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PLK1 Is Implicated in the Poor Prognosis of Hepatocellular Carcinoma

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Abstract

We aimed to identify if PLK1 could be used as a new diagnostic and therapeutic biomarker in hepatocellular carcinoma (HCC) patient. Expression of PLK1 in HCC was analyzed by using GEPIA (Gene Expression Profiling Interactive Analysis) and UALCAN databases. GEPIA and CBioPortal tools were applied to determine patients' survival and PLK1 mutations, respectively. PPI (Protein-Protein Interaction) networks were further built by STRING (Search Tool for the Retrieval of Interacting Genes) and Metascape Web portals. The data demonstrated that the expression of PLK1 in HCC was significantly enhanced when compared to normal liver tissues ($P < 0.001$). A higher PLK1 expression resulted in a remarkably shorter disease-free survival as well as overall survival. Moreover, the expression of PLK1 in HCC was related to HCC patients' grade and race, but not gender. The data also suggested that expression of PLK1 elevated gradually from stage 1 to 3 but decreased in stage 4. Three specific gene mutations K146R, S335Afs*120 and D429H of PLK1 occurred in HCC and these unique mutations were not seen in any other tumor tissues. Finally, PPI networks and GO enrichment analysis suggested that PLK1 might be associated with cell cycle and p53 signaling pathway etc. Taken together, our novel findings suggest that PLK1 is implicated in the poor prognosis of hepatocellular carcinoma.

Keywords

PLK1, HCC, TCGA, Biomarker, Cancer Therapy

1. Introduction

Clinical data showed that hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide due to its frequent metastasis and lack of curative treatment [1]. More than 580,000 new cases of liver cancer occur in Asia every year [2]. Progressive accumulation of alterations in cancer drives genes and dysregulation of their associated signaling pathways, causing the occurrence and progression of HCC [3]. HCC is insensitive to radiotherapy and chemotherapy, and there is no uniform standard for the optimal dose of radiotherapy [4]. Although liver transplantation is a potential therapy for HCC, its application is limited by the liver donor supply. For targeted therapy, it can block the effect of key molecules in the formation and progression of liver cancer. Targeted drugs affect liver cancer cells more than normal cells. However, only a few drugs are currently available for patients with advanced liver cancer. Therefore, it is significant to investigate the novel key genes and major signal pathways involving in the development of HCC. To date, serum alpha-fetoprotein (AFP) and PIVKA-II (protein induced by vitamin K absence or antagonist-II) are two best used biomarker for HCC in clinical screening [5]-[10]. The combination use of the both biomarkers has significantly improved HCC sensitivity detection despite that their sensitivity and specificity are far from satisfactory [11] [12] [13] [14]. However, numerous recent studies have shown that the cut-off value of PIVKA-II and AFP, the tumor size and etiology did not have significant effects on the liver cancer heterogeneity. Therefore, it is necessary to find new genes that are useful for screening, diagnosis and monitoring of HCC.

PLK1 (Polo-like kinase 1), a serine/threonine-protein kinase that belongs to the polo-like kinase family, participates in various biological processes, including cell cycle and RNA processing [15] [16]. At present, five polo-like kinsey family members (PLL1-5) have been identified in humans [17]. Bu *et al.* [18] tested high expression of PLK1 in the HCC tissues, and showed significantly worse effect in the hematological type. Study suggested that PLK1 promotes the degradation of SUZ12 and ZNF198 by protea-some, which is a major factor in liver cancer [19]. PLK1 phosphorylation of PTEN also caused a tumor promoting metabolic state [20]. Moreover, PLK1 is over expressed in many cancers and serves as a significant prognostic factor in cancers, such as small-cell lung cancer, colon cancer and ovarian cancer [21]. In addition, high expression levels of PLK1 in melanoma and breast cancer correlated well with the metastatic potential of these tumors [22] [23]. PLK1 over-expression might also contribute to the deregulation of cell proliferation during oncogenesis by overcoming mitotic checkpoints [24].

Therefore, in order to verify the value of PLK1 in the diagnosis and treatment of HCC, it is very important to analyze the expression and significance of PLK1 in liver cancer tissues.

2. Materials and Methods

2.1. UALCAN Analysis

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a web tool to profile gene expressions between tumor and non-tumor tissues and provides interactive data analyses [25]. In this study, we utilized this online tool to analyze the expression levels of PLK1 between HCC specimen and normal tissues.

2.2. 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 calculate 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.

