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Expression patterns and prognostic values of the *cyclin-dependent kinase 1* and *cyclin A2* gene cluster in pancreatic adenocarcinoma

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Abstract

Objective: Pancreatic adenocarcinoma (PAAD) is one of the most lethal malignant tumors worldwide. Various studies based on cell lines, preclinical mouse models, and human tissue samples have shown that cell cycle-associated proteins are involved in the tumorigenesis and progression of PAAD.

Methods: Herein, we analyzed the relationships between *CDK1* and *CCNA2* gene expression and prognosis in patients with pancreatic cancer, using information from the Oncomine, cBioportal, Kaplan–Meier Plotter, and GEPIA databases.

Results: Expression levels of *CDK1* and *CCNA2* were significantly higher in PAAD compared with control tissues, and were associated with more advanced tumor stage. Survival analyses using the Kaplan–Meier Plotter database further confirmed that increased expression levels of *CDK1* and *CCNA2* were associated with a poor prognosis in patients with pancreatic cancer.

Conclusions: The results of this study suggest that CDK1 and CCNA2 may be potential therapeutic targets and prognostic biomarkers in patients with PAAD.

Keywords

CDK1, CCNA2, pancreatic adenocarcinoma, gene expression, prognosis, database

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Introduction

Pancreatic adenocarcinoma (PAAD) is one of the most lethal malignant tumors worldwide. According to GLOBOCAN 2012 estimates, more than 331,000 people die from

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pancreatic cancer each year, accounting for 4.0% of all cancer-related deaths. PAAD is ranked as the seventh leading cause of cancer-related deaths in both sexes.[1,2] The incidence of pancreatic cancer is positively correlated with the level of economic development, and is higher in developed countries compared with developing countries. The 5-year survival rate for pancreatic cancer is estimated to be <5%.[3] Despite extensive studies aimed at improving surgery and targeted therapies, the overall survival (OS) rate for patients with pancreatic cancer is extremely low, and the treatment of recurrent pancreatic cancer still presents huge challenges. There is therefore an urgent need to explore the mechanisms of pancreatic cancer and to identify new tumor biomarkers with high specificity and sensitivity.

Cyclin-dependent kinase (CDK) 1 is the only essential CDK[4,5] required to facilitate the G2-M phase transition. It regulates G1 phase progression and G1-S transition.[6] Alterations in *CDK1* gene activity often lead to uncontrolled proliferation of tumor cells, which is a hallmark of malignant tumors. Cyclin A2 (CCNA2) is expressed in most human tissues and is a highly conserved member of the cyclin protein family. It plays a key role in controlling the G1-to-S and G2-to-M cell cycle transitions, in addition to being a key regulator of the hematopoietic lineage and in embryonic cells.[7] Although dysregulated expression levels of *CDK1* and *CCNA2* genes and their links to patient prognosis and features have been reported in some pancreatic cancer studies,[8] their expression profiles and prognostic relevance still remain unclear. In this study, we systematically evaluated the expression levels, potential functions, and prognostic relevance of *CDK1* and *CCNA2* in PAAD using a large number of data sets available in public databases.

Materials and methods

Ethics statement

This study was approved by the institutional review board of Shandong Provincial Third Hospital (Permit Number: No. 102) and was conducted according to the ethical guidelines of the Declaration of Helsinki. All the datasets were extracted from online public databases, for which written informed consent had been obtained.

Oncomine analysis

Oncomine (www.oncomine.org) is a public database that can be used to analyze gene expression in various cancers. It uses Student's *t*-tests for two-class differential expression analyses. In the current study, we entered the names of the genes of interest into Oncomine to analyze their expression levels in pancreatic cancer. The expression levels of *CDK1* and *CCNA2* in cancer specimens were compared with those in normal controls. The cut-off of *P*-value was set at 0.05.

GEPIA dataset

GEPIA (Gene Expression Profiling Interactive Analysis) is an online database that combines TCGA and GTEx data to compile RNA-seq results from 9,736 tumor and 8,587 normal samples, to allow assessment of differential expression profiles, patient outcomes, and other analyses.[9] We used these public bioinformatics platforms to evaluate the expression profiles of *CDK1* and *CCNA2* in pancreatic cancer.

