Comprehensive pan-cancer analysis reveals CDC6 as a potential immunomodulatory agent and promising therapeutic target in pancreatic cancer

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Background: CDC6 is critical in DNA replication initiation, but its expression patterns and clinical implications in cancer are underexplored. This study uses multi-omics data from The Cancer Genome Atlas (TCGA) to comprehensively analyze CDC6 across various cancers, aiming to evaluate its potential as a prognostic biomarker and explore its role in immunotherapy.

Methods: By leveraging multi-omics data from TCGA, we conducted a comprehensive analysis of CDC6 expression across a variety of cancer types. Least absolute shrinkage and selection operator (LASSO) regression was employed to assess the association of CDC6 with key molecules implicated in pancreatic cancer.

Results: CDC6 expression was found to be significantly upregulated across a broad spectrum of cancers. High levels of CDC6 expression were associated with poor prognosis in several cancer types. Notable associations were observed between CDC6 expression and tumor mutational burden (TMB), microsatellite instability (MSI), as well as immune cell infiltration. Co-expression analysis revealed significant associations between CDC6 and prevalent immune checkpoint genes. A risk model incorporating CDC6-related genes, including CCNA1, CCNA2, CCND1, CCND2, CDC25B, CDC6, and CDK2, was developed for pancreatic cancer.

Conclusions: CDC6 emerges as a promising prognostic biomarker and a potential target for immunotherapy across various cancers, including pancreatic cancer. It appears to modulate immune responses across cancer types, highlighting its regulatory role. Further exploration into the biological functions and clinical implications of CDC6 is warranted.

Keywords: CDC6; immune infiltration; pancreatic cancer; pan-cancer analysis; therapeutic target

Introduction

The rising incidence of cancer worldwide exerts substantial pressures on healthcare systems and economic stability (1-3). While diverse treatment modalities, including surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy, have achieved clinical successes, the prognosis and survival rates for cancer patients are often compromised by challenges such as drug resistance and adverse side effects (4-7). Consequently, the identification of early prognostic markers and reliable therapeutic targets is essential for enhancing cancer patient outcomes. Pan-cancer research plays a pivotal role in facilitating the application of diagnostic and therapeutic strategies across a broad spectrum of cancers by identifying molecular commonalities (8,9). Therefore, it is vital to undertake a detailed examination of the regulatory roles and molecular mechanisms of CDC6 within a pan-cancer context to unveil innovative strategies for clinical cancer therapy.

The regulation of cell cycle proteins in healthy cells is meticulously orchestrated through cell cycle-specific transcription and protein degradation mechanisms (10). However, tumor cells frequently exhibit dysregulation of these processes, leading to cell cycle abnormalities characterized by uncontrolled cell proliferation, which is a key driver of cancer development (11). Prior studies have established connections between genes involved in cell cycle

regulation and cancer initiation (12,13). CDC6, belonging to the AAA+ ATPase family, exhibits elevated expression in a variety of cancers, including lung, hepatocellular carcinoma, ovarian, glioma, and pancreatic cancers (14-19). Located on chromosome 17q21.3, CDC6 is instrumental in initiating DNA replication during the G1 and S phases of the eukaryotic cell cycle. It is involved in the assembly of the pre-replication complex at DNA replication origins during the early G1 phase, playing a critical role in synchronizing cell cycle progression with DNA replication (20-22).

Despite the growing body of literature highlighting CDC6's critical role in cancer progression, comprehensive pan-cancer analyses of CDC6 are scarce. In this study, we conducted an exhaustive analysis of CDC6 across various databases, including The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx), Gene Expression Profiling Interactive Analysis (GEPIA), Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), and Tumor Immune Estimation Resource (TIMER). Our investigations focused on gene expression, prognostic significance, correlations with immune infiltration, tumor mutational burden (TMB), and microsatellites. Furthermore, we explored the predictive value of CDC6-associated molecules in pancreatic cancer and established a novel seven-gene risk model for pancreatic cancer through Least absolute shrinkage and selection operator (LASSO) regression analysis. This study aims to provide valuable insights into the role of CDC6 in cancer development. We present this article in accordance with the TRIPOD reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-505/rc).

Methods

Data acquisition and processing

TCGA database harbors information from over 20,000 samples spanning 33 diverse cancer types. This rich dataset encompasses a wide array of molecular data, including transcriptomics (mRNA, lncRNA, miRNA), genomics [single-nucleotide variant (SNV), copy number variant (CNV)], epigenomics (DNA methylation), proteomics, and detailed clinical information. The TCGA database is renowned for its superior data quality, comprehensive omics coverage, extensive sample collection, and thorough clinical data. In our study, we utilized transcriptomic data and clinical information derived from the TCGA database (https://portal.gdc.cancer.gov/) for an analysis

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Highlight box

Key findings
• CDC6 has been identified as a significant prognostic biomarker and potential immunotherapeutic target across various cancers, including pancreatic cancer.

What is known and what is new?
• CDC6 is a cell cycle regulation protein known for its role in cell division.
• This study found significant upregulation of CDC6 across various cancer types and its association with crucial immunotherapeutic biomarkers such as tumor mutational burden (TMB), microsatellite instability (MSI), and immune cell infiltration. A new risk model based on CDC6 for pancreatic cancer has also been developed.

What is the implication, and what should change now?
• The findings reinforce CDC6's potential as a therapeutic target in cancer treatment, highlighting the need for further research into its biological functions and clinical significance across different cancers. This could guide future therapeutic strategies, especially in enhancing the effectiveness of immunotherapy.

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encompassing 33 cancer types. However, during our analysis, we encountered a notable challenge regarding the availability of transcriptome sequencing data for specific cancer types within the TCGA database. It was observed that transcriptome sequencing data for normal tissues were lacking for numerous cancer types, potentially compromising the precision of our analytical results. To mitigate this limitation, we explored additional resources and discovered the GTEx database (https://gtexportal.org/home/), which provides sequencing data from a vast array of normal samples across various tissues (23,24). Our aim was to enhance the reliability of our findings by integrating data from the GTEx database with that of the TCGA database, thereby compensating for the deficit of normal tissue sequencing data within the TCGA database. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Expression analysis of CDC6 across pan-cancer contexts

To ensure uniformity in gene expression data across samples, we initially transformed fragments per kilobase of transcript per million mapped reads (FPKM) values into transcripts per million (TPM) values, followed by normalization through Log2 conversion. Subsequently, we conducted a comprehensive analysis and portrayal of CDC6 expression variations across 33 distinct cancer types in comparison to their respective normal tissues. This approach allowed for a detailed examination of the differential expression patterns of CDC6, providing insights into its potential role and significance in a broad spectrum of cancers.

Survival analysis of CDC6 across pan-cancer contexts

The GEPIA platform, developed by researchers at Peking University, integrates data from public repositories, notably TCGA and the GTEx projects (http://gepia2.cancer-pku.cn/#index) (25,26). The platform utilizes a uniform pipeline and standardized processing workflows for the analysis of RNA-Seq expression data. The datasets available through GEPIA comprise 9,736 tumor samples and 8,587 normal samples from the TCGA and GTEx projects, ensuring cross-study compatibility. In our study, we utilized the GEPIA platform to investigate the associations between CDC6 expression and patient outcomes, specifically focusing on overall survival (OS) and disease-free survival (DFS) across various cancer types. This analysis aims to elucidate the prognostic value of CDC6 expression in a comprehensive range of cancers.