2.3. Construction of the PPI Networks

The STRING database (http://string-db.org/) and Metascape (http://metascape.org/) tool was used to analyze the PPI networks. In this study, the PPI networks of the PLK1 gene was constructed using STRING and Metascape database. The non-interacting genes were excluded in order to simplify the PPI network. The top 12 genes with the highest degree of connection to the others were presented.

2.4. GO and KEGG Analysis

The STRING database (http://string-db.org/) is an online tool for high-throughput functional analysis of genes. In this study, the potential associations between the 12 core genes and PLK1 were assessed through the GO annotation analysis and KEGG pathway enrichment analysis [26] [27]. P-values < 0.05 were considered as statistically significance.

2.5. Analysis of Genetic Alterations

cBioPortal (https://www.cbioportal.org/) is a database with integrated genetic data, including DNA mutations, gene amplifications and protein alterations. In our study, the cBioPortal database was used to analyze the association between genetic mutations and the development of HCC. The top 12 genes which are related to PLK1 were analyzed by using cBioPortal database. And we performed PLK1 gene mutations analysis across all tumor samples from the TCGA-HCC database.

3. Results

3.1. The Expression Levels of PLK1 in HCC Patients

To verify PLK1 expression levels in HCC tissues and the value to the diagnosis and surveillance of HCC, GEPIA database was applied. As shown in **Figure 1(a)**, the data showed that the expression level of PLK1 in the HCC group is significant higher than normal liver group (P < 0.001). Moreover, the relationship between PLK1 expression levels and HCC patients' clinicopathological parameters were further analyzed by UALCAN databases. The result demonstrated that

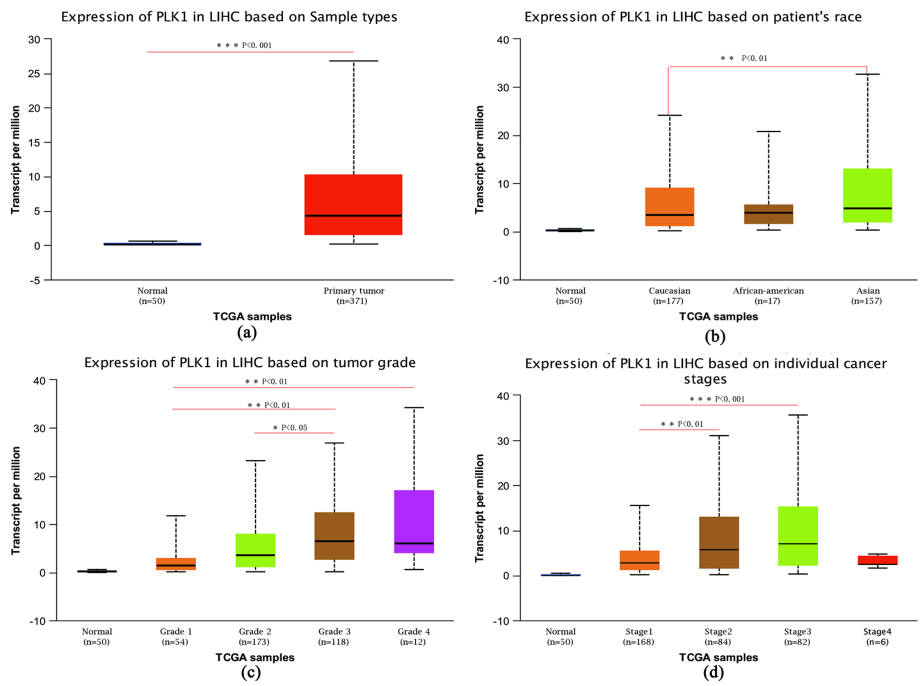


Figure 1. Over-expression of PLK is associated with malignancy of HCC. (a) PLK1 expression in normal and HCC tissues from TCGA data-sets; (b) The expressions of PLK1 was partially related to patients race; (c) The high expression of PLK1 was significantly related to cancer grade; (d) The expressions of PLK1 was partially related to cancer stages. * P < 0.05, ** P < 0.01, *** P < 0.001.

expression of PLK1 was higher in Asian HCC patients than Caucasian patients (P < 0.01, Figure 1(b)). The expression of PLK1 increased from grade 1 to grade 4 of HCC, suggesting PLK1 was remarkably correlated with HCC patients' grade (Figure 1(c)) (grade 1 vs grade 3, P < 0.01 ; grade 1 vs grade 4, P < 0.01; grade 2 vs grade 3, P < 0.05). As shown in Figure 1(d). we also found there are gradually increased expression of PLK1 from stage 1 to stage 3 but obviously declined in stage 4 (stage 1 vs stage 2, P < 0.01 and stage 1 vs stage 3, P < 0.001).

