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Research article

Pan-cancer analysis of polo-like kinase family genes reveals polo-like kinase 1 as a novel oncogene in kidney renal papillary cell carcinoma

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ABSTRACT

Background: Polo-like kinases (PLKs) are a kinase class of serine/threonine with five members that play crucial roles in cell cycle regulation. However, their biological functions, regulation, and expression remain unclear. This study revealed the molecular properties, oncogenic role, and clinical significance of PLK genes in pan-cancers, particularly in kidney renal papillary cell carcinoma (KIRP).

Methods: We evaluated the mutation landscape, expression level, and prognostic values of PLK genes using bioinformatics analyses and explored the association between the expression level of PLK genes and tumor microenvironment (TME), immune subtype, cancer immunotherapy, tumor stemness, and drug sensitivity. Finally, we verified the prognostic value in patients with KIRP through univariate and multivariate analyses and nomogram construction.

Results: PLK genes are extensively altered in pan-cancer, which may contribute to tumorigenesis. These genes are aberrantly expressed in some types of cancer, with PLK1 being overexpressed in 31 cancers. PLK expression is closely associated with the prognosis of various cancers. The expression level of PLK genes is related with sensitivity to diverse drugs and cancer immunity as well as cancer immunotherapy. Importantly, we verified that PLK1 was overexpressed in KIRP tissues and could be an unfavorable prognostic biomarker in patients with KIRP. Hence, PLK1 may serve as an oncogenic gene in KIRP and should be explored in future studies.

Conclusions: Our study comprehensively reports the molecular characteristics and biological functions of PLK family gens across human cancers and recommends further investigation of these genes as potential biomarkers and therapeutic targets, especially in KIRP.

1. Introduction

Cancers are the second leading cause of human deaths, posing a great challenge to global human health [1]. According to global

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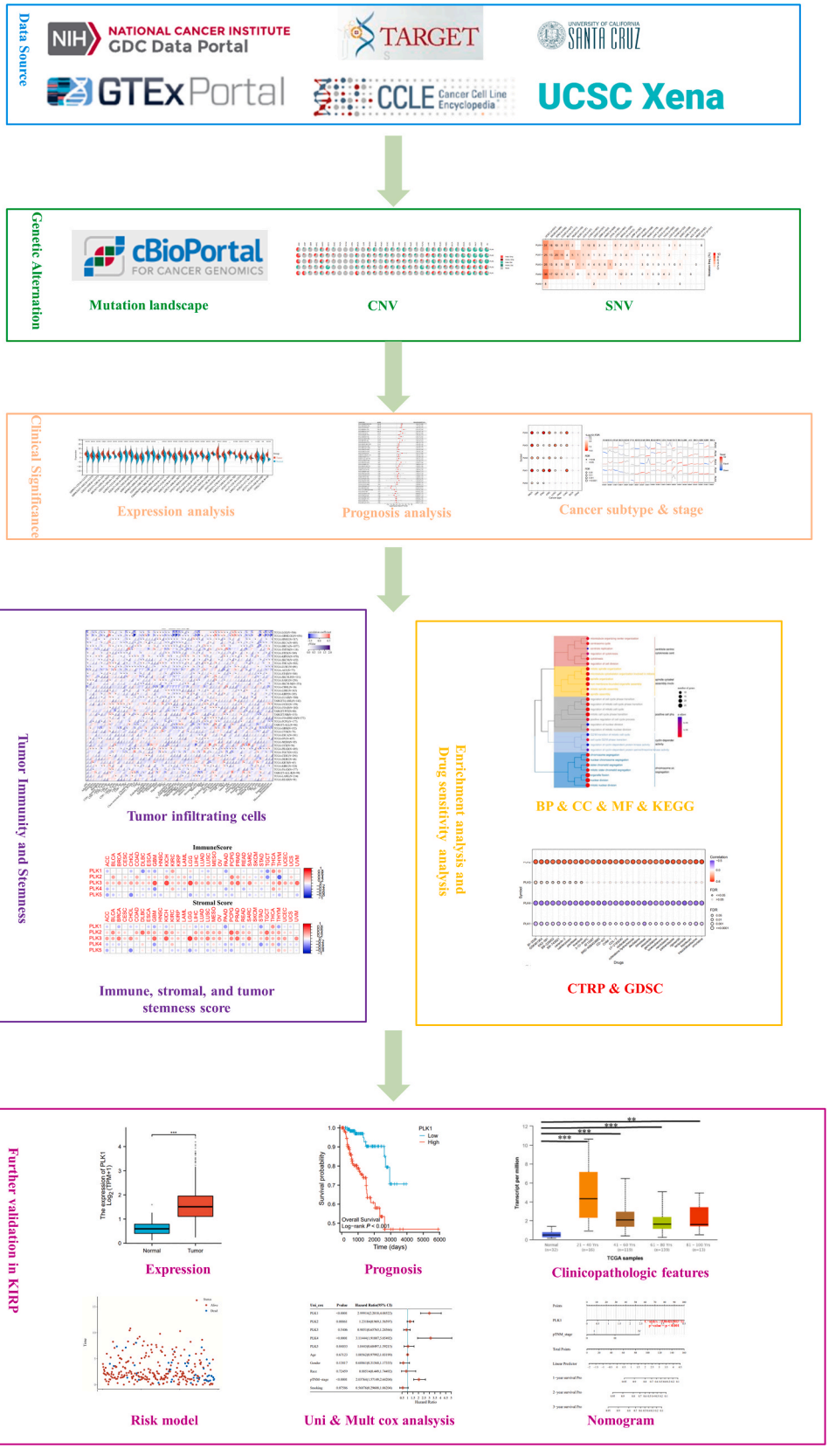

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Fig. 1. Flow chart of this study.

cancer report, there are nearly 19.3 million new cancer cases and 10 million cancer-related deaths worldwide in 2020 [2]. Cell proliferation is a complex and dynamic process involving changes in multiple cell cycle-dependent kinases and phosphatases [3]. Previous studies have revealed the molecular mechanisms regulating the cell cycle in normal and cancerous cells [4].

