	3	30	1.7E-3
GO-TERM-BP-DIRECT	regulation of signal transduction by p53 class mediator	3	30	1.9E-3
GO-TERM-BP-DIRECT	peptidyl-serine phosphorylation	3	30	1.9E-3
GO-TERM-BP-DIRECT	regulation of cell cycle G2/M phase transition	2	20	2.7E-3
GO-TERM-BP-DIRECT	protein ubiquitination involved in ubiquitin-dependent protein catabolic process	3	30	2.8E-3
GO-TERM-BP-DIRECT	DNA damage induced protein phosphorylation	2	20	4.3E-3
GO-TERM-BP-DIRECT	cellular response to DNA damage stimulus	3	30	5.2E-3
GO-TERM-BP-DIRECT	ventricular cardiac muscle cell development	2	20	5.3E-3
GO-TERM-BP-DIRECT	mitotic G2 DNA damage checkpoint	2	20	8.0E-3
GO-TERM-BP-DIRECT	DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator	2	20	8.5E-3
GO-TERM-BP-DIRECT	G2 DNA damage checkpoint	2	20	1.1E-2
GO-TERM-BP-DIRECT	positive regulation of protein localization to nucleus	2	20	1.1E-2
GO-TERM-BP-DIRECT	positive regulation of cardiac muscle cell proliferation	2	20	1.2E-2
GO-TERM-BP-DIRECT	cell aging	2	20	1.3E-2
GO-TERM-BP-DIRECT	positive regulation of mitotic cell cycle	2	20	1.5E-2
GO-TERM-BP-DIRECT	cell proliferation	3	30	1.5E-2
GO-TERM-BP-DIRECT	DNA damage checkpoint	2	20	1.6E-2
GO-TERM-BP-DIRECT	positive regulation of protein insertion into mitochondrial membrane involved in apoptotic signaling pathway	2	20	1.6E-2
GO-TERM-BP-DIRECT	intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	2	20	1.6E-2
GO-TERM-BP-DIRECT	response to gamma radiation	2	20	1.6E-2
GO-TERM-BP-DIRECT	peptidyl-threonine phosphorylation	2	20	2.0E-2
GO-TERM-BP-DIRECT	organ regeneration	2	20	2.5E-2
GO-TERM-BP-DIRECT	positive regulation of fibroblast proliferation	2	20	2.9E-2
GO-TERM-BP-DIRECT	cellular response to drug	2	20	3.6E-2
GO-TERM-BP-DIRECT	Ras protein signal transduction	2	20	3.7E-2
GO-TERM-CC-DIRECT	Nucleoplasm	10	100	4.5E-8
GO-TERM-CC-DIRECT	Centrosome	5	50	3.5E-5
GO-TERM-CC-DIRECT	Nucleus	9	90	4.0E-4
GO-TERM-CC-DIRECT	Cytosol	7	70	1.8E-3
GO-TERM-CC-DIRECT	condensed nuclear chromosome outer kinetochore	2	20	2.0E-3
GO-TERM-CC-DIRECT	replication fork	2	20	8.4E-3
GO-TERM-CC-DIRECT	Cytoplasm	7	70	2.0E-2
GO-TERM-CC-DIRECT	spindle microtubule	2	20	2.2E-2
GO-TERM-CC-DIRECT	chromosome, telomeric region	2	20	2.2E-2
GO-TERM-CC-DIRECT	Chromatin	2	20	4.3E-2
GO-TERM-CC-DIRECT	PML body	2	20	4.7E-2
GO-TERM-MF-DIRECT	protein kinase binding	6	60	6.3E-7
GO-TERM-MF-DIRECT	protein kinase activity	5	50	2.3E-5
GO-TERM-MF-DIRECT	cyclin-dependent protein serine/threonine kinase activity	3	30	1.4E-4
GO-TERM-MF-DIRECT	kinase activity	4	40	2.3E-4
GO-TERM-MF-DIRECT	ATP binding	6	60	5.0E-4
GO-TERM-MF-DIRECT	protein serine/threonine kinase activity	4	40	8.3E-4
GO-TERM-MF-DIRECT	histone kinase activity	2	20	2.1E-3
GO-TERM-MF-DIRECT	protein binding	10	100	2.8E-3
GO-TERM-MF-DIRECT	ubiquitin protein ligase binding	3	30	9.6E-3
KEGG-PATHWAY	Cell cycle	10	100	1.5E-16
KEGG-PATHWAY	p53 signaling pathway	6	60	9.2E-9
KEGG-PATHWAY	Progesterone-mediated oocyte maturation	5	50	2.9E-6
KEGG-PATHWAY	Oocyte meiosis	5	50	7.6E-6
KEGG-PATHWAY	Viral carcinogenesis	5	50	8.6E-5

(continued)

Table 1. (continued)

Category	Terms	Count	%	P
KEGG-PATHWAY	FoxO signaling pathway	3	30	1.2E-2
KEGG-PATHWAY	Hepatitis B	3	30	1.4E-2
KEGG-PATHWAY	HTLV-I infection	3	30	4.1E-2

Table 2. Specific Mutations of CDC25C Gene in TCGA-HCC Patients.