Correlation analysis of CDC6 with TMB and microsatellite instability (MSI) across pan-cancer contexts

TMB quantifies the total number of genetic mutations per megabase of the genome examined within a tumor, serving as a measure of the mutational landscape. MSI, on the other hand, refers to the phenotypic consequence of errors in DNA replication, specifically insertions or deletions, leading to variations in the length of microsatellite sequences. Both TMB and MSI have emerged as pivotal biomarkers in the realm of cancer immunotherapy, drawing significant scholarly interest due to their implications for patient response to treatment. The concept of TMB was notably highlighted in the seminal 2018 study, "The Immune Landscape of Cancer", led by Vesteinn Thorsson and colleagues (27). Concurrently, MSI was extensively characterized in the 2017 study, "Landscape of Microsatellite Instability Across 39 Cancer Types", conducted by Russell Bonneville and his team (28). In our study, we aimed to delineate the relationships between CDC6 expression and these two biomarkers (TMB and MSI) across a diverse array of cancer types, thereby contributing to the understanding of CDC6's potential role in cancer biology and its implications for immunotherapy.

Correlation analysis between CDC6 and immune response across pan-cancer contexts

The TIMER 2.0 database emerged from a collaborative initiative spearheaded by the West China Stomatological Hospital of Sichuan University, Harvard University, Tongji University, among other leading academic institutions. This endeavor culminated in a publication in Nucleic Acids Research in July 2020 (29). TIMER 2.0 integrates multiple algorithms to furnish a robust assessment of immune infiltration levels utilizing TCGA or user-uploaded datasets. The platform encompasses three primary modules: Immune Association, Cancer Exploration, and Immune Estimation (30,31). For our analysis, we employed three advanced algorithms, EPIC, TIMER, and xCell, from the "immunedeconv" R package, to conduct an extensive evaluation of the immune correlations. Additionally, we extracted expression data for eight pivotal immune checkpoint genes: SIGLEC15, IDO1, CD274 (PD-L1), HAVCR2 (TIM-3), PDCD1 (PD-1), CTLA4, LAG3, and PDCD1LG2 (PD-L2). Our

investigation delved into the association between CDC6 expression and these immune checkpoint genes across a spectrum of cancers. Furthermore, recognizing the crucial role of cancer-associated fibroblasts (CAFs) within the tumor microenvironment, we meticulously analyzed the correlation between CDC6 expression and the presence of CAFs across various cancer types, aiming to uncover insights into the interplay between CDC6 and the immune landscape in the context of cancer.

Identification of CDC6-related molecules and development of an innovative risk model for pancreatic cancer

The STRING database (https://string-db.org/) serves as a prolific repository for investigating interactions between known and predicted proteins, covering over 5,000 species and cataloging information on more than 24 million proteins alongside upwards of 20 million protein-protein interaction links (32,33). In our study, we harnessed the STRING database to identify the top 20 molecules related to CDC6. Subsequently, we retrieved STAR-counts data and clinical information pertaining to pancreatic cancer from TCGA database (https://portal.gdc.cancer.gov/). Only samples possessing both RNAseq data and clinical information were selected. The data, converted into TPM format, underwent normalization using log2(TPM+1) and were filtered accordingly. This process yielded a dataset comprising 179 pancreatic cancer samples, which formed the basis for further analysis.

For feature selection, the LASSO regression algorithm was utilized, incorporating 10-fold cross-validation executed via the glmnet package in R. Kaplan-Meier survival analysis, complemented by log-rank testing, facilitated the comparison of survival disparities among different groups. Additionally, the timeROC analysis was employed to ascertain the predictive accuracy of our model. Through this methodology, we devised a cutting-edge risk assessment model for pancreatic cancer, capitalizing on molecules intimately associated with CDC6, thereby paving the way for enhanced prognostic evaluation in this disease context.

Statistical analysis and visualization techniques

Statistical analyses within this study were executed utilizing R software version 4.0.3. Additionally, the integrated statistical analysis tools provided by the online platform were utilized to assess the data obtained from the respective database. The relationship between two variables was determined through Spearman's correlation test, while the rank sum test was applied to identify significant differences between groups. A P value of less than 0.05 was established as the threshold for determining statistical significance, ensuring rigor in the analysis. This methodological approach facilitated the robust examination and interpretation of our findings, contributing to the academic rigor of our research.

Results

### Expression of CDC6 mRNA across a spectrum of cancers and corresponding normal tissues

To investigate the expression of CDC6 across various cancer types, we commenced by analyzing its levels in both cancerous and non-cancerous tissues using gene expression data from the TCGA database. Violin plots were constructed to succinctly visualize these comparisons. Notably, cancer tissues from BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, PAAD, PRAD, READ, SARC, STAD, THCA, and UCEC exhibited significantly elevated CDC6 expression relative to their normal tissue counterparts (*Figure 1A-1D*). In light of the limited availability of normal tissue data within the TCGA database, we incorporated supplementary data from the GTEx database, enriching our comparative analysis. This integration revealed a pronounced increase in CDC6 expression in tumor tissues from ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, OV, PAAD, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, UCEC, and UCS when juxtaposed against normal tissues (*Figure 1E-1H*). In conclusion, our findings underscore a significant upregulation of CDC6 across a diverse array of cancers, suggesting its role as a potential oncogene in various malignancies.

Expression of CDC6 mRNA across diverse pathological stages of cancer

The stage of cancer is a critical determinant of prognosis for patients, indicative of the disease's progression (34). In our investigation, we analyzed the expression levels of the CDC6 gene across various cancer stages in a selection of cancer types. Our results revealed significant differences in CDC6 expression among the different stages of cancer in ACC, BRCA, KICH, KIRC, KIRP, LIHC, LUAD, and UCS (*Figure 2A-2H*). These findings indicate a