The process of cell cycle is controlled by many protein kinases, like cyclin-dependent kinases (CDKs), which are appealing therapeutic targets for cancers [5]. Other targets involved in mitosis, such as kinases, multi-protein complexes, and motor proteins, have also garnered attention as potential targets for cancer treatment [6]. Among these potential candidates, PLKs have emerged as promising targets because they are vital in regulating the cell cycle. PLKs are composed of five members in mammals: PLK1-5 [7]. PLK family members have a highly conserved N-terminus catalytic ATP-binding domain (except for PLK5) with two conserved non-catalytic regulatory domains at the C-terminus, termed polo-box domains (PBDs), except PLK4 which only has one PBD domain [8]. The PBD controls substrate recognition, binding, PLK phosphorylation, and subcellular localization [9], which is a different characteristics of PLKs compared to other protein kinases [10]. PLKs have multidimensional functions in mitosis, particularly ensuring the fidelity of checkpoint controls [10]. Among all PLK family member, PLK1 is the most extensively studied. PLK1 plays crucial roles in controlling cell cycle, including entry into mitosis, spindle formation, and cytokinesis, and inhibition of PLK1 can induce G2/M-phase arrest and apoptosis [11]. PLK1 is often upregulated in cancer cells, leading to mitotic dysregulation and enhanced chromosomal instability [12]. Recently, PLK1 inhibition was found recently to regulate the expression of programmed death ligand 1 (PD-L1) via activating the nuclear factor-kappa B (NF-κB) signaling. PLK1 inhibition sensitizes pancreatic ductal adenocarcinoma to immune checkpoint blockade (ICB) therapy, indicating the promising application of PLK1 inhibition in cancer immunotherapy [13]. And PLK1 could involve in the phosphorylation of Polteta and repair double-strand breaks in mitosis [14]. Additionally, PLK2, PLK3, PLK4, and PLK5 play important roles in carcinogenesis and can serve as biomarkers for patients with cancer. Currently, many drugs targeting the PLKs are under preclinical and clinical investigation [15]. Despite increasing studies on PLKs, their biological functions, regulation, expression and activation in human cancers remain unclear. A comprehensive analysis of the biological role and underlying mechanism of the PLKs family could help understand PLKs-mediated carcinogenesis and develop PLKs-targeting therapies.

With the evolution of bioinformatics and sequencing technologies, many public data and corresponding clinical information have been disclosed. We can analyze these data to explore the potential biomarkers for further study [16]. These advancements provide us more methods to understand the malignant behaviors of cancers from a perspective of genomics, proteomics, and single cell omics [17, 18].

In our study, we investigated the mutation profile of the PLKs across all human cancers. We next explored the expression levels of five PLKs and studied their relationship with cancer prognosis. Additionally, we evaluated the association between PLK expression and immune subtype, immunotherapy, TME score, cancer stemness, and drug sensitivity in human cancers. We constructed an integrated interaction network and enriched the pathways for PLKs and PLK-related proteins. We also verified the expression and prognostic value of PLK1 in KIRP. Overall, our study is the first one to describe and present the comprehensive insights on the functions of PLKs in pan-cancer. Our study offers an in-depth understanding into the potential therapeutic targets of PLKs for future drug development.

2. Materials and methods

2.1. Data acquisition and processing

The gene expression profile, gene mutation data, drug sensitivity data, stemness score, and clinical data were obtained from The Cancer Genome Atlas (TCGA) using UCSC Xena (https://xenabrowser.net/) [19]. Genotype-Tissue Expression (GTEx) (https://gtexportal.org/home/index.html) was used to download normal tissue data as para-cancerous tissues. A list of the cancer types is provided in Supplementary Table 1. In addition, Therapeutically Applicable Research to Generate Effective Treatments (TARGET) (https://www.cancer.gov/ccg/research/genome-sequencing/target) was applied to verify the prognostic role of PLKs in pan-cancer. The detailed flow chart of this study is displayed in Fig. 1.

2.2. Genomic alteration analysis of PLKs

Pan-cancer genomic alterations of PLK genes were explored by cBioPortal (http://www.cbioportal.org/), a comprehensive tumor genome study platform [20]. All cancer types in the TCGA Pan-Cancer Atlas were selected for analysis. Mutations, including missense, amplification, truncation, deep deletion, splicing, fusion, and in-frame were calculated within the coding sequence of every PLK gene.

All expression and methylation analyses and plotting were performed using R software ggplot2 or generated from the website of the data source. Single nucleotide variations (SNV) plots were obtained using maftools [21]. Copy number variations (CNV) data was processed using GISTICS2.0 [22]. Data were analyzed using *t*-test or ANOVA. To compare the data, the integrated expression level of the PLK gene set was calculated by gene set variation analysis (GSVA) [23]. We also accessed the SNV dataset of level4 processed using MuTect2 software [24] from GDC (https://portal.gdc.cancer.gov/) and integrated the mutation information of all samples. Structural domain information of the PLKs was downloaded using the R package maftols.

2.3. Gene expression and survival analysis of PLKs

We obtained standardized pan-cancer datasets from the UCSC website (https://xenabrowser.net/): TCGA TARGET GTEx (PANCAN, Number = 19131, gene = 60499). To obtain more comprehensive data, we used the TARGET database to identify additional cancer types. Subsequently, 41 types of cancers were selected in our analysis including adrenocortical carcinoma (ACC), acute lymphoblastic leukemia (ALL), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), colon adenocarcinoma/rectum adenocarcinoma (COADREAD), diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), glioma (GBMLGG), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), pan-kidney cancer (KIPAN), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), neuroblastoma (NB), osteosarcoma (OSA), ovarian cancer (OV), pancreatic adenocarcinoma (PAAD), pheochromocytoma and paraganglioma (PCPG), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), sarcoma (SARC), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), stomach and esophageal carcinoma (STES), testicular germ cell tumors (TGCT), thyroid carcinoma (THCA), thymoma (THYM), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS), uveal melanoma (UVM), and Wilms tumor (WT) (Table S1). PLK gene expression levels were processed using Perl tool. "Wilcox.test" was applied to explore the differential expression of the PLK genes in pan-cancer. For pan-cancer expression analysis of PLKs, cancer types with less than five adjacent tissues as controls (15 cancer types) were excluded. Box plot, heatmap, and violin plot were generated by the R packages "pheatmap" and "ggpubr." Correlation analysis of PLK genes was analyzed using R-package "corrplot." We also analyzed PLK family members in cancer cell lines using the Cancer Cell Line Encyclopedia (CCLE) database (https://sites.broadinstitute.org/ccle/). We used the clinical information of samples from nine cancer types for expression and subtype analyses. We used the pathological stage and clinical information of tumor samples from 27 cancer types for expression and pathological stage analyses. The mRNA expression profile, clinical subtype, and pathologic stage data were merged using a sample barcode to generate a line chart.

For survival analysis, the genetic regulation of PLK family gene expression was analyzed using the cBioPortal (http://www.cbioportal.org/). We then obtained and curated each sample's survival information from the TCGA and TARGET databases and next analyzed the association between PLK gene expression and overall survival (OS), disease-free interval (DFI), disease-specific survival (DSS) or progression-free interval (PFI) of patients. We further performed a Cox analysis to identify the relationship between expression of PLK genes and OS in all cancers. Finally, a forest plot was generated by the R package "forestplot" and "survival."

2.4. Tumor microenvironment, immunotherapy and tumor stemness analysis of PLKs

We obtained the expression profiles of PLK genes in each sample and performed a log2(x+0.001) transformation for each expression data point. Then we used deconvo_xCell of the IOBR algorithm, a multi-omics immuno-oncology tool [25,26]. We integrated and visualized 64 types of immune cell infiltration, including $CD8^+$_T-cells, $CD4^+$_T-cells, Macrophages, B-cells, and three types of immune scores (Immune Score, Stroma Score, and Microenvironment Score), and analyzed PLKs family genes, immune checkpoint genes, and immune regulatory genes.