Cancer Type	Sample ID	Protein Change
HCC	H072059	V41A
HCC	TCGA-DD-AADB-01	X309-splice
HCC	TCGA-DD-AAE2-01	N222K
HCC	TCGA-DD-AACY-01	L87H

Table 3. Mutations in the CDC25C Gene in Other TCGA Cancers Patients.

Cancer Type	Sample ID	Protein Change
LUAD	LUAD-D02085-Tumor	X289-splice
LUAD	LUAD-RT-S01774-Tumor	D94H
LUAD	TCGA-50-7109-01	P215Q
LUAD	TCGA-78-7536-01	E81D
LUAD	TCGA-49-AARN-01	F419L
LUAD	TCGA-55-A493-01	D100N
LUAD	TCGA-73-A9RS-01	P68Qfs*15
LUAD	TCGA-97-A4LX-01	Q430P
LUAD	TCGA-MN-A4N4-01	S277C
LUAD	TCGA-44-4112-01	CDC23-CDC25C
LUSC	TCGA-66-2789-01	P421L
LUSC	TCGA-94-7943-01	D135Tfs*29
SCLC	JHU-LX108	D276Y
SCLC	JHU-LX108-R	D276Y
COAD	HCT-15	G343*
COAD	Coadread-dfci-2016_593	E219D
COAD	Coadread-dfci-2016_2269	E219D
COAD	Coadread-dfci-2016_546	Y245Vfs*22
COAD	Coadread-dfci-2016_3535	Y245Vfs*22
COAD	Coadread-dfci-2016_3646	X124-splice
COAD	Coadread-dfci-2016_102	G210R
COAD	Coadread-dfci-2016_2766	E382*
COAD	Coadread-dfci-2016_3643	X124-splice
COAD	Coadread-dfci-2016_3181	X124-splice
COAD	Coadread-dfci-2016_4505	R389C
COAD	Coadread-dfci-2016-55	V50A
COAD	587376	R448Q
COAD	TCGA-AA-3678-01	G339R
COAD	TCGA-AA-A010-01	K303N
COAD	01CO022	K244Sfs*15
PRAD	09-006F1_RENAL	D301V
PRAD	6115250	L158M
PRAD	PROS01448-6115250	L158M
ICC	TCGA-WD-A7RX-01	H436N
ICC	TCGA-HQ-A2OF-01	S263F
ICC	TCGA-K4-A4AC-01	A91P
BRCA	TCGA-BH-A18J-01	H437N
BRCA	TCGA-A2-A3Y0-01	Q202E
BRCA	TCGA-A7-A13G-01	S7L
BRCA	TCGA-AC-A5XS-01	E352Q
GBM	Mayo-PDX-Sarkaria-12	L194S
GBM	Mayo-PDX-Sarkaria-181	D234N
CSCC	TCGA-JW-A5VL-01	E393K
CSCC	TCGA-C5-A3HE-01	L54V
STAD	PFG181T	P407Qfs*39
STAD	TCGA-BR-7851-01	K244Sfs*15
STAD	TCGA-BR-4184-01	R392C
STAD	TCGA-BR-6452-01	D73N
STAD	TCGA-BR-8687-01	K317T
STAD	TCGA-BR-8081-01	Y245Vfs*22
SCC	TCGA-F7-A624-01	S17*
SCC	TCGA-CV-A6K2-01	E4K
SCC	TCGA-CV-A45Q-01	P180S
SCC	TCGA-CV-A468-01	L221*
DLBCL	TCGA-G8-6326-01	R461Q
DLBCL	TCGA-GR-7351-01	P218A
DLBCL	DLBCL-139	E203V

(continued)

applied. Ten CDC25C interacted proteins: CDK1, CCNB1, CHEK2, CHEK1, PLK1, CCNA2, YWHAZ, TP53, CCNB2 and WEE1 were identified. GO enrichment analysis suggested these genes are enriched in cell cycle, CHK1/CHK2(cds1) mediated inactivation of cyclin B: CDK1 complex, cyclin A/B1/B2 associated events during G2/M transition, FOXO pathway, p53 signaling pathway and PID-ATR pathway.