3.2. Survival Analysis of HCC Patients Based on PLK1 Expression

Here, PLK1 expression of HCC patients was divided into low-expression group and high-expression group (cutoff-high is 50%, cutoff-low is 50%). As shown in Figure 2, the overall survival (Figure 2(a)) and disease free survival (Figure 2(b)) were significant better in low PLK1 expression group than high PLK1 expression group (P < 0.001). Survival curves analysis showed that PLK1 was suitable for predicting liver cancer patients' prognosis.

3.3. PPI Networks and GO Enrichment Analysis of PLK1

The functional interactions between proteins can provide us some information in molecular mechanism. In this study, PPI network was constructed by the Metascape database. PPI network analysis indicated that PLK1 has more interactions with other 12 proteins, including CDC20, ERCC6L, CCNB1, CCNB2,

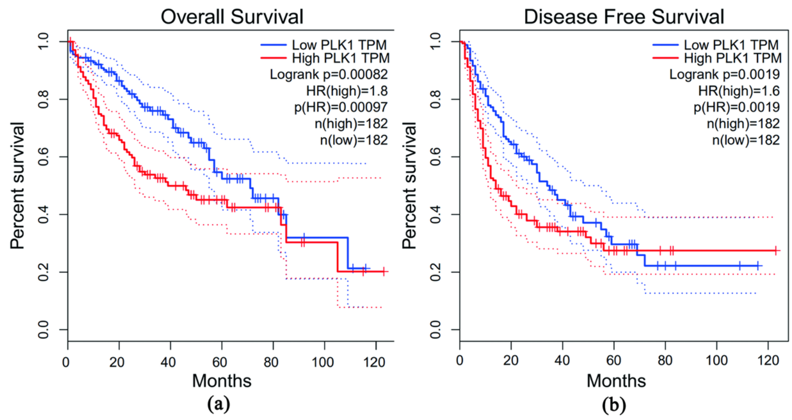


Figure 2. Prognostic value of PLK1 in liver cancer patients. Higher expressions of PLK1 was associated with poorer OS (a) and DFS (b) in HCC patients.

KIF2C, BUB1, MAD2L1, CENPE, INCENP, CDK1, CDCA8 and NDC80 (**Figure 3(a)**). To predict the biological functions and signaling pathways in which PLK1 were involved in HCC, GO enrichment and KEGG pathway analyse were further performed (**Figure 3(b)** and **Table 1**). The results showed that those proteins were biologically closely associated with cell cycle, p53 signaling pathway, oocyte meiosis and progesterone-mediated oocyte maturation etc.

3.4. Specific Mutations of PLK1 Genes in HCC Patients

In order to analyze the mutations of PLK1 gene in HCC patient's tissues, the CBioPortal database analysis was employed. As shown in **Figure 4**, we performed PLK1 gene mutations analysis across all tumor samples from the TCGA-HCC database (https://www.cbioportal.org/). Intriguingly, there were three specific mutations K146R, S335Afs*120 and D429H (**Figure 4(a)** and **Figure 4(b)**) in the HCC samples that were not present in any other tumor samples. These particular mutations of PLK1 in HCC patients might contribute greatly to HCC clinical diagnosis and monitoring. Moreover, the mutations between PLK1 and its interacted genes (CDC20, ERCC6L, CCNB1, CCNB2, KIF2C, BUB1, MAD2L1, CENPE, INCENP, CDK1, CDCA8 and NDC80) were analyzed through the cBioPortal dataset. The alteration statuses of 12 key genes were analyzed using TCGA HCC patients' data of cBioPortal database. The genetic alteration of PLK1 genes was altered in 52 (13%) of 377 HCC patients (**Figure 4(c)**).

3.5. Prediction of Relevance Genes to PLK1

To predict the biological functions and signaling pathways in which PLK1 were involved in HCC, we found that the 12 genes were considered to be relevant genes and the scatter plots were shown in **Figures 5(a)-(l)**.