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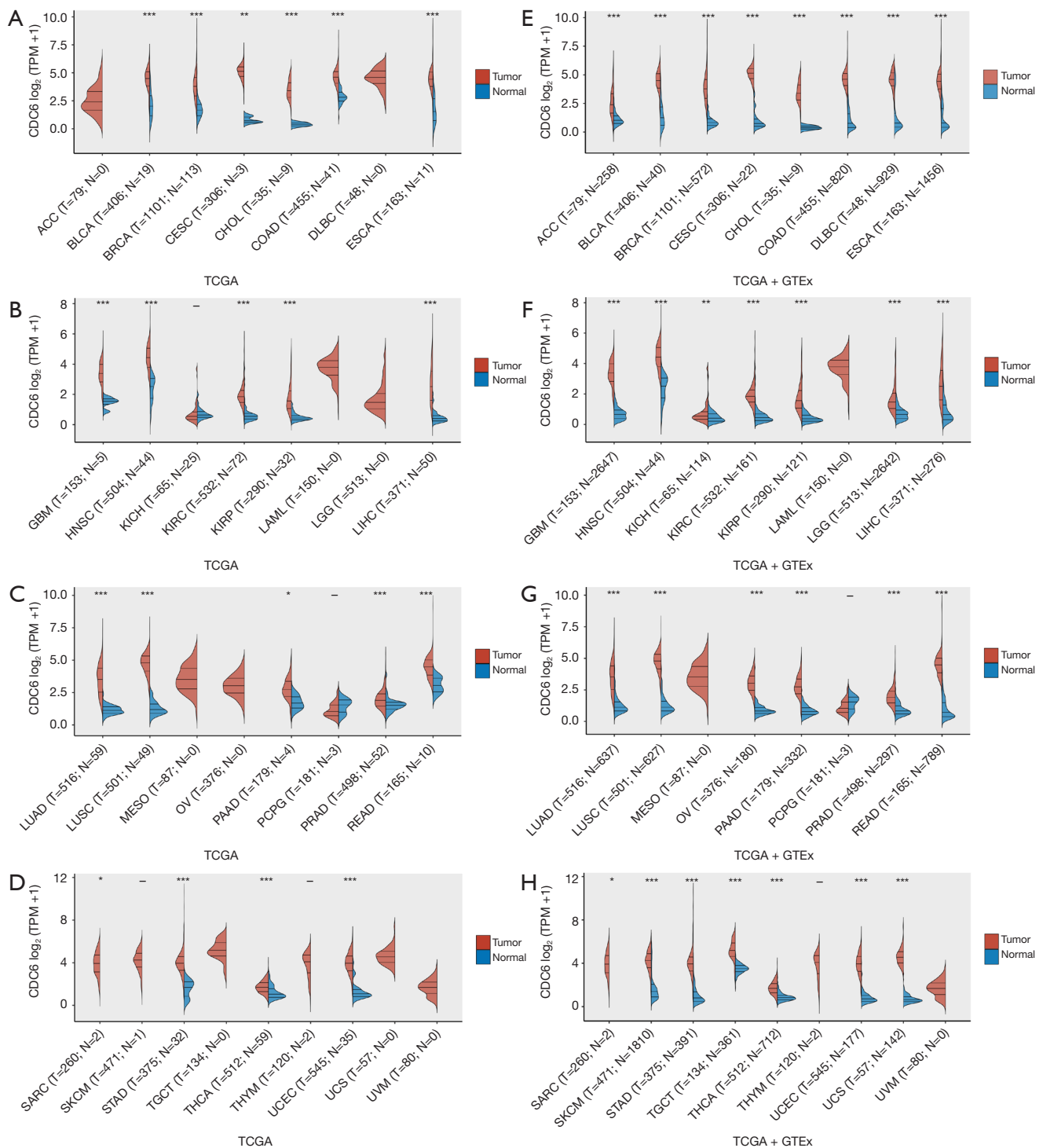
Figure 1 The expression of CDC6 mRNA was analyzed in pan-cancer pathological tissues and normal tissues as follows: (A-D) with the help of the TCGA database, 33 types of cancer tissues and normal tissues were examined for CDC6 expression, and the results are presented in violin plots; (E-H) the expression data of CDC6 in cancer tissues and normal tissues, obtained from the TCGA and GTEx databases, was visualized using the R language. Tumor tissues are represented in red, while normal tissues are represented in blue. Indicated statistical significance is by asterisks (*, P<0.05; **, P<0.01; ***, P<0.001; "-" indicates no statistical difference). TPM, transcripts per million; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression.
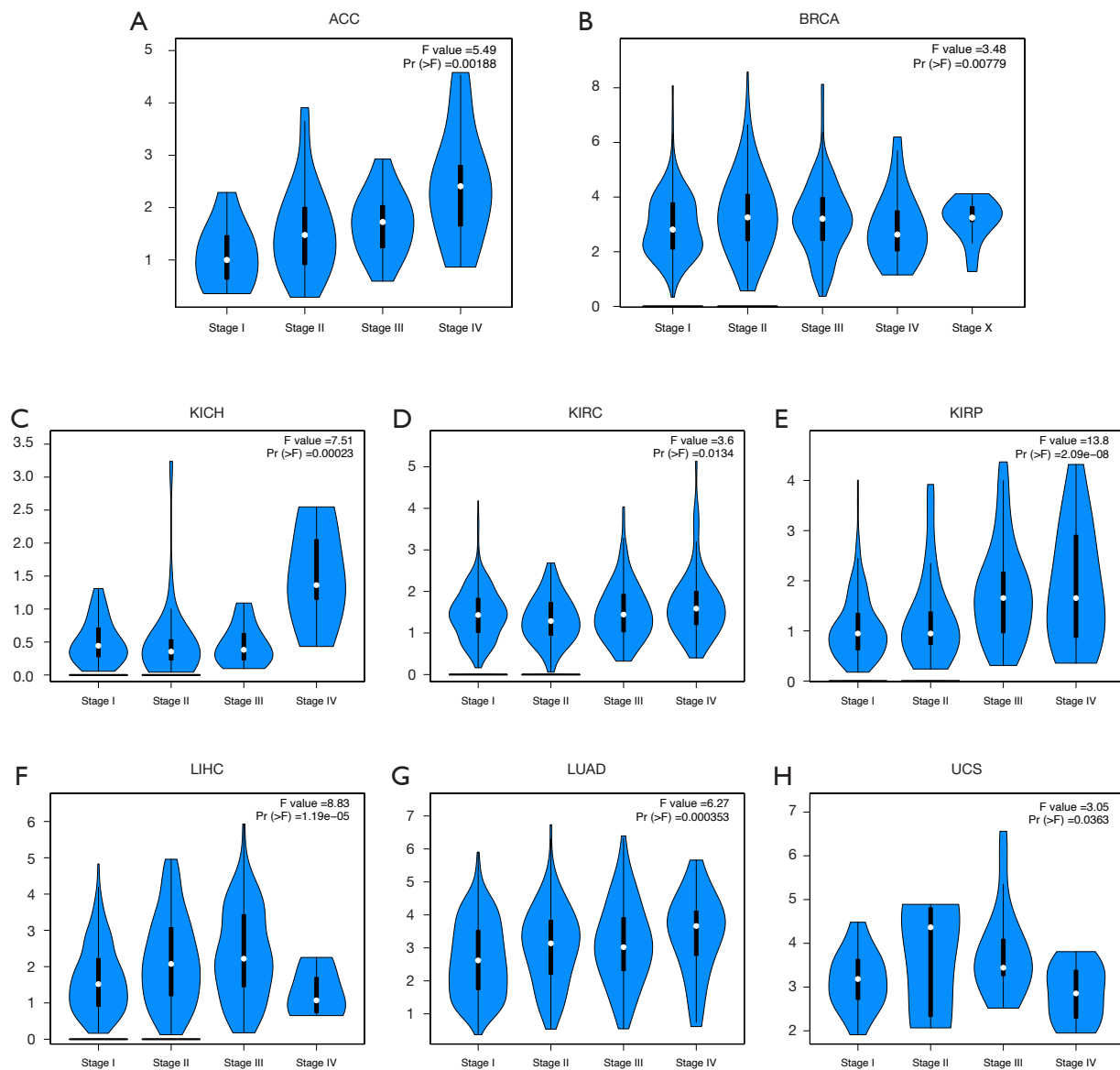
Figure 2 The mRNA expression of CDC6 was analyzed in different pathological stages of various cancer types, including: (A) ACC, (B) BRCA, (C) KICH, (D) KIRC, (E) KIRP, (F) LIHC, (G) LUAD, and (H) UCS. In the cancer staging system, "X" is commonly used as a suffix to indicate that the situation cannot be assessed.

potential association between CDC6 expression and the progression of malignancy, suggesting its relevance in the pathophysiological development of cancer.

OS implications of CDC6 expression across multiple cancer types

OS, the period from randomization to death from any cause, stands as the definitive measure of clinical efficacy for anticancer therapies in randomized controlled trials. Its reliance solely on survival events makes it the unequivocal standard for assessing anticancer drug performance in clinical research (35). To explore the relationship between CDC6 gene expression and OS across various cancers, we utilized RNA sequencing and corresponding clinical data from the TCGA database (*Figure 3*). Univariate Cox regression analysis was performed, with findings illustrated through forest plots created with the "forestplot" package

in R (*Figure 3A*). To corroborate our findings, additional analyses using the GEPIA database assessed CDC6's impact on OS in different cancer types (*Figure 3B*). This investigation identified a significant correlation between higher CDC6 expression and reduced OS in cancers such as ACC, KICH, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PRAD, SARC, and SKCM (*Figure 3C-3K,3M,3N*). Conversely, in READ and THYM cancers, elevated CDC6 expression was distinctly linked to poorer OS outcomes (illustrated in *Figure 3L,3O*), indicating its prognostic significance across a diverse array of malignancies.