UALCAN database

The UALCAN database (http://ualcan.path.uab.edu) is an interactive web resource including RNA-seq and clinical data for 31 cancer types from the TCGA database. It can be used to compare expression of

the gene of interest between tumor and normal samples, and to analyze the relationships between gene expression and clinicopathologic parameters.[10] In this study, we used UALCAN to analyze the mRNA expression levels of *CDK1* and *CCNA2* in pancreatic cancer tissues and their association with clinicopathologic parameters. The significance threshold was $P < 0.05$.

RNA extraction and quantitative reverse transcription-polymerase chain reaction (PCR)

We verified the mRNA expression levels of *CDK1* and *CCNA2* in 10 pairs of PAAD tissues and adjacent non-tumor tissues collected during surgery from patients at Shandong Provincial Third Hospital, between June and November 2019. Written informed consent was obtained from all participating patients. Approximately 20 mg of tissue was obtained from each sample and subjected to RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. All PCR reactions were performed using an ABI Prism 5700 Sequence Detection System (PerkinElmer Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

Human Protein Atlas

The Human Protein Atlas database (https://www.proteinatlas.org) contains immunohistochemical expression data for

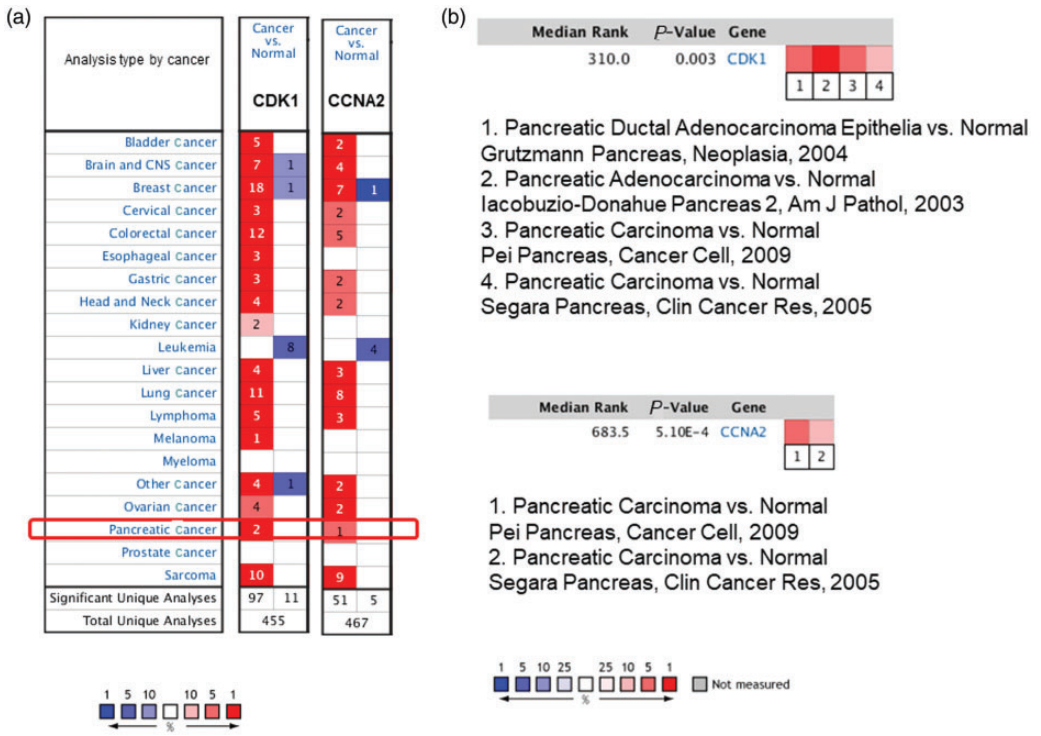

Figure 1. Transcription levels of *CDK1* and *CCNA2* in different types of cancers (Oncomine). (a) Expression levels of *CDK1* and *CCNA2* in different types of cancers. Red, over-expression; blue, under-expression. (b) Expression levels of *CDK1* and *CCNA2* in several pancreatic cancer studies. Red, over-expression. Significance threshold $P < 0.05$.

nearly 20 common kinds of tumors, including 12 individual tumor subtypes for each tumor.[11] The database allows researchers to identify the expression patterns of certain proteins in particular types of tumors. We directly compared CDK1 and CCNA2 expression levels between normal and pancreatic cancer tissues based on immunohistochemistry images.