As we known, CDC25C plays an important regulatory role in the G2/M phase transformation of the cell cycle. Lindqvist[25] conducted an experimental study verified that CDC25C could activate the Cyclin B/CDC2 complex through dephosphorylation and facilitate the smooth transition from G2 phase to M phase during mitosis.The cell cycle pathway is one of the most important cellular signaling pathways that regulates both cell division and apoptosis. DNA damage readily leads to dysregulation of the cell cycle, which is an essential step in the initiation and development of human malignancies.[26-28] It has been reported that cell cycle arrest has been confirmed to be an effective approach in controlling tumor growth.[29,30] There were more cells in the G2 /M phase, whereas cells in the G1 or S phase were reduced. The expression of CDC25C might contribute to the HCC cell cycle by regulating the G2/M phase. By inhibiting the expression level of the proteins CDC25C, HCC cells proliferation and the G2/M phase cell cycle arrest can significantly inhibited. It has been reported that checkpoint is an important regulatory node of the cell cycle,[31-33] and cell can only passing a checkpoint test can cell enter the next cell cycle.[34]

It is widely believed that the CDK1/Cyclin B complex is a key regulatory factor of checkpoint in the G2/M phase.[35] The complex is synthesized in large quantities when the cell passes through the G2/M phase.[36] Therefore, CDK1 /Cyclin B complex is decreased with G2/M cycle arrest. The study showed that the cancer cell arrest at the G2/M phase when the content of the CDK1/Cyclin B complex decresed. It is widely believed that increasing the phosphorylation of CDC25C, can lead to inhibition of the formation CDK1/Cyclin B complex and cell cycle arrest at the G2/M phase.[37,38] The protein kinase

Table 3. (continued)

Cancer Type	Sample ID	Protein Change
DLBCL	DLBCL-038	S78G
UEC	TCGA-AX-A0J1-01	R331H
UEC	TCGA-B5-A11E-01	H436Y
UEC	TCGA-B5-A11E-01	L33P
UEC	TCGA-D1-A174-01	V374M
UEC	TCGA-D1-A17Q-01	E378*
UEC	MM18 T	Q430H
HCC	H072059	V41A
HCC	TCGA-DD-AADB-01	X309-splice
HCC	TCGA-DD-AAE2-01	N222K
HCC	TCGA-DD-AACY-01	L87H

CDC25C, which belongs to the dual-specificity CDC25 phosphatase family, dephosphorylates the 2 inhibitory residues of CDK1 (Tyr15 and Thr14) to activate the CDK1-Cyclin B complex at the G2 phase.[39] Therefore, CDC25C might contribute to the HCC cell cycle by activating the CDK1-Cyclin B complex at the G2 phase. In turn, by inhibiting the expression level of CDC25C, HCC cells proliferation and the G2/M phase cell cycle arrest can be significantly inhibited.