DFS associated with CDC6 expression across multiple cancer types

DFS is defined as the time from randomization to the initial event of either disease recurrence or death from any cause. It primarily measures the recurrence of disease and is commonly utilized to evaluate the efficacy of adjuvant treatments post-surgery or radiation therapy. This study explored the DFS associated with the CDC6 gene across a spectrum of cancers. Employing a methodology analogous to our investigation of OS, we conducted univariate Cox regression analysis and visualized the results using forest plots generated via the "forestplot" package in R (*Figure 4A*). To substantiate our initial findings, we further analyzed DFS in relation to CDC6 expression using the GEPIA database across different cancer types (*Figure 4B*). Our analysis revealed a significant link between increased expression of the CDC6 gene and reduced DFS in patients with cancers such as ACC, KICH, KIRP, LGG, LIHC, MESO, PAAD, and THCA (*Figure 4C-4J*), indicating the prognostic value of CDC6 expression in predicting disease recurrence and patient survival following treatment.

Association between CDC6 expression, gene variation, and immune response across various cancers

TMB quantifies the number of gene mutations within a specific tumor tissue, defined as mutations per megabase of the coding sequence in the genome of tumor samples (36,37). MSI, on the other hand, refers to the alterations in the length of microsatellite sequences resulting from insertion or deletion mutations during DNA replication. MSI emerges due to the accumulation of replication errors in microsatellites when the DNA mismatch repair system (MMR) is deficient, often caused by pathogenic mutations in MMR genes (MLH1, MSH2, MSH6, PMS2, and EPCAM)

or by the hypermethylation of the MLH1 promoter region, which leads to MLH1 expression loss (38,39). TMB and MSI serve as pivotal biomarkers for predicting the response to cancer immunotherapy. Leveraging the TCGA database, we assessed the TMB and explored the relationship between CDC6 expression and TMB across 33 cancer types. Our findings revealed a significant positive correlation between CDC6 and TMB in 13 cancer types (ACC, LUAD, STAD, UCS, PAAD, LGG, KICH, PRAD, SARC, UCEC, BRCA, CHOL, and BLCA), and a negative correlation in THYM (*Figure 5A*). Subsequently, we analyzed the correlation between CDC6 expression and MSI. In cancers such as UCEC, UCS, CHOL, STAD, UV, and MESO, a positive correlation was observed with CDC6, whereas a negative correlation was noted in DLBC (*Figure 5B*).

Immune checkpoints, which are immunosuppressive molecules crucial for regulating immune responses and maintaining tissue integrity, play a significant role in immune tolerance and tumor formation processes. Notably, in cancers like THYM, TGCT, LUSC, LAML, and CESC, the majority of immune checkpoint genes were positively correlated with CDC6 expression, positioning them as potential targets for immune checkpoint inhibitors (*Figure 5C*). The complexity of the tumor microenvironment, particularly the presence of tumor-infiltrating immune cells, has garnered considerable attention in recent studies. We delved into the relationship between CDC6 expression and the infiltration of immune cells in tumors using three sophisticated algorithms (EPIC, TIMER, and xCell) (*Figure 5D-5F*). These insights enhance our understanding of the tumor microenvironment and are crucial for future investigations into tumor immunotherapy.