Prognostic value analyses

We assessed the prognostic relevance of *CDK1* and *CCNA2* expression in pancreatic cancer using the OncoLnc (www.oncolnc.org) and GEPIA databases. For each gene, the median expression value was used to stratify patients into low- and high-expressing groups, and the OS of pancreatic cancer patients was

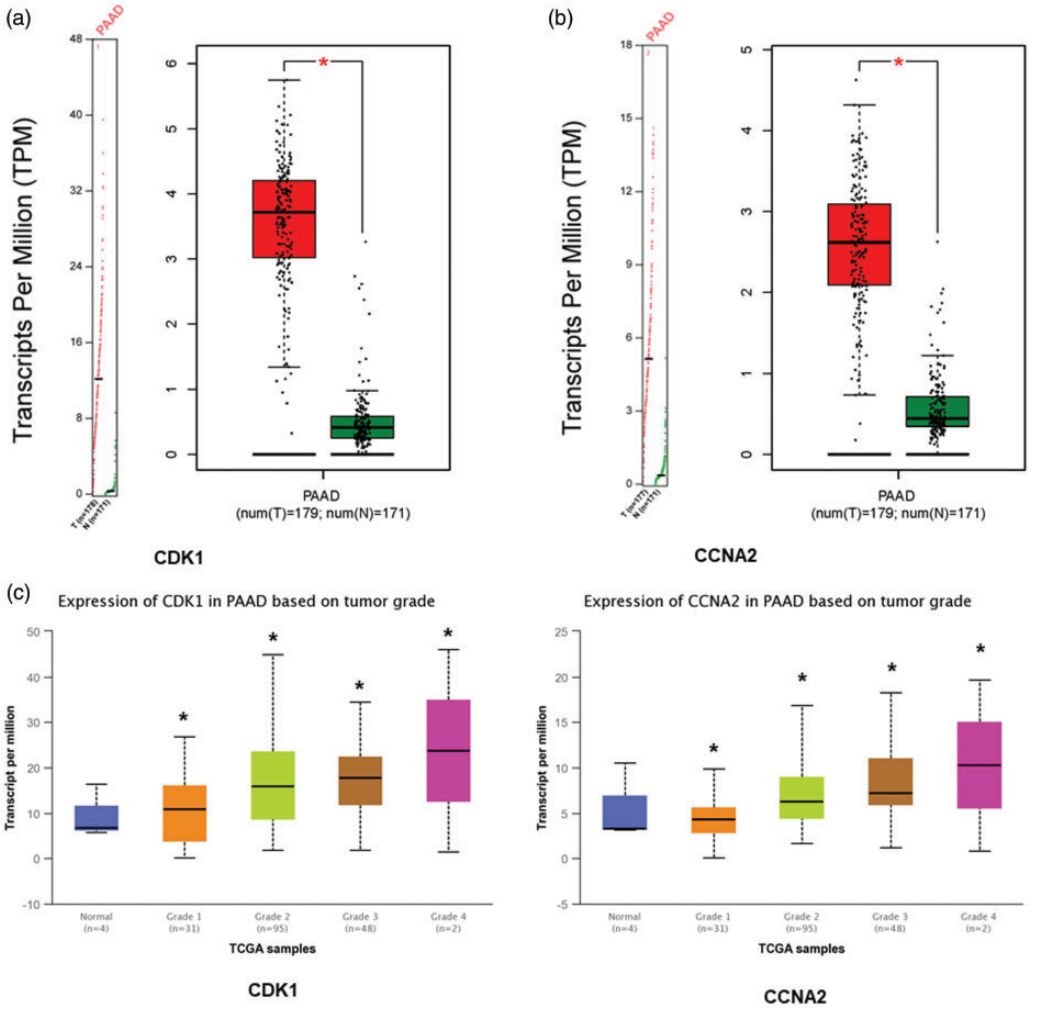

Figure 2. Correlation between *CDK1*/*CCNA2* expression and tumor stage in patients with pancreatic adenocarcinoma (PAAD). Expression profiles of *CDK1* (a) and *CCNA2* (b) in patients with PAAD. (c) Correlation between *CDK1* and *CCNA2* expression levels and tumor stage in patients with PAAD (UALCAN database). X-axis indicates PAAD tumor stage; y-axis indicates expression level of *CDK1*/*CCNA2*. *$P < 0.05$ compared with normal control. PAAD, pancreatic adenocarcinoma; T, tumor; N, normal.

assessed, with corresponding 95% confidence intervals and *P*-values. $P < 0.05$ was considered statically significant.