4. Discussion

Bioinformatics methods can provide us with gene expression levels and predict

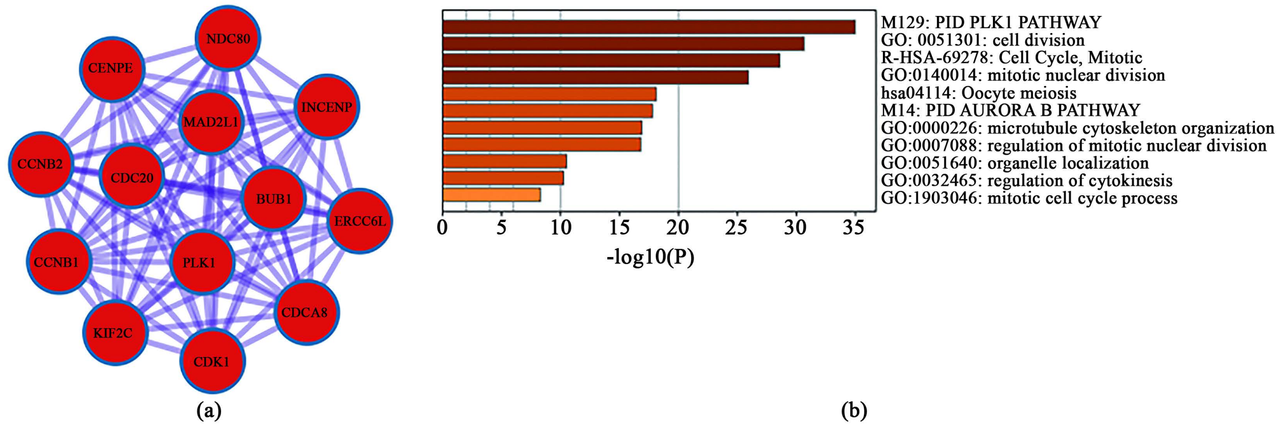


Figure 3. PPI network and GO enrichment analysis of twelve hub genes related to PLK1. (a) PPI network and MCODE components identified in the gene lists; (b) GO enrichment analysis of the 12 PLK1 related genes.

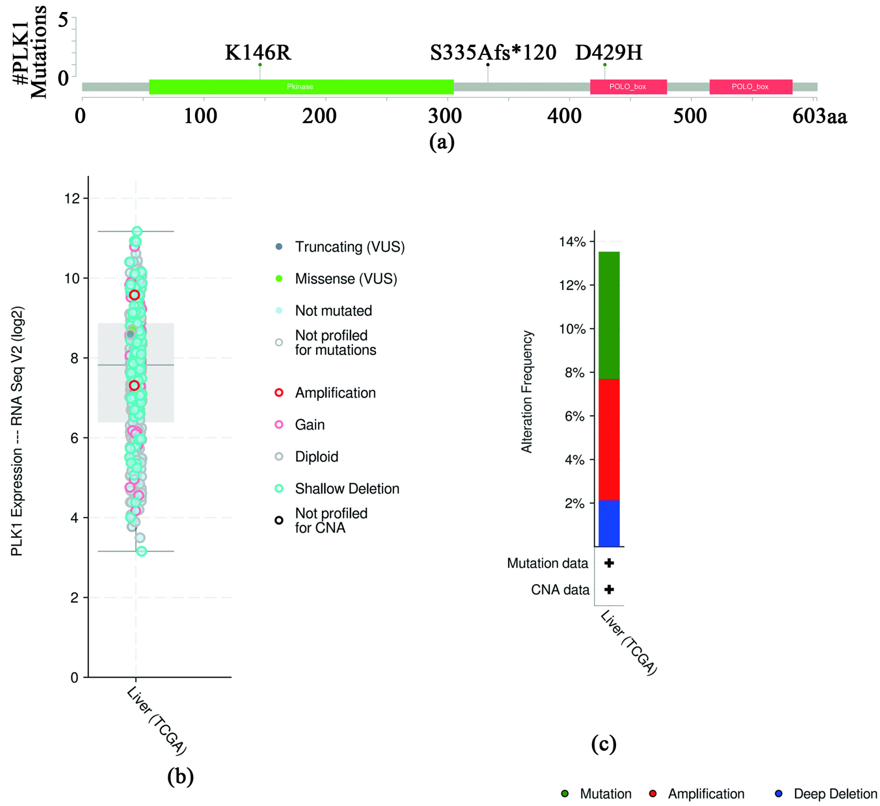


Figure 4. (a) and (b) The particular mutations (K146R, S335Afs*120 and D429H) of PLK1 in HCC patients. (c) A visual summary of genetic alterations (data from HCC in TCGA) shows the genetic alteration of PLK1 genes which were altered in 52 (13%) of 377 HCC patients.