To explore the relationship between PLK family and cancer immunotherapy, we collected 21 datasets of cancer immunotherapy, including the expression profile in cancer patients receiving ICB therapy. The immunotherapy datasets include six types of cancer, including the GBM, KIRC, LGG, SKCM, non-small cell lung cancer (NSCLC), and STAD. The difference of PLKs between responded and non-responded groups were analyzed using "wilcox" test. And the area under the curve (AUC) was applied to reflect the diagnostic efficiency of PLKs between responded and non-responded groups.

Then, we performed the correlation analysis between immune subtypes and expression of PLK genes using R package "ggplot2," "limma," and "reshape2." Immune and stromal and cell scores were performed by using the ESTIMATE algorithm [27] to assess the correlation of PLK gene expression and infiltrating immune/stromal cells in cancers. A correlation analysis between RNA stemness score (RNAss) or DNA stemness score (DNAss) and PLK family genes expression was performed using Spearman's method. Both stemness plots were generated by R-package "corrplot."

2.5. Pathway and enrichment analysis of PLKs

First, we evaluated the role of PLKs in ten cancer-related pathways, including cell cycle, apoptosis pathways, tuberous sclerosis 1 protein (TSC)/mechanistic target of rapamycin (mTOR), phosphatidylinositol-4,5-bisphosphate-3-kinase (PI3K)/protein kinase B (AKT), receptor tyrosine kinase (RTK), RAS/mitogen-activated protein kinase (MAPK), hormone estrogen receptor (ER), hormone androgen receptor (AR), DNA Damage Response, and epithelial-mesenchyme transition (EMT). The pathway score was the sum of the relative protein levels of all positive regulatory components minus those of the negative regulatory components in a particular pathway. The pathway activity score (PAS) was calculated as before [28], where PAS (Gene A group High) > PAS (Gene A group Low); the gene had an activating effect; otherwise, it had an inhibitory effect. Next, we predicted potential functional interaction network of PLKs using GeneMANIA (http://genemania.org/), which is an online website for analysis of gene-gene interaction and enrichment [29].

To explore the interaction network of PLKs and correlated genes, GEPIA2.0 was applied to obtain the top 20 PLK1-5 correlated genes based on all human tumors and corresponding normal tissues. We merged the five datasets (PLK1-5) to generate the Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses. Then, we visualized the

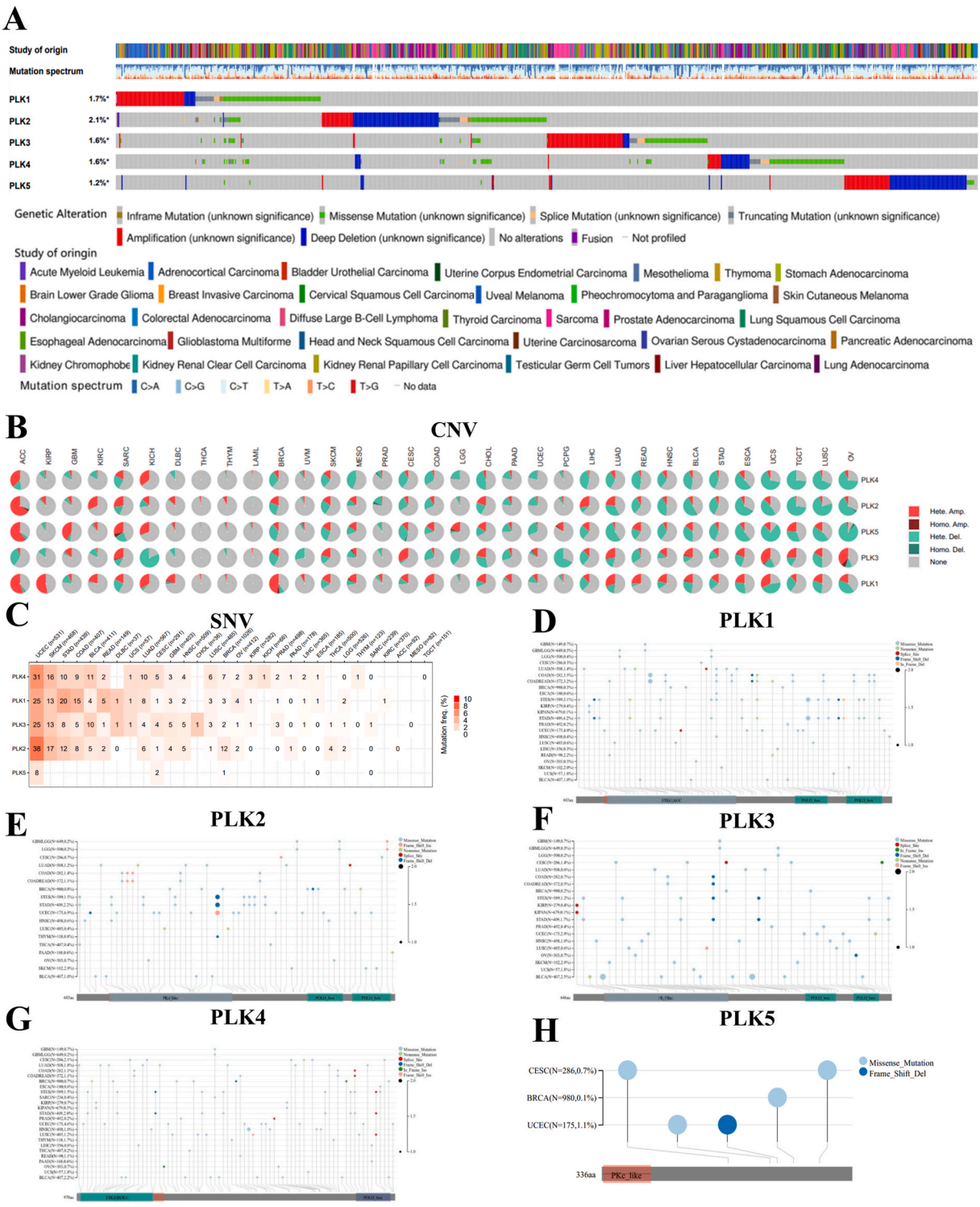


Fig. 2. Genomic profiles of PLK family genes. A. An OncoPrint plot showing PLK genetic alterations across human cancers. Each sample was represented as a column and each PLK gene was represented as a row. The alterations were represented in different colors; **B.** CNV distribution pie chart across cancers. Hete Amp represents heterozygous amplification; Hete Del represents heterozygous deletion; Homo Amp represents homozygous amplification; Homo Del represents homozygous deletion; None = no CNV; **C.** The SNV landscaped plot of PLK genes in pan-cancer; **D-H.** The mutation landscape of PLK 1 (**D**), PLK2 (**E**), PLK3 (**F**), PLK4 (**G**), and PLK5 (**H**) in pan-cancer.

biological process (BP), cellular component (CC), and molecular function (MF), and KEGG pathway as bubble plots using R packages, "clusterProfiler," "tidyr," and "ggplot2."