Functional pathway enrichment analyses

The Gene Ontology (GO) resource allows the identification of attributes that are significantly overrepresented in a set of genes, including cellular component, molecular function, and biological process attributes.[12] In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database is used to understand the high-level functions and utilities of biological systems. The online STRING (https://string-db.org/) tool is used to provide investigators with systematic and comprehensive functional annotation tools to reveal the biological meaning behind an extensive list of genes. In our study, GO and KEGG enrichment analyses were conducted for *CDK1* and *CCNA2* using STRING. The significance threshold was $P < 0.05$.

Results

CDK1 and *CCNA2* gene expression in various cancers

We compared *CDK1* and *CCNA2* gene expression levels in all cancer and normal tissues using the Oncomine database. *CDK1* mRNA levels were significantly elevated in pancreatic cancer in two datasets, and *CCNA2* was significantly upregulated in one pancreatic cancer dataset ($P < 0.05$) (Figure 1a). *CDK1* and *CCNA2* were expressed in most cancers, indicating that

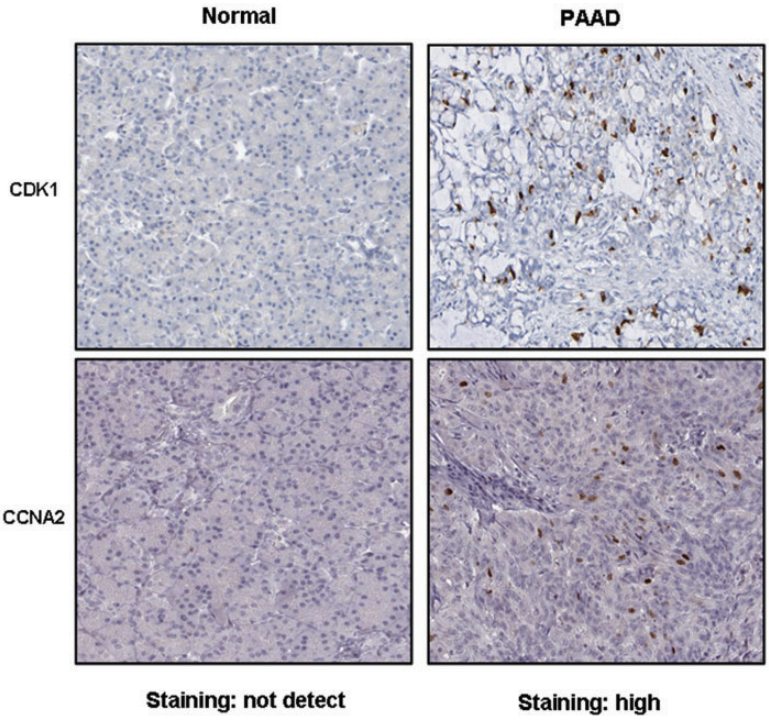

Figure 3. CDK1 and CCNA2 expression in pancreatic adenocarcinoma (PAAD) and normal tissues from the Human Protein Atlas. CDK1 and CCNA2 proteins were highly expressed in PAAD but not in normal pancreatic tissues, according to immunohistochemistry. PAAD, pancreatic adenocarcinoma.

these genes may be cancer-promoting genes. We further queried the Oncomine database to analyze the expression levels of *CDK1* and C*CNA2* in several pancreatic cancer studies, and found that the transcription levels of both genes were significantly higher in pancreatic cancer compared with normal pancreatic tissues ($P < 0.05$) (Figure 1b).