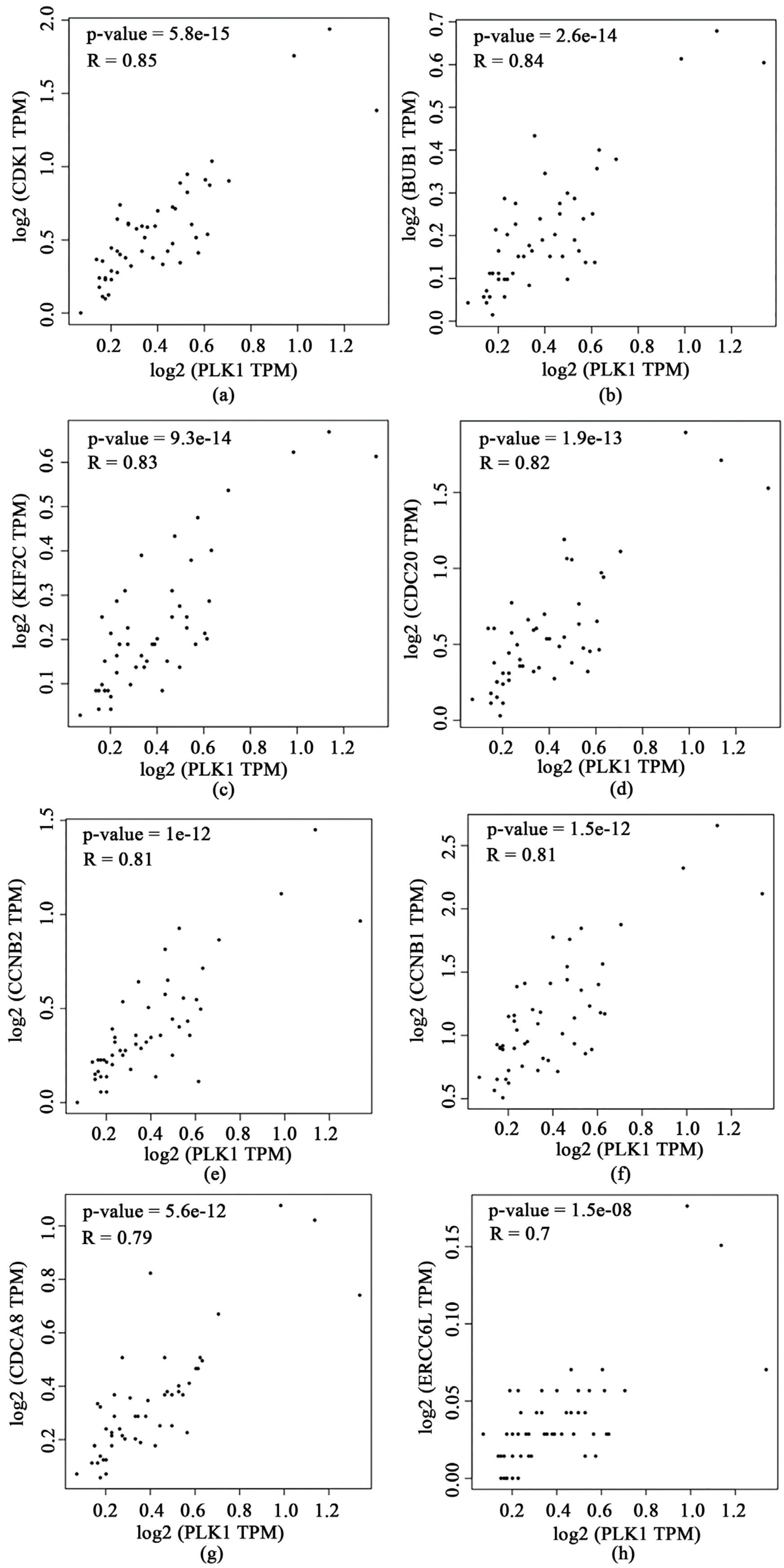
potential therapeutic targets. A large number of clinical data showed that the death rate of liver cancer is very high. One of the best ways to reduce mortality is to detect accurately and treat successfully. Identifying key genes associated with the development and the progression of HCC is crucial for its diagnosis and treatment.

Table 1. Significantly enriched GO terms and KEGG pathways of PLK1.