2.6. Drug sensitivity analysis of PLKs

We first downloaded the processed drug sensitivity information from the Genomics of Drug Sensitivity in Cancer (GDSC) [30] and the Cancer Therapeutics Response Portal (CTRP) database [31]. We then visualized the results through bubble plots using the R package "limma," "impute," "ggpubr," and "ggplot2." To make our analysis more comprehensive, we accessed CellMiner (https://discover.nci.nih.gov/cellminer/) [32] on 60 cancer cell anticancer drugs. We then applied the "NCI-60 Analysis Tool" function, which can search the activity reports of 20,503 small molecule compounds and transcripts of 22,379 genes. We then analyzed the correlation between 50 % growth inhibitory concentrations of the drugs and PLK gens by Pearson's correlation coefficient using R software.

2.7. Validation of PLK1 in kidney renal papillary cell carcinoma (KIRP)

Based on prognosis analysis of PLK1 in pan-cancer, the highest hazard ratios (HR) of PLK1 in KIRP caught our attention (HR = 2.31, P = 3.0e-12), so we selected the KIRP as the subsequent validation tumor. The TCGA KIRP data was downloaded to evaluate PLK1 expression in patients with KIRP. We first evaluated the expression level of PLK1 in paired and unpaired KIRP samples. To further evaluate the prognostic potential of PLK1 in KIRP, we performed the receiver operating characteristic (ROC) plot by R packages "pROC" and "ggplot2". We then analyzed the association between PLK1 expression and clinical parameters (including patient age, cancer stage, histologic subtype, and nodal metastasis status) and promoter methylation in KIRP samples using the UALCAN tool [33]. Clinical information and corresponding RNA-sequencing expression profiles and for KIRP were accessed from the TCGA database. The log-rank test was applied to compare differences in survival between groups. Time timeROC analysis was applied to calculate the predictive accuracy of PLK1 mRNA expression. For the Kaplan-Meier (K-M) plots, p-values and HR with 95 % confidence intervals (CI) were obtained using univariate Cox proportional hazards regression and log-rank tests. Furthermore, univariate and multivariate Cox regression analyses were conducted to select the clinical parameters for establishing the nomogram. The foresting analysis was used to show the HR, 95 % CI, and P value of each variable via R package "forestplot." PLK1 expression level and pTNM_stage were integrated to build the nomogram, based on the results of multivariate Cox regression analysis, to predict the 1-, 2-, and 3-year OS using the "rms" R package.

2.8. Statistics

All statistical examinations were performed by database derived tools and R software. All plots in this paper were constructed in database derived tools and R studio. All datasets were standardized using zero-mean normalization. The Kaplan-Meier analysis was applied to assess the survival outcomes. And various test methods were used to determining the statistical significance of differential groups. P value < 0.05 was statistically significant for all analysis.

3. Results

3.1. Pan-cancer genetic alternation profiles of PLK family genes

Genetic alterations, such as copy number variations (CNVs) and single nucleotide variants (SNVs) are associated with tumorigenesis, tumor progression, and treatment outcomes [34]. Fig. 2A shows the genomic alteration of PLKs in pan-cancers. The overall alteration frequency of each PLKs gene was 1.2–2.1 %. PLK2 (2.1 %), PLK1 (1.7 %), PLK3 (1.6 %), and PLK4 (1.6 %) showed higher gene alteration frequencies than PLK5 (1.2 %). The CNV profile demonstrated various functional patterns of PLK genes in all types of cancers. The PLK gene set's pan-cancer pie chart demonstrated that the majority of CNVs were heterozygous, whereas a minority represented homozygous CNVs. ACC, OV, and LUSC were the top three cancer types most closely associated with the CNV alteration frequency of PLK genes. Interestingly, heterozygous amplification of PLK1 seemed to play a crucial role in KIRP (Fig. 2B). Furthermore, the SNV profile revealed that PLK1 and PLK2 were the most frequently mutated PLK genes in 32 human cancers, particularly in UCEC (PLK1:31 %, PLK2:38 %). In the context of UCEC, a comprehensive analysis was conducted to detect SNVs across the five PLK genes. The findings revealed a significantly elevated mutation rate in UCEC as compared to other types of cancers for all PLK genes. (Fig. 2C). The mutation profile of PLKs revealed that most mutations were almost evenly distributed in the coding sequences of PLK genes. Furthermore, Fig. S1 showed that protein expression of PLK1 was increased in UCEC, LUAD, LSCC, HNSCC, COAD, while decreased in KIRC. Notably, 25 % and 15 % mutation rate of PLK1 are found in UCEC and COAD, respectively. Our analysis showed that the mutations of PLK1 could lead to activation of the protein in UCECA and SKCM. In pancreatic cancer, the most common mutations in PLK genes are missense mutations. Of the 37 cancer types, genomic alterations in UCEC, BRCA, and CESC occurred in all PLKs; 23 cancer types (62 %) had genomic alterations in the structural domain of PLK1 (Fig. 2D–H).

3.2. Pan-cancer expression profiles of PLK family genes

We first analyzed the expression levels of PLK family members in various cancer cell lines and found that all PLKs, except PLK5, had

higher expression in cancer cell lines, including DLBC, SARC, BLCA, KIRC, and OV (Figs. S2A–E). To explore the expression pattern of PLKs in pan-cancer, a comprehensive analysis was conducted on the expression levels of five PLK family members in 33 human cancers utilizing TCGA pan-cancer data. PLK2 showed the highest expression level, whereas PLK5 showed the lowest expression in all cancers (Fig. 3A). Overall, PLK family genes were more likely to be elevated compared to those in adjacent normal tissues. In the selected 14 cancer types, PLK1 was upregulated in 12 types of cancer and PLK2 was upregulated in 13 types of cancer (Fig. 3B). However, inter- and intra-cancer heterogeneity was observed in the expression levels of the corresponding genes for all five PLK members. There is no unified intrinsic mode of PLK gene expression. Additionally, the expression levels of the PLK family members were associated with each other in pan-cancer studies. However, the absolute value of the correlation index ranged from 0.03 to 0.61, indicating a weak or