Relationship between CDK1 and CCNA2 expression levels and clinicopathological findings in patients with PAAD

Expression levels of *CDK1* and *CCNA2* were significantly higher in PAAD compared with normal control tissues, according to GEPIA ($P < 0.05$) (Figure 2a, b). There was also a clear correlation between *CDK1* and *CCNA2* expression and PAAD tumor grade, with more advanced grades associated with higher expression of *CDK1* and *CCNA2* (Figure 2c). We also examined CDK1 and CCNA2 protein expression patterns in PAAD using the Human Protein Atlas, and confirmed that CDK1 and CCNA2 protein levels were elevated in PAAD relative to normal controls (Figure 3). We further verified the mRNA expression levels of *CDK1* and *CCNA2* in 10 pairs of PAAD and adjacent non-tumor tissues collected during surgery from

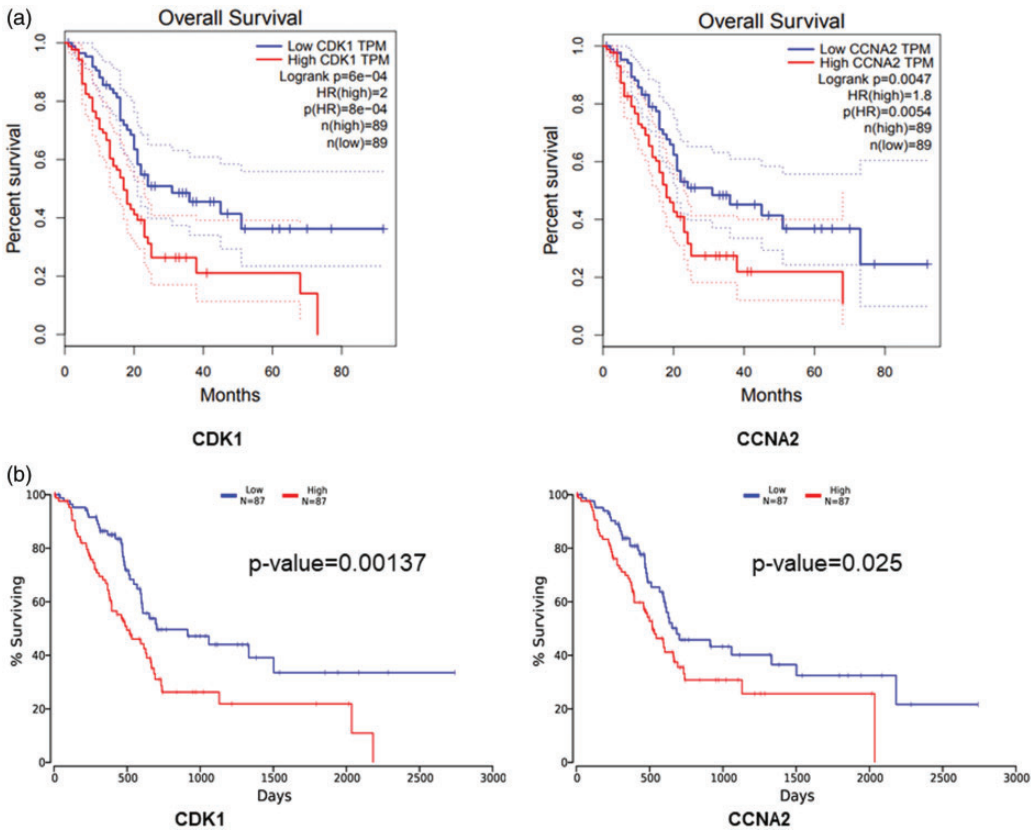

Figure 4. Prognostic values of *CDK1* and *CCNA2* expression in patients with pancreatic adenocarcinoma. Overall survival curves for patients with high (red) and low (blue) *CDK1* and *CCNA2* expression levels were plotted using the GEPIA and OncoLnc databases, with a threshold of $P < 0.05$. TPM, transcripts per million.

patients at Shandong Provincial Third Hospital (aged 55–70 years, 5 male and 5 female), and showed that *CDK1* and *CCNA2* mRNA levels were significantly higher in PAAD compared with normal tissues ($P < 0.05$) (Appendix 1). Taken together, our results showed that transcriptional and proteomic expression levels of *CDK1* and *CCNA2* were increased in patients with PAAD.

Prognostic values of CDK1 and CCNA2 in PAAD patients

We evaluated the prognostic values of *CDK1* and *CCNA2* in PAAD patients using the GEPIA and OncoLnc databases, and showed that increased expression levels of both genes were significantly associated with poor OS ($P = 0.001$ and $P = 0.025$, respectively) (Figure 4).