Category	Terms	Count	P-Value
GOTERM_BP_DIRECT	cell division	21	2.35E−31
GOTERM_BP_DIRECT	mitotic cell cycle process	19	6.36E−25
GOTERM_BP_DIRECT	cell cycle	21	2.57E−23
GOTERM_BP_DIRECT	nuclear division	14	1.80E−19
GOTERM_BP_DIRECT	sister chromatid segregation	12	2.37E−19
GOTERM_BP_DIRECT	chromosome segregation	13	6.97E−18
GOTERM_BP_DIRECT	regulation of cell cycle process	16	8.80E−18
GOTERM_BP_DIRECT	regulation of nuclear division	12	1.51E−17
GOTERM_BP_DIRECT	mitotic nuclear division	11	6.31E−17
GOTERM_BP_DIRECT	regulation of cell cycle	17	2.87E−16
GOTERM_BP_DIRECT	mitotic sister chromatid segregation	10	4.30E−16
GOTERM_BP_DIRECT	anaphase-promoting complex-dependent catabolic process	8	2.87E−15
GOTERM_BP_DIRECT	regulation of mitotic nuclear division	10	3.03E−14
GOTERM_BP_DIRECT	microtubule cytoskeleton organization	12	6.12E−14
GOTERM_BP_DIRECT	regulation of cell cycle phase transition	11	2.65E−12
GOTERM_BP_DIRECT	chromosome organization	14	3.17E−12
GOTERM_BP_DIRECT	regulation of chromosome segregation	8	4.06E−12
GOTERM_BP_DIRECT	negative regulation of cell cycle process	10	4.06E−12
GOTERM_BP_DIRECT	regulation of mitotic metaphase/anaphase transition	7	4.42E−12
GOTERM_BP_DIRECT	negative regulation of cell cycle phase transition	9	4.89E−12
GOTERM_MF_DIRECT	anaphase-promoting complex binding	3	9.51E−6
GOTERM_MF_DIRECT	protein serine/threonine kinase activity	7	1.24E−5
GOTERM_MF_DIRECT	micro-tubule binding	6	1.24E−5
GOTERM_MF_DIRECT	ATP binding	10	2.44E−5
GOTERM_MF_DIRECT	protein kinase binding	7	2.44E−5
GOTERM_MF_DIRECT	histone kinase activity	3	2.44E−5
GOTERM_MF_DIRECT	ubiquitin-protein transferase regulator activity	3	2.44E−5
GOTERM_MF_DIRECT	microtubule motor activity	4	4.46E−5
GOTERM_MF_DIRECT	cyclin-dependent protein serine/threonine kinase activity	3	4.96E−5
GOTERM_MF_DIRECT	ATPase activity	5	0.0002
GOTERM_MF_DIRECT	ubiquitin-protein transferase activator activity	2	0.0002
GOTERM_MF_DIRECT	catalytic activity, acting on a protein	8	0.004

Continued

GOTERM_MF_DIRECT	enzyme binding	8	0.004
GOTERM_MF_DIRECT	catalytic activity	13	0.004
GOTERM_MF_DIRECT	protein binding	14	0.005
GOTERM_MF_DIRECT	binding	18	0.03
GOTERM_MF_DIRECT	protein-containing complex binding	4	0.04
GOTERM_CC_DIRECT	spindle	14	2.13E−18
GOTERM_CC_DIRECT	chromosome, centromeric region	11	1.40E−17
GOTERM_CC_DIRECT	condensed chromosome, centromeric region	12	1.40E−17
GOTERM_CC_DIRECT	microtubule cytoskeleton	17	1.40E−16
GOTERM_CC_DIRECT	condensed chromosome kinetochore	10	2.79E−16
GOTERM_CC_DIRECT	cytoskeletal part	17	1.37E−14
GOTERM_CC_DIRECT	mid-body	9	1.05E−12
GOTERM_CC_DIRECT	condensed nuclear chromosome, centromeric region	6	3.00E−12
GOTERM_CC_DIRECT	condensed chromosome outer kinetochore	5	3.96E−11
GOTERM_CC_DIRECT	condensed nuclear chromosome kinetochore	5	6.72E−11
GOTERM_CC_DIRECT	condensed nuclear chromosome	7	8.66E−11
GOTERM_CC_DIRECT	intracellular non-membrane-bounded organelle	19	9.47E−11
GOTERM_CC_DIRECT	cytosol	20	1.44E−10
GOTERM_CC_DIRECT	condensed nuclear chromosome outer kinetochore	4	4.54E−10
GOTERM_CC_DIRECT	spindle midzone	5	1.99E−9
GOTERM_CC_DIRECT	microtubule	8	2.41E−8
GOTERM_CC_DIRECT	nuclear lumen	17	3.27E−8
GOTERM_CC_DIRECT	nucleoplasm	16	4.07E−8
GOTERM_CC_DIRECT	microtubule associated complex	6	4.85E−8
GOTERM_CC_DIRECT	spindle pole	6	5.91E−8
KEGG_PATHWAY	cell cycle	10	3.64E−16
KEGG_PATHWAY	oocyte meiosis	10	3.64E−16
KEGG_PATHWAY	progesterone-mediated oocyte maturation	9	2.60E−15
KEGG_PATHWAY	p53 signaling pathway	3	0.00019
KEGG_PATHWAY	HTLV-I infection	4	0.00036
KEGG_PATHWAY	foxo signaling pathway	3	0.00081
KEGG_PATHWAY	cellular senescence	3	0.0012
KEGG_PATHWAY	ubiquitin mediated proteolysis	2	0.0150
KEGG_PATHWAY	microRNAs in cancer	2	0.0163
KEGG_PATHWAY	viral carcinogenesis	2	0.0215