Zou Y, et al found that CDK1, CCNB1 and CCNB2 are potential prognostic biomarkers and associated with immune cell infiltration in HCC.[40] The genes may be utilized to predict the reaction of immunotherapy. Combining inhibitors of these
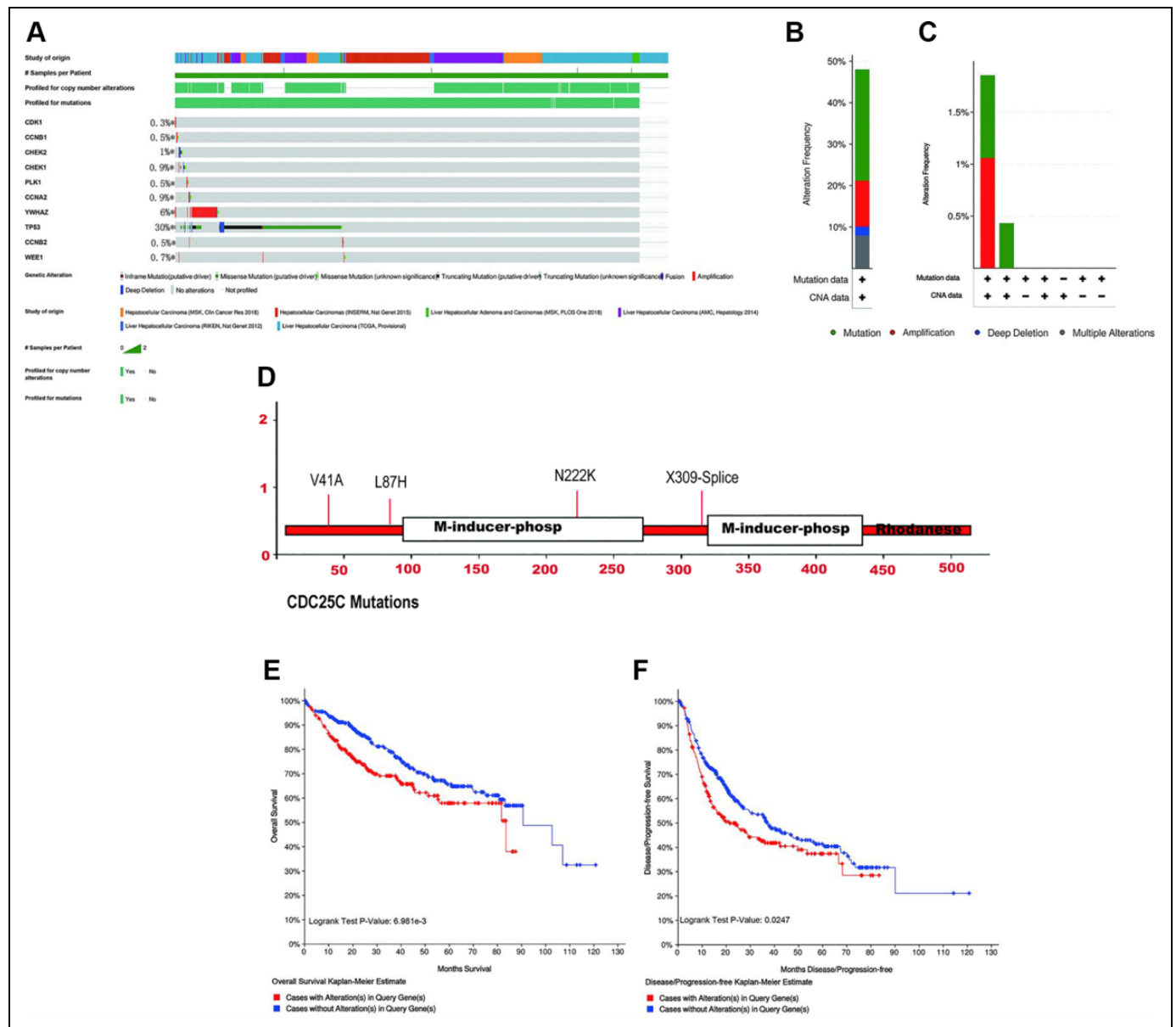
Figure 5. (A) A visual summary of Genetic alterations (data from HCC in TCGA) shows the genetic alteration of 10 key genes which were altered in 184 (49%) of 377 HCC patients. (B) The total alteration frequency of 10 key genes is illustrated. (C) the genetic alteration of CDC25C genes which were altered in 7 (2%) of 377 HCC patients. (D) The particular mutations (V41A, L87 H, N222 K, X309-splice) of CDC25C in HCC patients. (E) Overall survival Kaplan Meier estimate. (F) Progression free survival Kaplan Meier estimate.
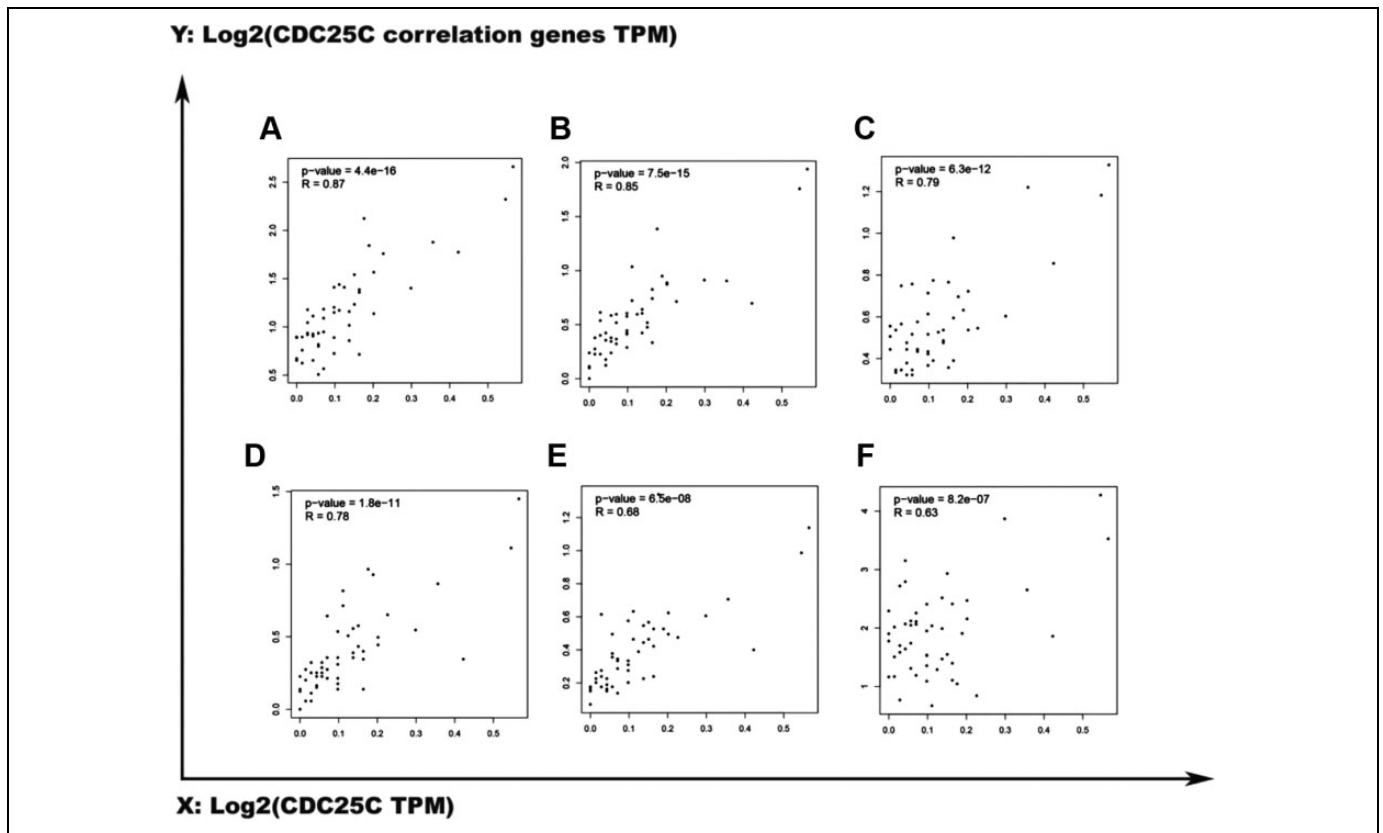
Figure 6. The plots of 6 pair-wise correlation genes. (A) CDC25C and CCNB1, (B) CDC25C and CDK1, (C) CDC25C and CHEK1, (D) CDC25C and CCNB2, (E) CDC25C and PLK1, (F) CDC25C and CHEK2.