Functional enrichment of CDK1 and CCNA2 in PAAD patients

We explored the functions of *CDK1* and *CCNA2* by GO and KEGG analyses. According to GO analyses, *CDK1* and *CCNA2* and their neighboring genes were primarily enriched for signal transduction involved in DNA damage checkpoint, histone phosphorylation, cellular protein metabolic process, protein phosphorylation, G2/M transition of mitotic cell cycle, mitotic cell cycle checkpoint, nuclear division, negative regulation of mitotic cell cycle, regulation of cell cycle, and cell division (Figure 5a). Enriched molecular functions included transcription regulation by protein domain-specific binding, enzyme binding, protein binding, enzyme regulator activity, ATP binding, protein kinase binding, cyclin binding, histone kinase activity, anaphase-promoting complex binding, and cyclin-dependent protein serine/threonine kinase activity (Figure 5b). Cellular component annotations for these genes included

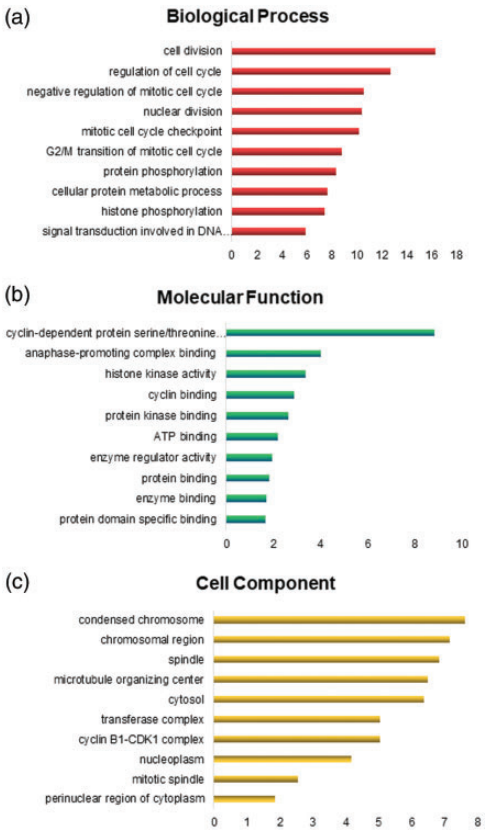

Figure 5. Functional Enrichment Analysis of *CDK1* and *CCNA2* in patients with pancreatic adenocarcinoma. GO enrichment analysis predicted the functions of the target genes from three aspects: biological processes (a), cellular components (b), and molecular functions (c).

perinuclear region of cytoplasm, mitotic spindle, nucleoplasm, cyclin B1-CDK1 complex, transferase complex, cytosol, microtubule organizing center, spindle, chromosomal region, and condensed chromosome (Figure 5c). The KEGG pathways for these two genes are shown in Table 1. Among these pathways, cell cycle, oocyte meiosis, cellular senescence, p53 signaling pathway, viral carcinogenesis, human T-lymphotropic virus type-I infection, FoxO signaling pathway, Epstein–Barr virus infection, phosphoinositide 3-kinase-Akt signaling

Table 1. KEGG pathway analysis of CDK1 and CCNA2 in pancreatic cancer.

Pathway ID	Pathway name	Gene count	False discovery rate	Genes
Hsa04110	Cell cycle	12	1.61E-25	BUB1, BUB1B, CCNA2, CCNB1, CCNB2, CCNE1, CDC20, CDK1, CDK2, MAD2L1, PLK1, PTTG1
Hsa04114	Oocyte meiosis	10	6.36E-20	BUB1, CCNB1, CCNB2, CCNE1, CDC20, CDK1, CDK2, MAD2L1, PLK1, PTTG1
Hsa04218	Cellular senescence	6	1.49E-09	CCNA2, CCNB1, CCNB2, CCNE1, CDK1, CDK2
Hsa04115	p53 signaling pathway	5	2.24E-09	CCNB1, CCNB2, CCNE1, CDK1, CDK2
Hsa05203	Viral carcinogenesis	5	2.23E-07	CCNA2, CCNE1, CDC20, CDK1, CDK2
Hsa05166	HTLV-I infection	5	8.71E-07	BUB1B, CCNB2, CDC20, MAD2L1, PTTG1
Hsa04068	FoxO signaling pathway	4	2.86E-06	CCNB1, CCNB2, CDK2, PLK1
Hsa05169	Epstein–Barr virus infection	3	0.00047	CCNA2, CDK1, CDK2
Hsa04151	PI3K-Akt signaling pathway	2	0.0239	CCNE1, CDK2
hsa05200	Pathways in cancer	2	0.0467	CCNE1, CDK2