p-value = 5.8e-15
R = 0.85
log2 (CDK1 TPM)
log2 (PLK1 TPM)
(a)
p-value = 2.6e-14
R = 0.84
log2 (BUB1 TPM)
log2 (PLK1 TPM)
(b)
p-value = 9.3e-14
R = 0.83
log2 (KIF2C TPM)
log2 (PLK1 TPM)
(c)
p-value = 1.9e-13
R = 0.82
log2 (CDC20 TPM)
log2 (PLK1 TPM)
(d)
p-value = 1e-12
R = 0.81
log2 (CCNB2 TPM)
log2 (PLK1 TPM)
(e)
p-value = 1.5e-12
R = 0.81
log2 (CCNB1 TPM)
log2 (PLK1 TPM)
(f)
p-value = 5.6e-12
R = 0.79
log2 (CDCA8 TPM)
log2 (PLK1 TPM)
(g)
p-value = 1.5e-08
R = 0.7
log2 (ERCC6L TPM)
log2 (PLK1 TPM)
(h)

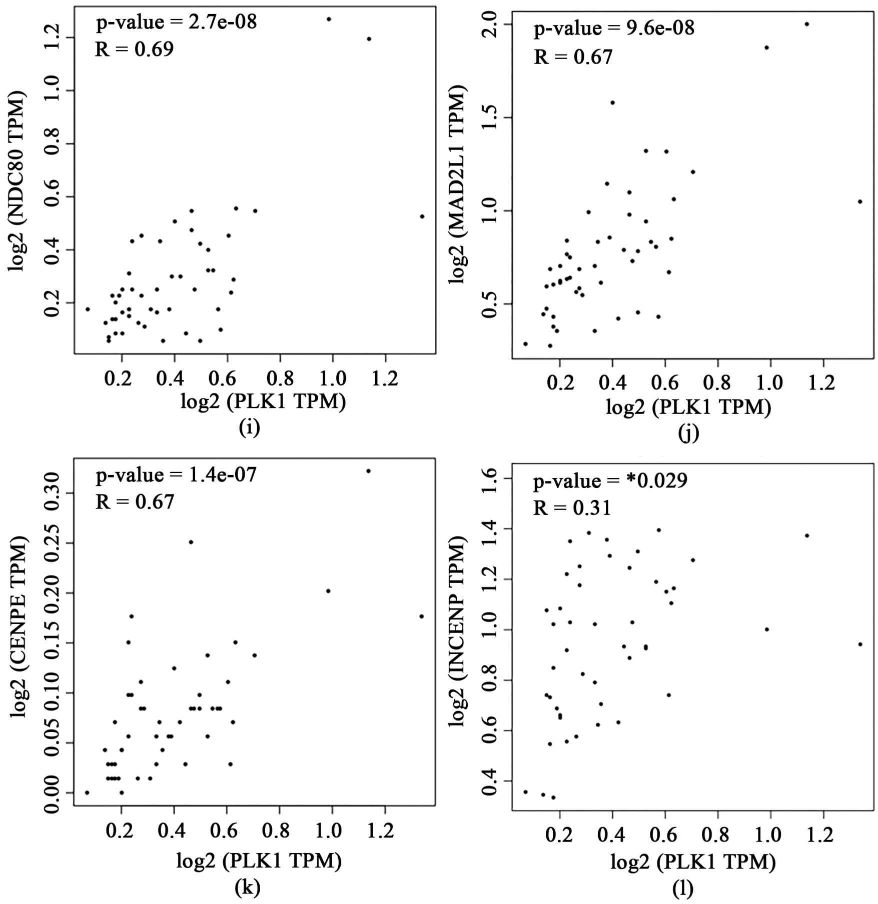


Figure 5. The plots of 12 pair-wise correlation genes. (a) PLK1 and CDK1; (b) PLK1 and BUB1; (c) PLK1 and KIF2C; (d) PLK1 and CDC20; (e) PLK1 and CCNB2; (f) PLK1 and CCNB1; (g) PLK1 and CDCA8; (h) PLK1 and ERCC6L; (i) PLK1 and NDC80; (j) PLK1 and MAD2L1; (k) PLK1 and CENPE; (l) PLK1 and INCENP.