Association between CDC6 expression and CAF infiltration across cancers

CAFs are dynamic, plastic, and robust cells that are integral to both primary and metastatic tumor environments (40). Engaging in multifaceted interactions within the tumor microenvironment, they significantly contribute to cancer progression. Beyond their role in synthesizing extracellular matrix components that constitute the tumor stroma, CAFs undergo epigenetic alterations, which result in the release of substances, exosomes, and metabolites impacting tumor angiogenesis, immune responses, and metabolism. Given their critical involvement in cancer progression, CAFs represent a compelling therapeutic target (41-43). In our study, we explored the relationship between CDC6
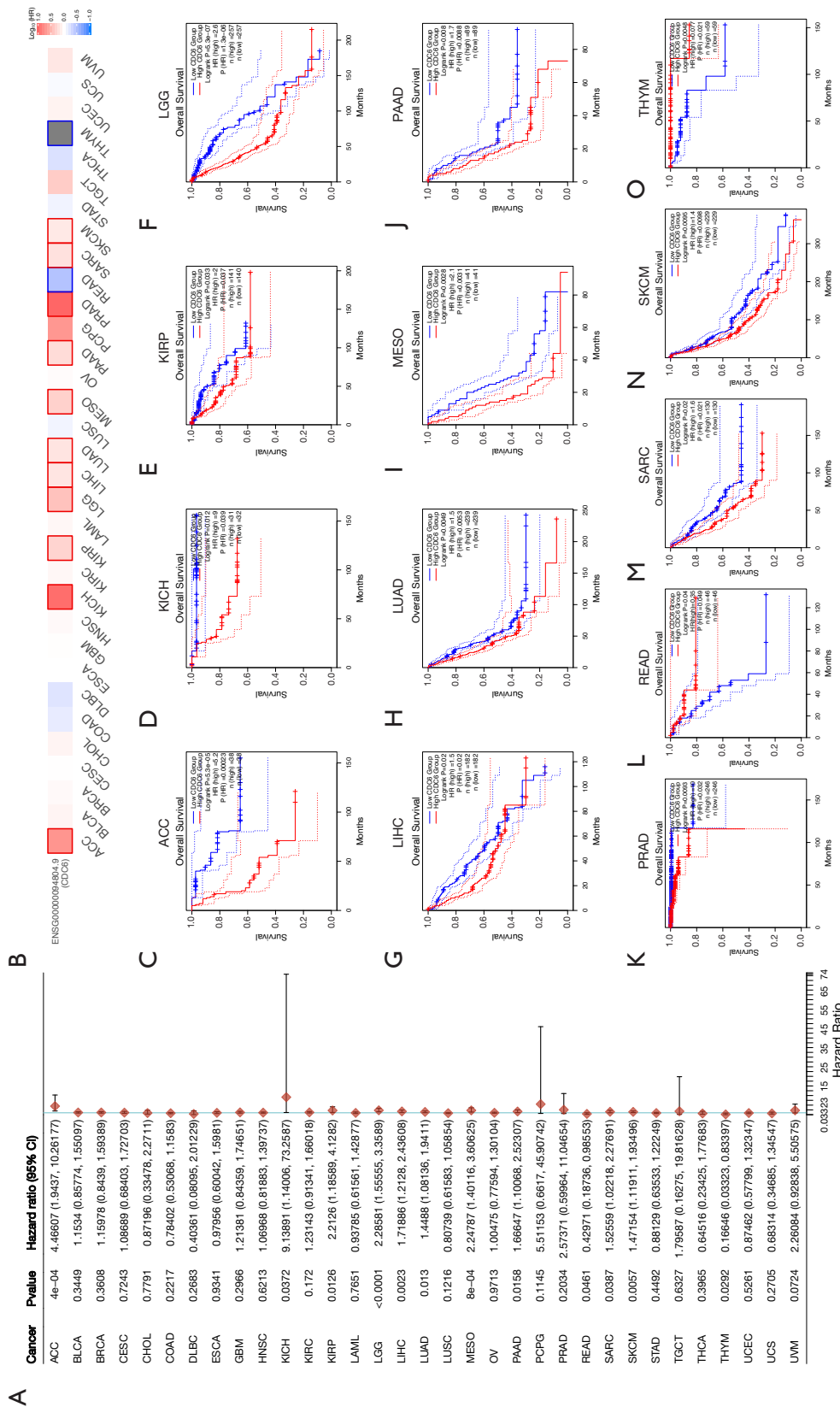
Figure 3 The overall survival analysis of CDC6 in pan-cancer was performed as follows: (A) CDC6 gene expression and OS of patients in 33 cancer types were combined in forest plots using univariate Cox analysis; (B) the OS analysis results of CDC6 in 33 cancer types were presented in a heatmap based on the GEPIA database; (C–O) Kaplan-Meier curves were used to illustrate the overall survival of CDC6 in specific cancer types, including ACC, KICH, KIRP, LGG, LIHC, LUAD, MESO, PAAD, PRAD, READ, SARC, SKCM, and THYM. The low expression group of CDC6 was represented by blue, while the high expression group was represented by red. OS, overall survival; GEPIA, Gene Expression Profiling Interactive Analysis.
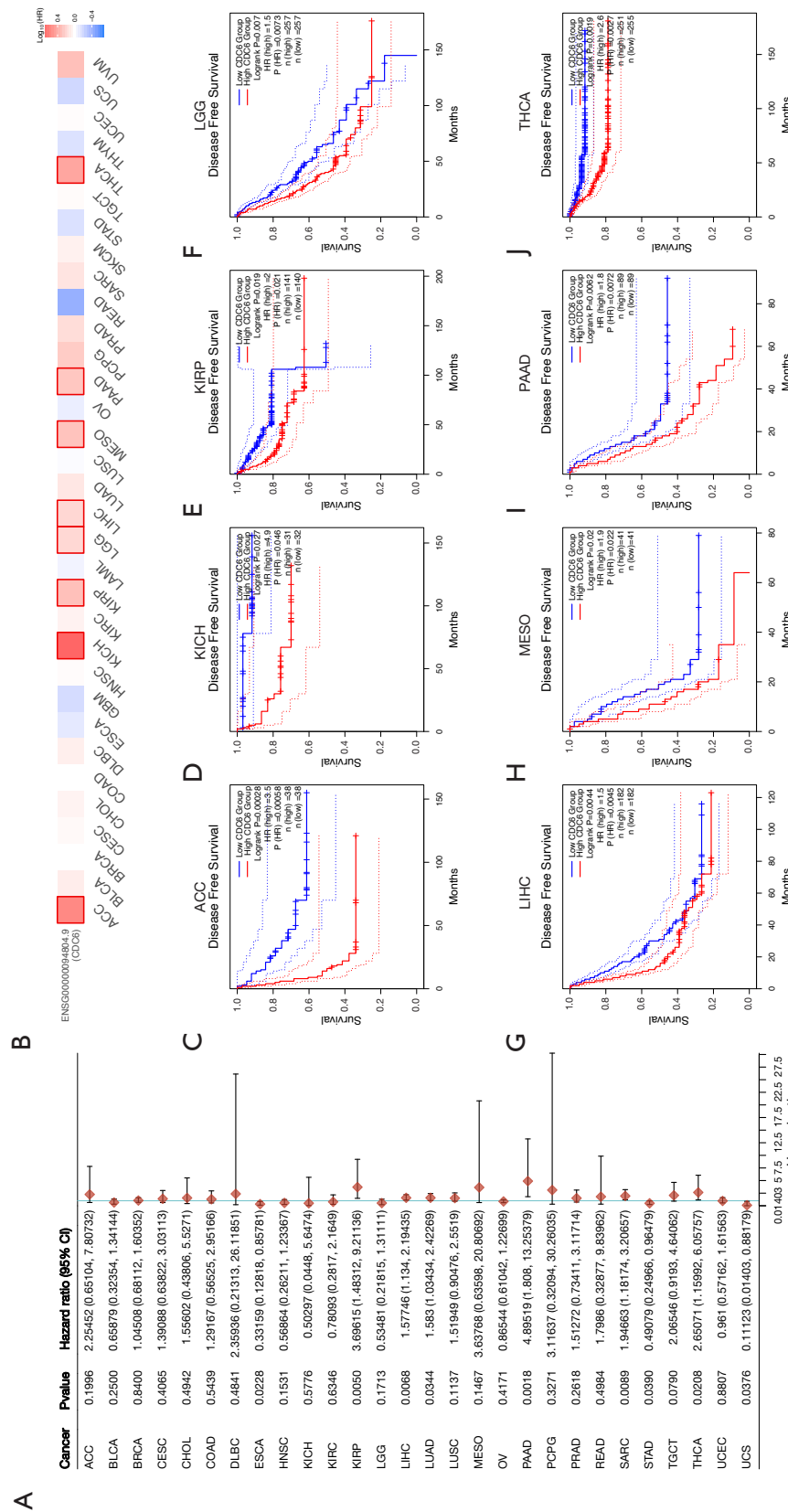
Figure 4 The DFS analysis of CDC6 in pan-cancer was conducted as follows: (A) univariate Cox analysis results of CDC6 in 33 cancer types were presented using forest plots, combining CDC6 gene expression and patients' DFS; (B) the results of the DFS analysis of CDC6 in 33 cancer types were displayed in a heatmap based on the GEPIA database; (C-J) Kaplan-Meier curves were utilized to illustrate the DFS of CDC6 in specific cancer types, including ACC, KICH, KIRP, LGG, LIHC, MESO, PAAD, and THCA. The low-expression group of CDC6 was represented by blue, while the high-expression group was represented by red. DFS, disease-free survival; GEPIA, Gene Expression Profiling Interactive Analysis.

expression and the presence of CAFs using the TIMER database (*Figure 6A*). Our findings revealed a pronounced positive correlation between CDC6 expression and CAF infiltration in a variety of cancers, including ACC, KICH, MESO, THYM, BRCA, DLBC, LUAD, and STAD (illustrated in *Figure 6B-6M*).

Development of a novel risk model for pancreatic cancer using LASSO regression analysis and CDC6-related molecules

In this analysis, we initially leveraged the STRING database to identify the top 20 molecules associated with CDC6, including CCNA1, CCNA2, CCNB1, CCND1, CCND2, CCNE1, CDC25B, CDK2, CDKN1B, GINS1, GINS2, MCM2, MCM4, MCM5, MCM6, ORC2, ORC3, ORC5, ORC6, and POLE2. Following this, we utilized LASSO regression analysis to formulate a 7-gene risk model for pancreatic cancer, incorporating CDC6-associated molecules: CCNA1, CCNA2, CCND1, CCND2, CDC25B, CDC6, and CDK2 (*Figure 7A,7B*).

The risk model is calculated as follows: Risk Score = $(-0.1277 \times \text{CCNA1}) + (0.2778 \times \text{CCNA2}) + (0.0048 \times \text{CCND1}) + (-0.1706 \times \text{CCND2}) + (-0.1138 \times \text{CDC25B}) + (0.1846 \times \text{CDC6}) + (0.0205 \times \text{CDK2})$.

Applying this risk assessment model, we stratified patients with pancreatic cancer into high-risk and low-risk groups. Survival analysis revealed a significantly worse prognosis for patients in the high-risk group compared to those in the low-risk group (P=0.00112) (*Figure 7C-7F*). Moreover, the area under the curve (AUC) values of the receiver operating characteristic (ROC) curve were 0.695, 0.747, and 0.797 for one-year, three-year, and five-year survival predictions, respectively (*Figure 7G*), indicating the model's robust predictive capacity. This model offers valuable insights into the prognostic landscape of pancreatic cancer, potentially guiding therapeutic decisions and improving patient outcomes.