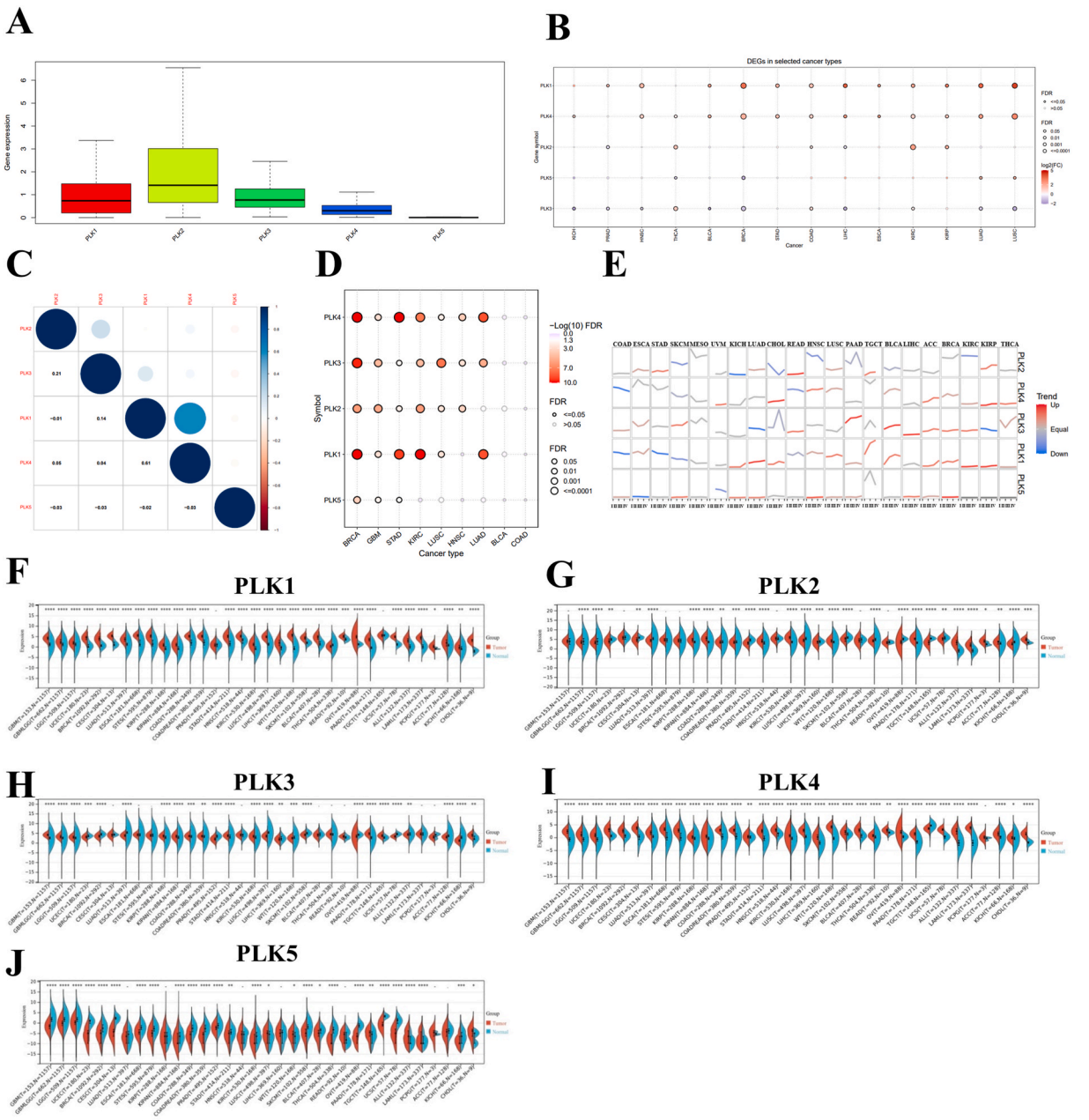
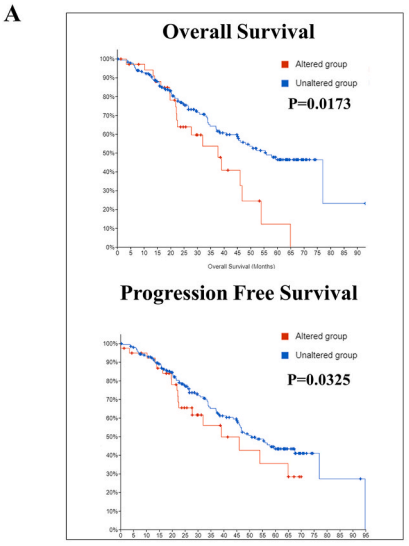
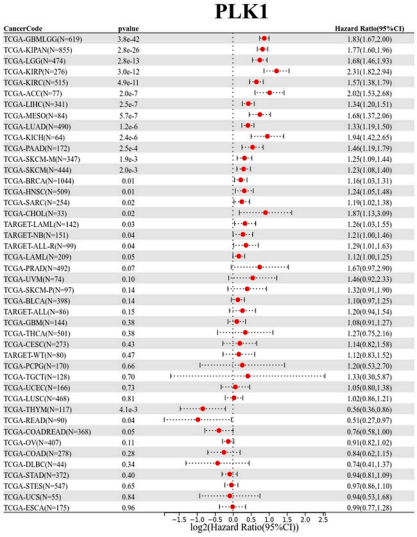
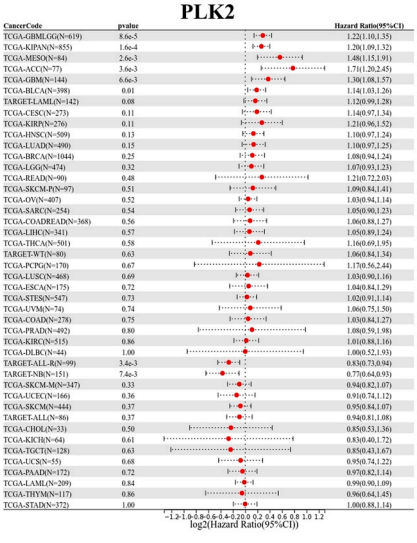
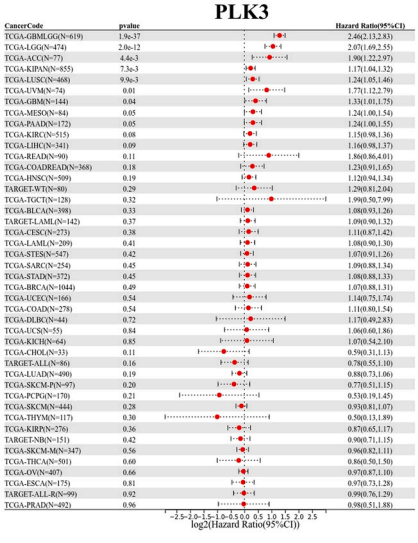


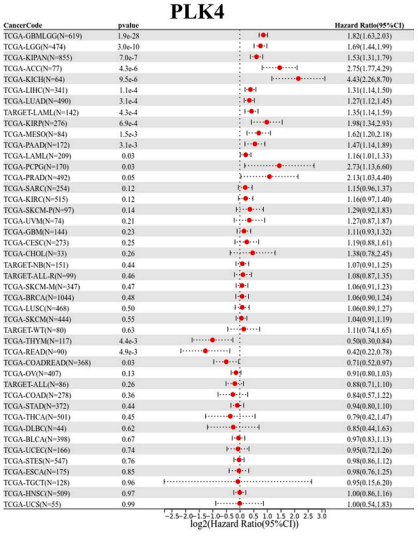
Fig. 3. Pan-cancer expression profiles of PLK family genes. A. Box plots represent the relative expression of PLK genes in pan-cancer. **B.** Expression difference between cancer and non-cancer tissues from TCGA cohorts; **C.** Matrix graph of Pearson's correlation of PLK genes expression in pan-cancer. Blue dots indicate positive correlation and red dots indicate negative correlation; **D.** Expression differences between subtypes of cancers; **E.** Expression differences between stages of cancers; **F-J.** The expression landscape of PLK 1 (**F**), PLK2 (**G**), PLK3 (**H**), PLK4 (**I**), and PLK5 (**J**) in pan-cancer. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.