In this study, our data showed that PLK1 expression was higher in HCC patients than that in normal tissues. PLK1 expression was remarkably correlated with HCC patients' grade. The results demonstrated that PLK1 expression enhanced gradually from stage 1 to stage 3 but decreased in stage 4. A higher PLK1 expression resulted in a significant shorter disease free survival as well as overall survival in HCC patients, suggesting that PLK1 may play an important role in the prognosis of HCC. By mutation analysis, CBioPortal tool unveiled three specific mutations (K146R, S335Afs*120 and D429H) unique presented in the HCC samples that were not occurred in any other tumor types. These characteristic mutations of PLK1 in HCC patients might facilitate to HCC clinical diagnosis and monitoring.

To determine the probably pathogenic mechanism of PLK1 in HCC, PPI networks were further applied. The twelve interacted proteins were identified by using PPI network analysis. Go enrichment analysis suggested these genes are enriched in cell cycle and p53 signaling pathway etc. Recent study indicated that cell cycle dysregulation plays an important role in the liver tumorigenesis [1].

It's been reported that disruption of the cell cycle pathway can result in cell cycle arrest and has previously been related to the prognosis of human cancers [28]. Furthermore, cell cycle arrest has been confirmed to be an effective approach in controlling tumor growth [29] [30]. Boxuan Li *et al.* [31] found that PLK1 plays a crucial role in the disruption of the cell cycle pathway by dramatically induced apoptosis. Shen, L.Y. found that PLK1 could arrest cell cycle in G2/M phase and then block cell cycle pathway [32]. High expression level of PLK1 was also identified in HCC tissues [33]. Taken together, our novel findings suggest that PLK1 might play a crucial role in regulating the middle of cell cycle pathway.

Recently, new findings have pointed that PLK1 is able to inhibit apoptosis in a p53-dependent manner in a variety of carcinomas [34]. Wei Sun [35] reported that the p53 tumor-suppressor protein is phosphorylated by PLK1, which can inhibit the proapoptotic function of p53.The inhibition of PLK1 leads to a failure to complete mitosis, eventually resulting in cell death [36]. PLK1 could interact with the DNA binding domain of p53, thereby decreasing its stability and transcriptional activity [37]. Thus, p53 is a major target for PLK1 controlling the growth of carcinoma cells. PLK1 is a cell cycle protein that plays multiple roles in promoting cell cycle progression. Among the many roles, the most prominent role of PLK1 is to regulate the mitotic spindle formation checkpoint at the M-phase [38]. Robert D. Van Horn [39] thought that CDK1 and PLK1 are likely to act in a positive feedback activation loop for CDK1 activation through CDC25-mediated dephosphorylation during G2/M transition. CDK1 and PLK1 could form a positive feedback activation loop in human cells. Activation of CDK1 initiates the entry into mitosis and activation of PLK1. PLK1 then further feedback-activate CDK1 to promote rapid and timely entry into mitosis and coordinately regulate various aspects of mitosis, such as bipolar mitotic spindle formation and checkpoint response [40].

Sharon I. King *et al.* have demonstrated a striking association between cancer and K146R mutation of PLK1 by immunohistochemical analysis and DNA sequencing analysis of 215 primary breast tumours [41]. Targeting PLK1 mutant at K146R site breast cancer might offer therapeutic opportunities. Although no related mutations have been reported in HCC tissues, we suggest that these particular mutations of PLK1 in HCC patients might contribute greatly to HCC clinical diagnosis and monitoring.

5. Conclusion

In summary, this study has novelly identified the elevated expression of PLK1in HCC patients when compared to that in normal tissue, and it is negatively correlated with patients' survival time. The results from this study may push forward the mechanism underlying PLK1 progression, and provide the high prognostic value of HCC. However, further studies are needed to intensively disclose the molecular mechanism and implication of PLK1 in HCC tumorigenesis and therapy.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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