HTLV, human T-lymphotropic virus; PI3K, phosphoinositide 3-kinase.

pathway, and pathways in cancer were involved in the tumor development and pathogenesis of PAAD (Figure 6a, b).

Discussion

PAAD remains an important unresolved global health problem, largely because of a lack of early diagnosis and effective treatment targets.[13] Stratifying patients based on biological markers and exploring the prognostic roles of such biomarkers can help to identify novel therapeutic approaches and appropriate treatment strategies. In the current study, we investigated the mRNA expression patterns and prognostic values of *CDK1* and *CCNA2* in PAAD. These results will support efforts to improve the diagnosis, prognostic analysis, and treatment of patients with PAAD.[14,15]

CDK1 is a cell cycle regulator associated with tumor growth.[4] CDK1 can execute all the events required for cell cycle division, indicating that it is sufficient for the proliferation of mammalian cells. CDK1 expression was shown to be significantly increased in colorectal cancer, hepatocellular carcinoma, and human melanoma,[16–18] while another study found that up-regulation of CDK1 expression was associated with a poor prognosis in patients with breast cancer and epithelial ovarian cancer.[19–21] Furthermore, CDK1 inhibitors significantly reduced the proportion of cells in the S and G2/M stages in a pancreatic ductal adenocarcinoma (PDAC) tumor cell model, and inhibition of CDK1 expression also showed promising anti-cancer activity in pancreatic cancer cells.[22] All these findings were consistent with the current results. We found that *CDK1* expression was significantly increased in patients with pancreatic cancer, and its up-regulation was significantly associated with a poor prognosis.