Discussion

With its increasing incidence and mortality rates, cancer constitutes a formidable challenge to public health. Among the most widespread globally are breast, lung, pancreatic,
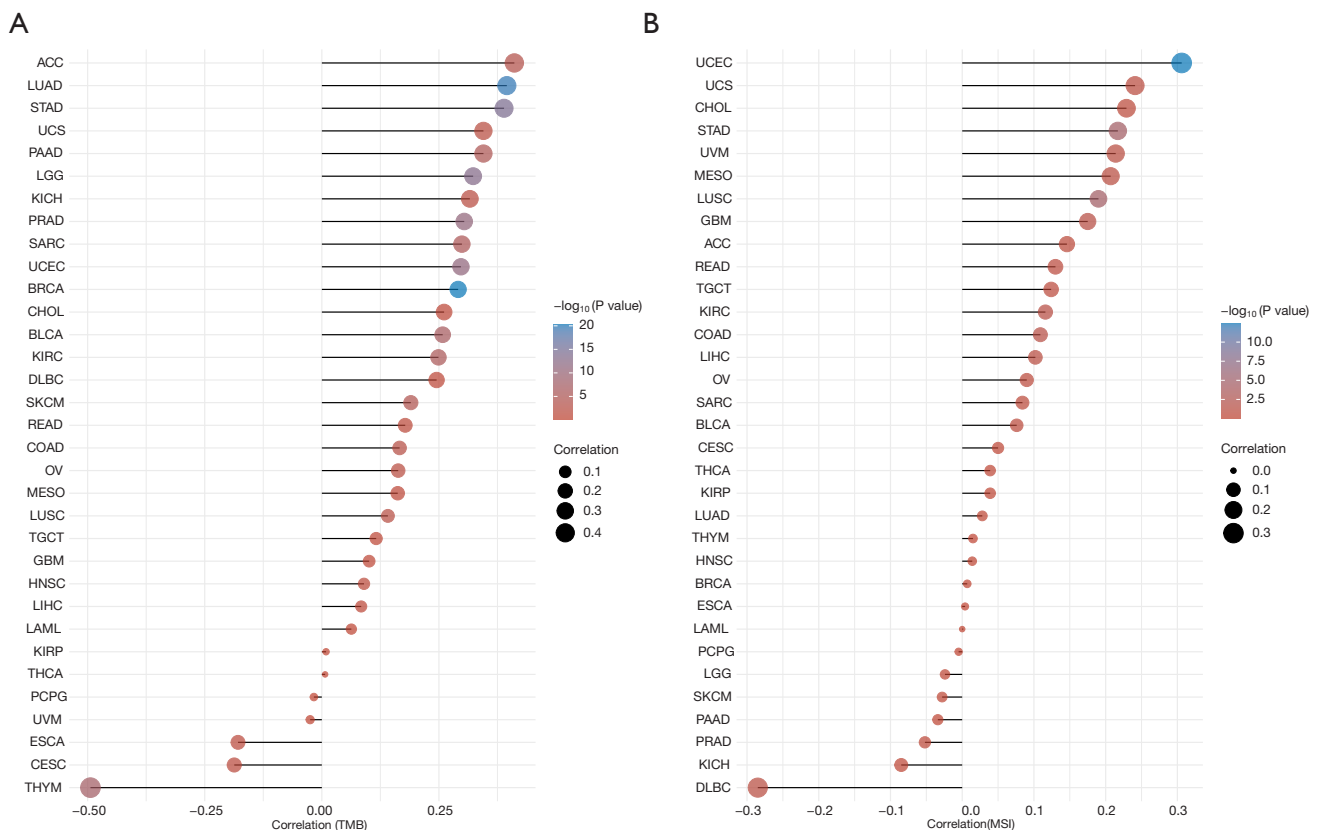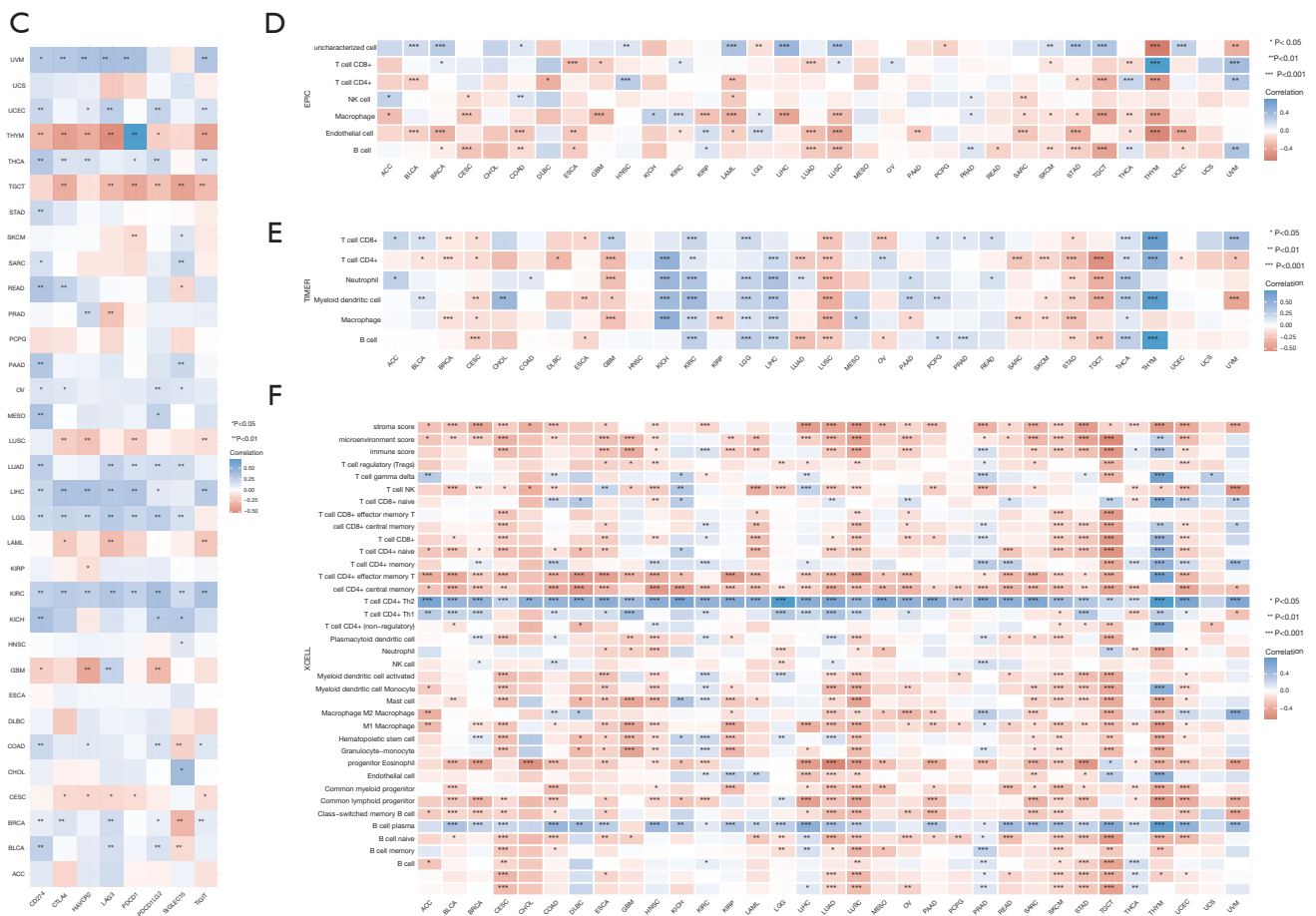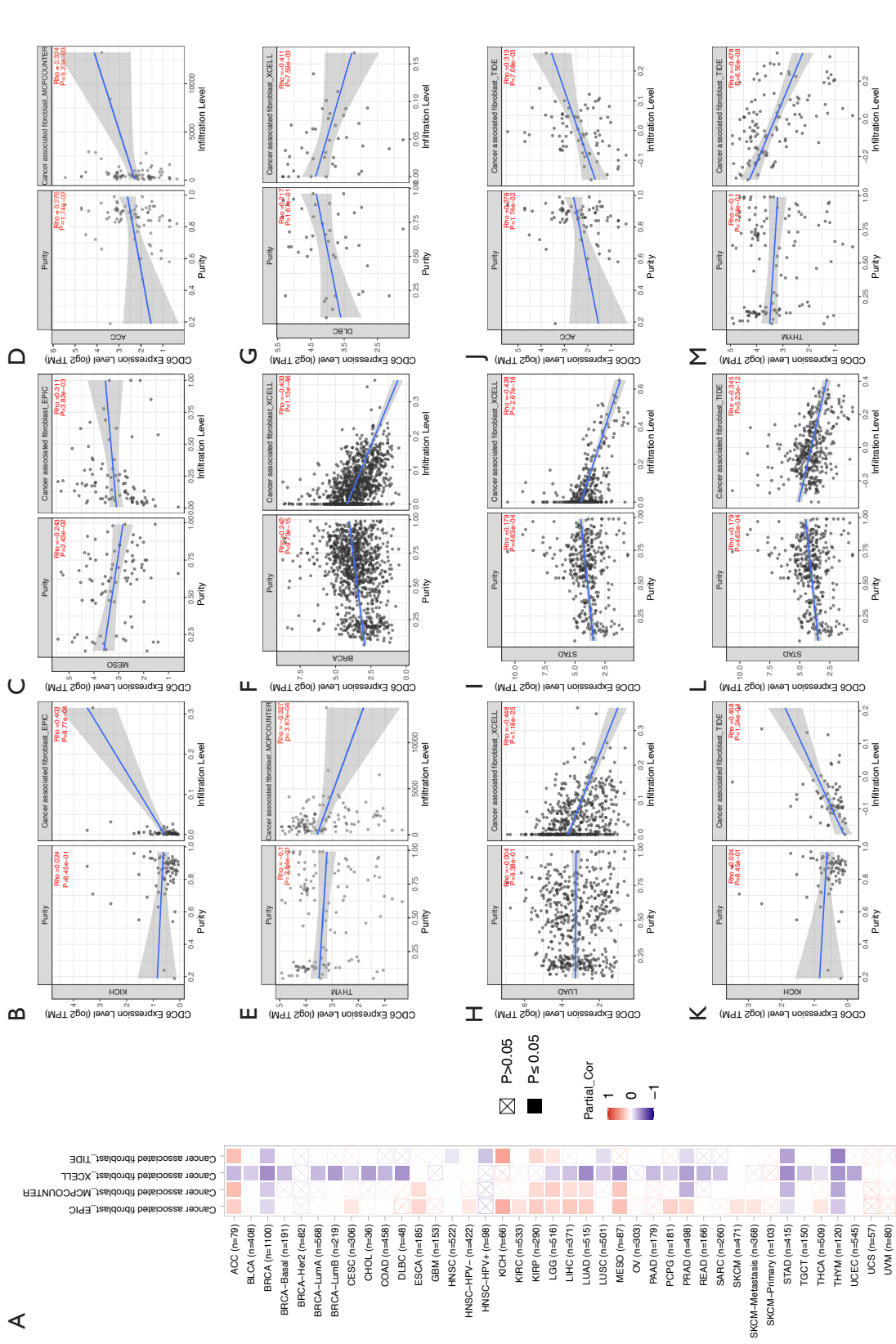
Figure 5 The correlation between CDC6 and gene variation and immune response in pan-cancer was analyzed as follows: (A) Spearman correlation analysis was conducted to examine the relationship between CDC6 gene expression and TMB; (B) Spearman correlation analysis was performed to assess the correlation between CDC6 gene expression and MSI. The size of the dots in the chart represents the correlation coefficient, while the color represents the significance of the P value, with bluer colors indicating smaller P values; (C) a heatmap shows the correlation between CDC6 expression in pan-cancer and immune checkpoints, such as SIGLEC15, IDO1, CD274, HAVCR2, PDCD1, CTLA4, LAG3, and PDCD1LG2; (D-F) heatmaps were generated to display the correlation between CDC6 expression and immune cell infiltration using three different algorithms: EPIC, TIMER, and xCell. Indicated statistical significance is by asterisks (*, P<0.05; **, P<0.01; ***, P<0.001). NK, natural killer; TMB, tumor mutational burden; MSI, microsatellite instability.