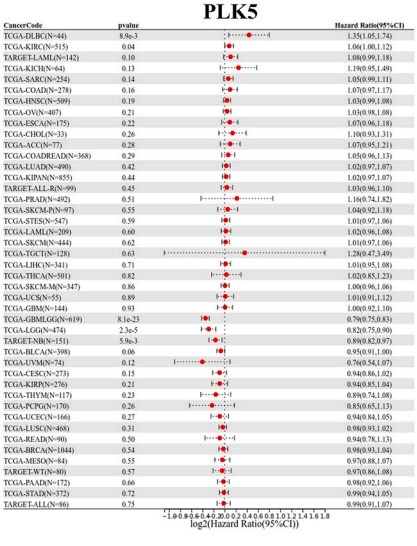


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Fig. 4. Correlation of PLK genes expression with patient's survival. A. Kaplan–Meier survival curves for PLK genes alteration associated with overall survival and progression free survival. PLK genes altered group associated with poor prognosis in pan-cancer. **B–F.** The forest plots showing the univariate Cox proportional hazards model for correlation between PLK1 **(B)**, PLK2 **(C)**, PLK3 **(D)**, PLK4 **(E)**, and PLK5 **(F)** expression and overall survival of various patients with cancer.

negligible correlation (Fig. 3C). These results show intrinsic differences in the expression patterns of PLK genes between different PLK family members and various cancer types. Whether a specific PLK gene is an oncogene or a tumor suppressor cannot be determined in different cancer types. Therefore, the complicacy of the expression spectrum requires further investigation, with each PLK gene as an individual entity.

A gene may have a disparate expression pattern in different cancer subtypes [35]. Analysis of subtypes revealed a significant correlation between the manifestation of PLK genes and three types of malignancies, namely, BRCA, GBM, and STAD. (Fig. 3D). In addition, with an increase in tumor stage, the expression of PLK family members tended to increase, indicating the potential of PLKs as cancer biomarkers (Fig. 3E). We further verified our results by combining TCGA, GTEx, and TARGET cohorts. The verification cohort showed that all five PLK genes were significantly dysregulated in various cancer types (Fig. 3F–J). PLK1 was overexpressed in 31 cancer types and downregulated in 1 (only in THCA) (Fig. 3F). The highest expression of PLK2 was observed in 11 cancer types, whereas the lowest expression was observed in 16 (Fig. 3G). Additionally, PLK3 was highly expressed in 12 cancer categories and decreased in 13 cancer types (Fig. 3H). PLK4 was upregulated in 32 cancer types and downregulated only in TGCT (Fig. 3I). PLK5 exhibited a different expression pattern compared to other PLK family members; it was only upregulated in 6 types of cancer and significantly downregulated in 21 types of cancer (Fig. 3J).

3.3. Patient survival correlated with the expression of PLK family genes

Due to the wide range of genetic modifications and variations in expression among PLK genes in various forms of human malignancies, an investigation was conducted to examine the association between alterations and expression patterns within the PLK family and the survival outcomes of patients, encompassing OS, DSS, DFI, and PFI. Our findings indicated a significant correlation between altered PLK genes and poorer OS and progression-free survival in pan-cancer cases (Fig. 4A). According to the univariate Cox model, the survival risk displayed a significant correlation with the expression levels of PLK genes. Furthermore, using a univariate Cox proportional hazards model, we observed a significant association between the expression levels of PLK genes and the risk of patient survival (Fig. 4B–F, Figs. S3–4). However, identifying specific PLK genes as risk factors for high or low survival varies depending on the type of cancer. For example, high PLK1 expression increased the survival risk in 20 types of cancer and decreased the survival risk in

Table 1
Correlation of PLKs expression and overall survival in pan-cancer.

Cancer	PLK1	PLK2	PLK3	PLK4	PLK5
ACC	P	P	P	P	
ALL	P	N			
BLCA		P			
BRCA	P				
CHOL	P				
COADREAD	N			N	
DLBC					P
GBM		P	P		
GBMLGG	P	P	P	P	N
HNSC	P				
KICH	P			P	
KIPAN	P	P	P	P	
KIRC	P				P
KIRP	P			P	
LAML	P			P	
LIHC	P			P	
LUAD	P			P	
LUSC			P		
LGG	P		P	P	N
MESO	P	P	P	P	
NB	P	N			N
PAAD	P		P	P	
PCPG				P	
PRAD				P	
READ	N			N	
SARC	P				
SKCM	P				
THYM	N			N	
UVM			P		

N, negatively correlated; P, positively correlated.