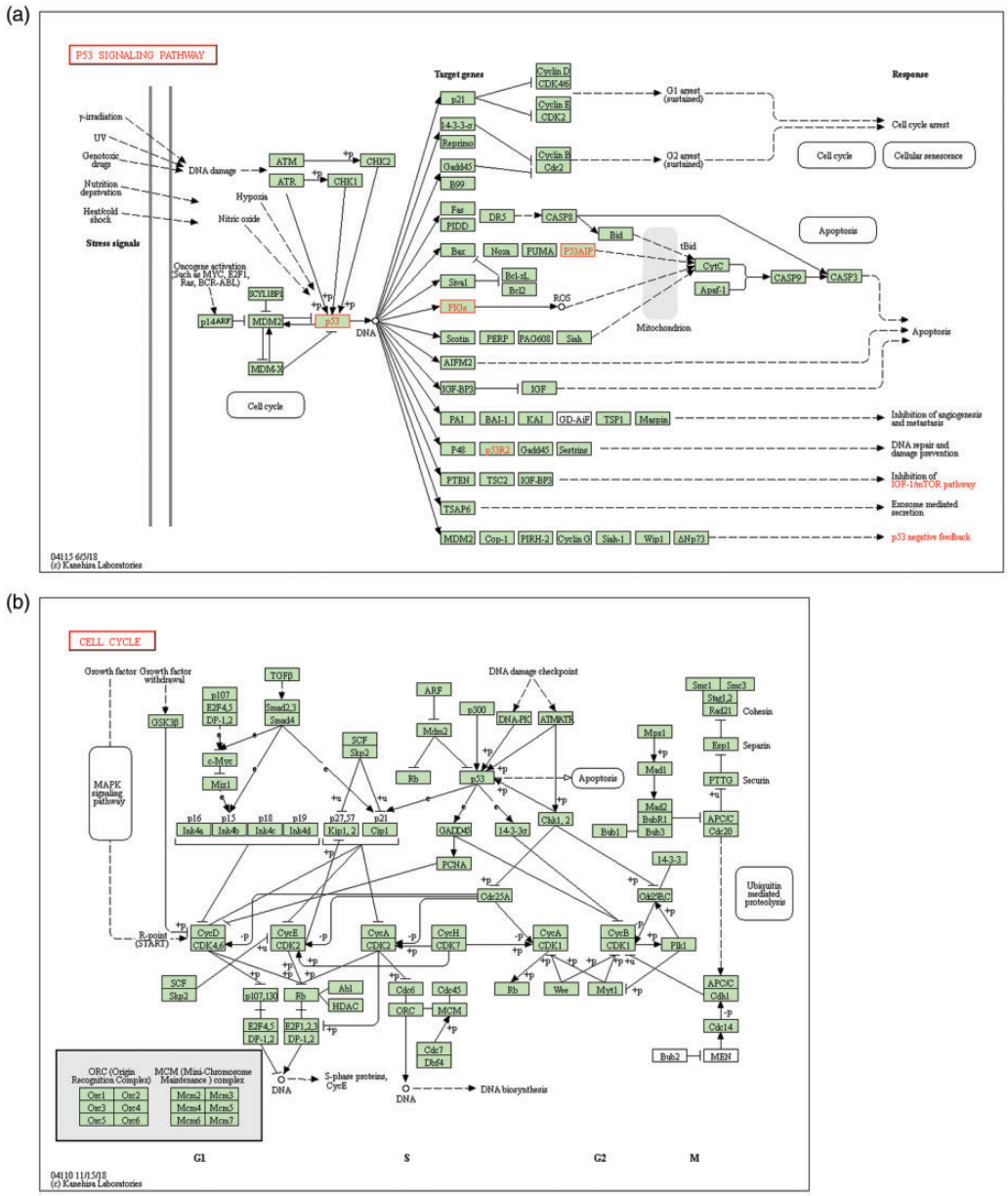

Figure 6. p53 signal pathway and cell cycle pathway regulated by *CDK1* and *CCNA2* in pancreatic adenocarcinoma (PAAD). p53 signal pathway (a) and cell cycle pathway (b) regulated by the *CDK1* and *CCNA2* gene cluster in PAAD.

CCNA2 is a member of the highly conserved cyclin family, and has been found to be up-regulated in a variety of tumors, indicating potential roles in tumor progression and transformation.[23] High expression of *CCNA2* was associated with increased proliferation of hepatocellular and breast cancer cells, which might be

helpful in monitoring the efficacy of chemotherapy.[24,25] Bioinformatics analysis showed that over-expression of *CCNA2* was associated with the development of PDAC, which was consistent with our results. We demonstrated that over-expression of *CCNA2* was significantly associated with a poor prognosis in patients with pancreatic cancer. Based on the above findings, we concluded that the *CDK1* and *CCNA2* gene cluster may provide a novel combined prognostic biomarker for PAAD.

This study had several limitations. First, the data used in this study were provided by various public databases, and were not generated by us. Second, the results were web-based and were not verified by biological experiments. Further mechanistic studies based on our findings are therefore needed.

In conclusion, this study provides a comprehensive bioinformatics analysis of the roles of *CDK1* and *CCNA2* in the progression and development of PAAD. The results suggest that the *CDK1* and *CCNA2* gene cluster may provide potential therapeutic targets for PAAD, and as well as biomarkers for identifying high-risk populations among pancreatic cancer patients. These results might also contribute to further studies of the therapeutic potential of various cell cycle inhibitors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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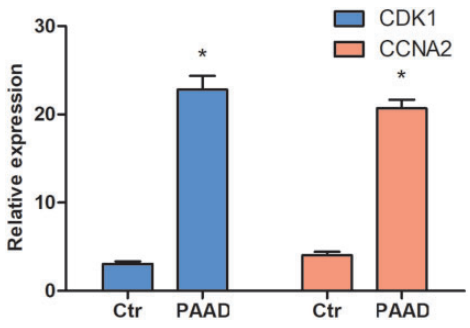
Appendix 1.

CDK1 and *CCNA2* mRNA expression levels in 10 pairs of pancreatic adenocarcinoma and adjacent non-tumor tissues.



*$P < 0.05$. Ctr, control; PAAD, pancreatic adenocarcinoma.