and colorectal cancers (CRCs) (44). Despite the prevalent adoption of surgical excision, radiation therapy, and adjunct chemotherapy, the effectiveness of these treatments remains constrained (4). Consequently, the early detection and intervention are imperative for improving patient outcomes in oncology. Pan-cancer analysis, through comprehensive evaluation across diverse cancer types, facilitates the identification of both shared and distinct molecular signatures, thereby offering improved avenues for cancer prevention and the development of individualized treatment protocols. In recent years, genome-wide pan-

cancer studies have drawn heightened attention, uncovering RNA variations and gene mutations integral to cancer's onset and progression (45). These insights are indispensable for the early diagnosis of cancer and the selection of appropriate therapeutic strategies. Therefore, it is critical to pursue further research to discover more effective cancer biomarkers. The development of cancer is intricately linked to the aberrant expression of proteins that regulate the cell cycle, a reflection of the rapid growth and division characteristic of cancer cells (46,47). Numerous studies have highlighted the pivotal role of CDC6 in cancer progression.

Figure 6 Correlation between CDC6 and cancer-associated fibroblast infiltration in pan-cancer was analyzed and presented as follows: (A) the heatmap illustrates the correlation between CDC6 expression and cancer-associated fibroblasts in 33 types of cancer. Positive correlation is represented by red, while negative correlation is represented by blue; (B,C) scatterplots display the correlation between KICH and MESO with Cancer associated fibroblast_EPIC; (D,E) scatterplots demonstrate the correlation between ACC and THYM with Cancer associated fibroblast_MCPCOUNTER; (F-I) scatterplots show the correlation between BRCA, DLBC, LUAD, and STAD with Cancer associated fibroblast_XCELL; (J-M) scatterplots exhibit the correlation between ACC, KICH, STAD, and THYM with cancer associated fibroblast_TIDE.