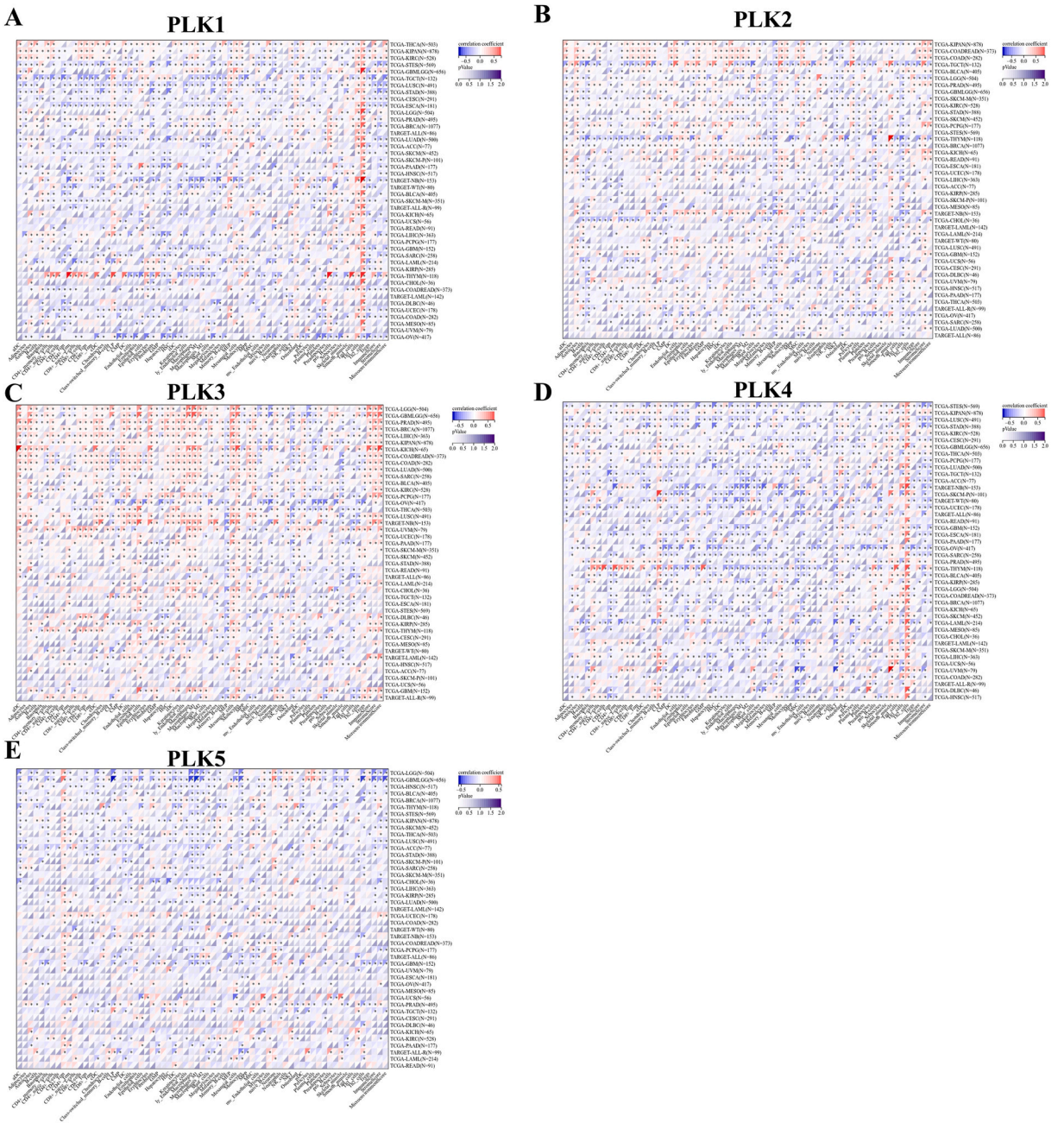

Fig. 5. Correlation of PLK genes expression with various immune cells in TME using xCELL. A-E. Correlation of PLK1 (**A**), PLK2 (**B**), PLK3 (**C**), PLK4 (**D**), and PLK5 (**E**) with various immune cells in TME. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

THYM and READ (Fig. 3B). Additionally, OS benefited from high PLK2 expression in NB and ALL-R and low expression in GBMLGG, KIPAN, BLCA, GBM, MESO, and ACC (Fig. 4C). High PLK3 expression was associated with the survival risk in the GBMLGG, KIPAN, GBM, LGG, LUSC, MESO, UVM, and ACC groups (Fig. 4D). Furthermore, high PLK4 expression predicted poor OS in 14 types of cancer but better OS in COADREAD, THYM, and READ (Fig. 4E). The overexpression of PLK5 was also linked to unfavorable (OS in cases of KIRC and DLBC; however, it was associated with improved OS in GBMLGG, LGG, and NB (Fig. 4F). Moreover, PLK expression exhibited correlations with PFI, DFI, and DSS in cancer patients (Figs. S3–5). Overall, the expression patterns of PLK genes demonstrated strong associations with patient survival, depending on the particular cancer type (Table 1).

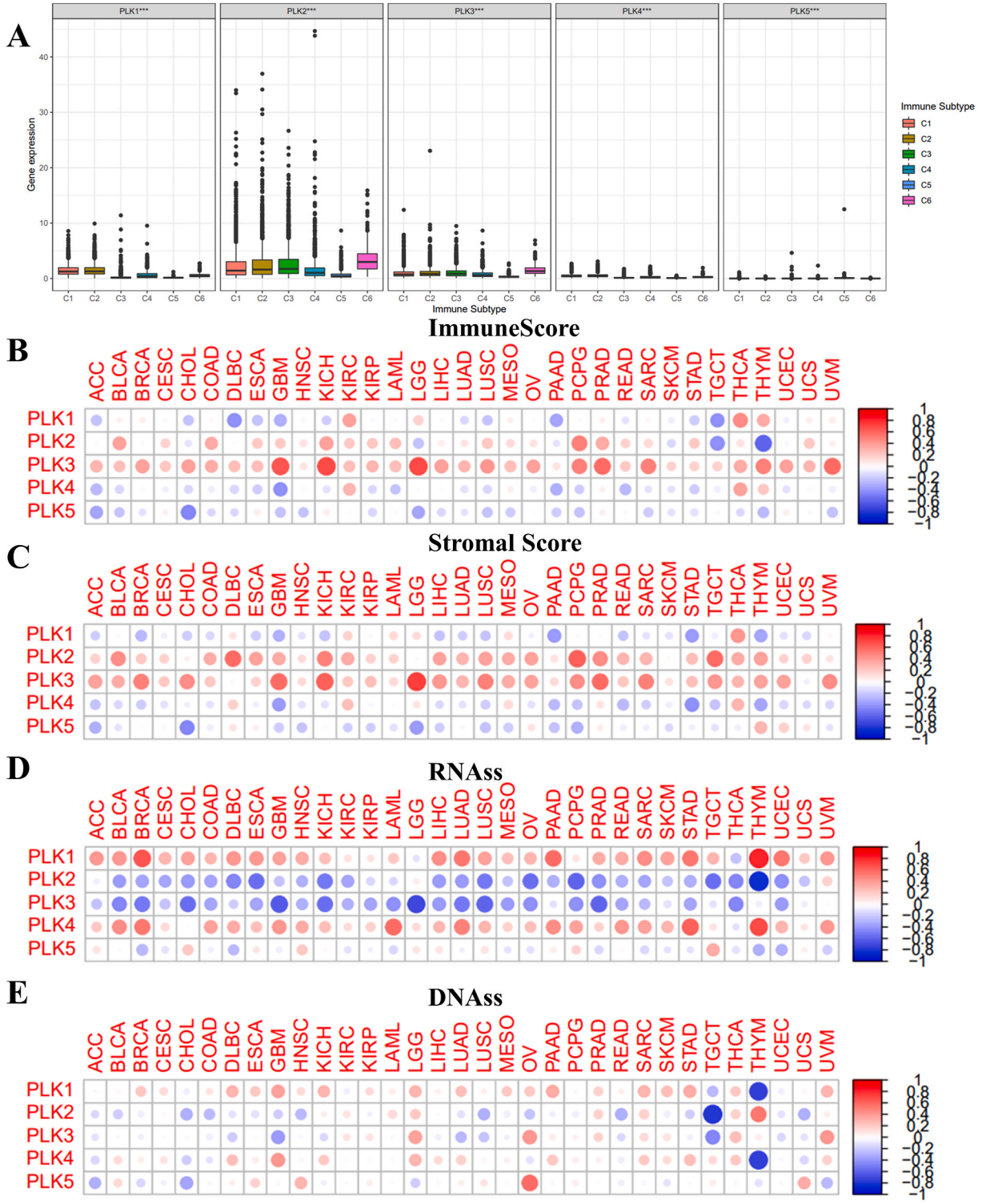

Fig. 6. The differential expression of PLK genes across immune subtypes and the correlation between PLK genes expression and TME/DNAss/RNAss in pan-cancer. A. The distribution of PLK expression in six immune subtypes. C1 (wound healing); C2 (IFN-gamma dominant); C3 (infammatory); C4 (lymphocyte depleted); C6 (TGF-β dominant); **C, D.** Correlation matrix between PLK genes expression and Immunescore/Stromascore in diverse cancers; **E, F.** Correlation matrix between PLK genes expression and cancer stemness scores RNAss and DNAss in diverse cancers. Cancer types and PLK genes were shown on the horizontal and vertical axis respectively. Red represents the positive correlation, while blue represents negative correlation. RNAss, RNA stemness score; DNAss, DNA stemness score. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.4. PLK family genes were associated with immune subtypes, tumor microenvironment, immunotherapy, and tumor stemness