genes with immunotherapy may improve the survival time of HCC patients. mRNA expression of CDK1, CCNB1 and CCNB2 was up-regulated in various tumor tissues including HCC. Higher expression of these genes was associated with poorer prognosis in HCC patients. Notably, expression levels of CDK1, CCNB1 and CCNB2 were positively correlated with infiltrating levels of CD4^{+} T cells, CD8^{+} T cells and dendritic cells in HCC. Malki A, Elbayaa RY, et al found that CCNB1, CCNB2 and CHEK1 were involved in p53 signaling pathway.[41] Abundant studies found that upregulating CCNB1, CCNB2 and CHEK1 could regulate p53 signaling pathway, thereby promoting the progression of HCC.[42,43] Liu S, et al[44] found that mRNA levels of 3 p53 markers including CCNB1, CCNB2 and CHEK1 were significantly upregulated in 20 HCC tumor tissues versus nontumor adjacent tissues by bioinformatics analysis and experimental verification, and their mRNA expression levels were all negatively correlated with HCC patients' survival rates. In this study, GO and KEGG analyses revealed that CDC25C was involved in pathways including p53 signaling pathway, which might be associated with invasive characteristics of HCC. According to the report, CDC25C can suppress p53-induced growth arrest,[45] and deregulation of p53 signaling pathway might to be principal mechanisms in liver tumors.

In summary, this study has novelly identified that the elevated expression of CDC25C in HCC patients when compared to that in normal tissue.The results from this study may push forward the mechanism underlying CDC25C progression, and provide the high prognostic value of HCC. However, further studies are needed to intensively disclose the molecular mechanism and implication of CDC25C in HCC tumorigenesis and therapy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Statement

The data we utilized were all drawn from TCGA dataset, which were not obtained from our own clinical samples. The current study was approved and consented by the ethics committee of The Second School of Clinical Medicine & Jingzhou Central Hospital of Yangtze University.

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