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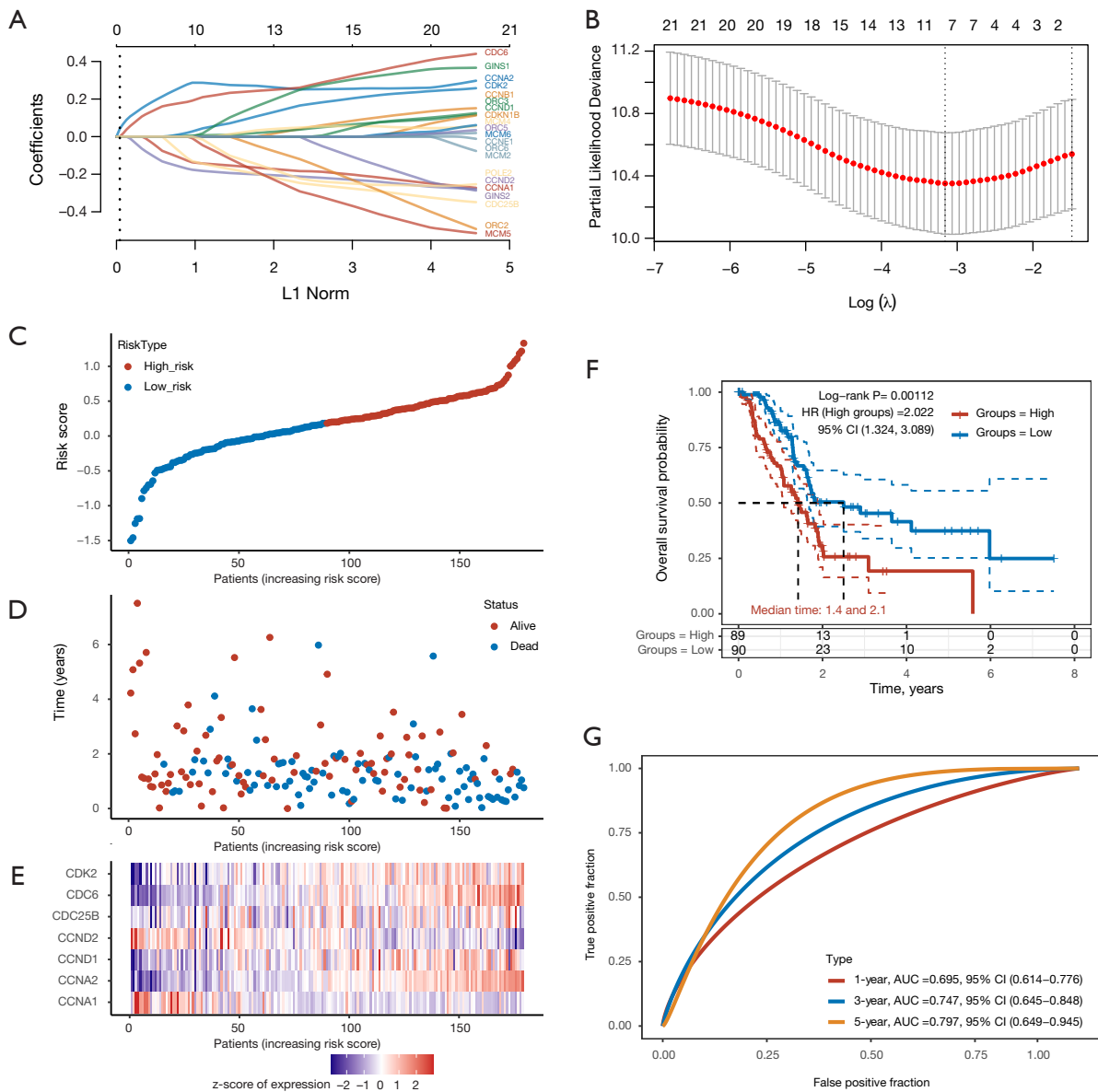
Figure 7 Based on LASSO regression analysis, a new risk model was established in pancreatic cancer using CDC6-related molecules. The process and results are presented as follows: (A,B) feature selection was performed using the LASSO regression algorithm with 10-fold cross-validation to identify relevant molecules; (C,D) two scatterplots display the ranking of pancreatic cancer patients based on the risk model, distinguishing between high and low-risk groups, and showing their corresponding survival outcomes; (E) a heatmap illustrates the expression levels of CDK2, CDC6, CDC25B, CCND2, CCND1, CCNA2, and CCNA1 in pancreatic cancer patients. The x-axis represents samples with increasing risk scores from left to right, and the risk scores are calculated based on the risk model from this study; (F) survival curves were plotted to depict the prognosis of pancreatic cancer patients based on the established risk model; (G) the ROC curve was utilized to assess the accuracy of the risk model in predicting patient outcomes. HR, hazard ratio; CI, confidence interval; AUC, area under the curve; LASSO, least absolute shrinkage and selection operator; ROC, receiver operating characteristic.

Its overexpression has been associated with adverse treatment outcomes, highlighting its potential as both a prognostic marker and a therapeutic target (16,48-50).

This study was designed to examine the variations in CDC6 expression across a range of cancer types. Initially, we evaluated the mRNA expression levels of CDC6 in cancerous and normal tissues using the TCGA database and observed heightened expression in more than ten cancer types. However, we encountered limitations within the TCGA database, notably the lack of sequencing data for normal or adjacent tissues in several cancers, such as ACC, DLBC, LAML, LGG, MESO, OV, TGCT, and UCS. To address this challenge, we utilized data from the GTEx database, which offers an expansive collection of normal tissue expression data. By integrating data from both TCGA and GTEx, we were able to attain a more comprehensive insight into the transcriptomic landscapes. Our analysis identified a significant increase in CDC6 mRNA expression across nearly all the cancer types examined. Furthermore, using the GEPIA database, we assessed the prognostic relevance of CDC6 in various cancers. Our OS analysis indicated that CDC6 overexpression might act as a predictive biomarker for several cancers, associated with a worse prognosis in patients with high levels of CDC6 expression.

Previous studies have underscored the pivotal involvement of CDC6 in cancer development and progression. Mahadevappa *et al.* investigated the role and physiological significance of CDC6 in breast cancer, demonstrating that breast cancer cell lines exhibited increased CDC6 expression relative to normal mammary epithelial cells, and high CDC6 expression was associated with worse clinical outcomes. Notably, estrogen receptor (ER)-negative breast cancers showed higher CDC6 expression than ER-positive cancers, suggesting a potential link to increased aggressiveness (48). The suppression of CDC6 expression disrupts DNA replication, leading to cell cycle arrest in the G1/S phase and inducing apoptosis (51-53). Furthermore, CDC6 serves as a critical regulatory target for the androgen receptor, influencing the G1-S phase transition in prostate cancer cell proliferation (54). Research by Kim *et al.* revealed that CDC6 mRNA expression was higher in prostate cancer tissues than in benign prostatic hyperplasia (BPH) tissues, correlating with higher Gleason scores, elevated PSA levels, and advanced disease (55).

Other investigations, such as those by Deng *et al.*, found elevated CDC6 protein levels in epithelial ovarian cancer (EOC) tissues compared to normal ovarian tissues,

with CDC6 expression associated with various clinical and pathological parameters (16). In CRC, tumor tissues displayed significantly higher CDC6 mRNA and protein levels than adjacent normal tissues, with high CDC6 expression correlating with advanced TNM stage and tumor metastasis (50). Zhang *et al.* reported that lower CDC6 expression was associated with improved OS in lung cancer patients (56). Similarly, research by Feng and colleagues found that CDC6 mRNA and protein expression was significantly elevated in precancerous lesions and oral squamous cell carcinoma (OSCC), linking higher CDC6 levels to OSCC progression and dissemination (57).

However, it is crucial to recognize certain constraints in this research. Initially, the comparatively limited sample sizes of rarer tumor types could potentially cause overall impacts or produce less precise outcomes. Furthermore, the present discoveries offer initial understanding into the correlation between CDC6 and cancer advancement in different types of tumors, necessitating additional experimental research to clarify the exact molecular role of CDC6 in the development of tumors.

Conclusions

In this study, we have generated comprehensive data underscoring the prognostic relevance and immunological significance of CDC6 across a wide spectrum of cancers. However, our research is subject to certain limitations. Primarily, the data analyzed were sourced exclusively from publicly available databases, necessitating further clinical data to robustly assess the reliability of the constructed risk model. Moreover, the pivotal gene CDC6 requires further validation through *in vivo* and *in vitro* experiments. In future investigations, we aim to explore in greater depth the biological function and underlying mechanisms of CDC6 in the context of pan-cancer, in order to enhance our understanding of its role in cancer progression and treatment outcomes. In conclusion, this investigation provides valuable insights and robust evidence that may inform future research endeavors.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-24-505/rc

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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