Tumors have evolved into ecosystems consisting of stromal, tumor cells, and infiltrating immune cells. According to the findings in Fig. 5A–E, the expression of PLK1 was found to be strongly and positively linked to both Th1 and Th2 cells in the majority of human cancers. Similarly, PLK4 expression displayed a similar pattern to that of PLK1 expression, as it was also found to be associated with

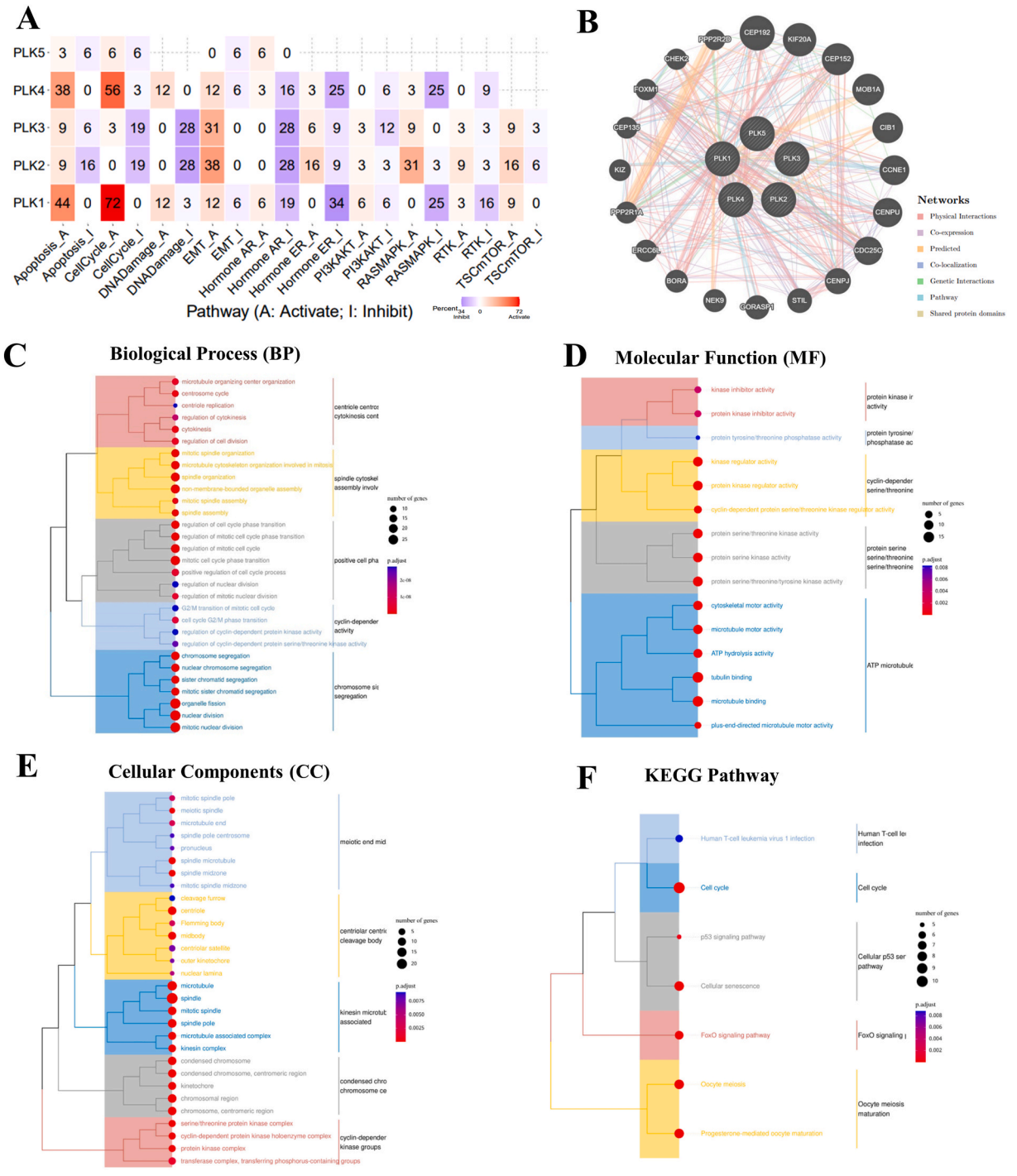

Fig. 7. The cross-talk profile and drug sensitivity analysis enrichment analysis of PLK genes. A. Heatmap of the percentage of the effect of PLK genes on cancer pathway activity. Each pathway (activate or inhibit) was represented as a column and each PLK gene was represented as a row; **B.** The interaction networks of PLK genes; **C–F.** The GO (**C-E**) and KEGG (**F**) enrichment analysis of PLK and PLK-associated genes.

diverse infiltrating immune cells in pan-cancer cases. This discovery indicates a potential correlation between PLK1 and PLK4 within the TME. Additionally, PLK3 was observed to have a positive correlation with various immune cells in select cancers, including LGG, GBM, PRAD, BRCA, and LUSC. Interestingly, further analysis was conducted to explore the relationship between the expression of PLK genes and immune checkpoints in pan-cancers. Illustrated in Fig. S6, the data reveals a positive association between PLK expression and multiple immune checkpoints, encompassing both immune inhibitory and immune stimulatory checkpoints. Furthermore, it was observed that members of the PLK family were significantly linked to a wide range of immunomodulators, including immunoinhibitors, immunostimulators, MHC molecules, chemokines, and chemokine receptors, in all forms of cancer (Figs. S6–7). These outcomes underscore the importance of comprehending the immunological roles of PLK family members in order to identify the types of cancer that could potentially benefit from anti-PLK immunotherapy.

A comprehensive transcriptomic immune classification of solid tumors has successfully identified six distinct immune subtypes, identified as C1–C6. These subtypes are categorized as the following: C1 (associated with wound healing), C2 (characterized by IFN-γ dominance), C3 (linked to inflammation), C4 (known for lymphocyte depletion), C5 (described as immunologically quiet), and C6

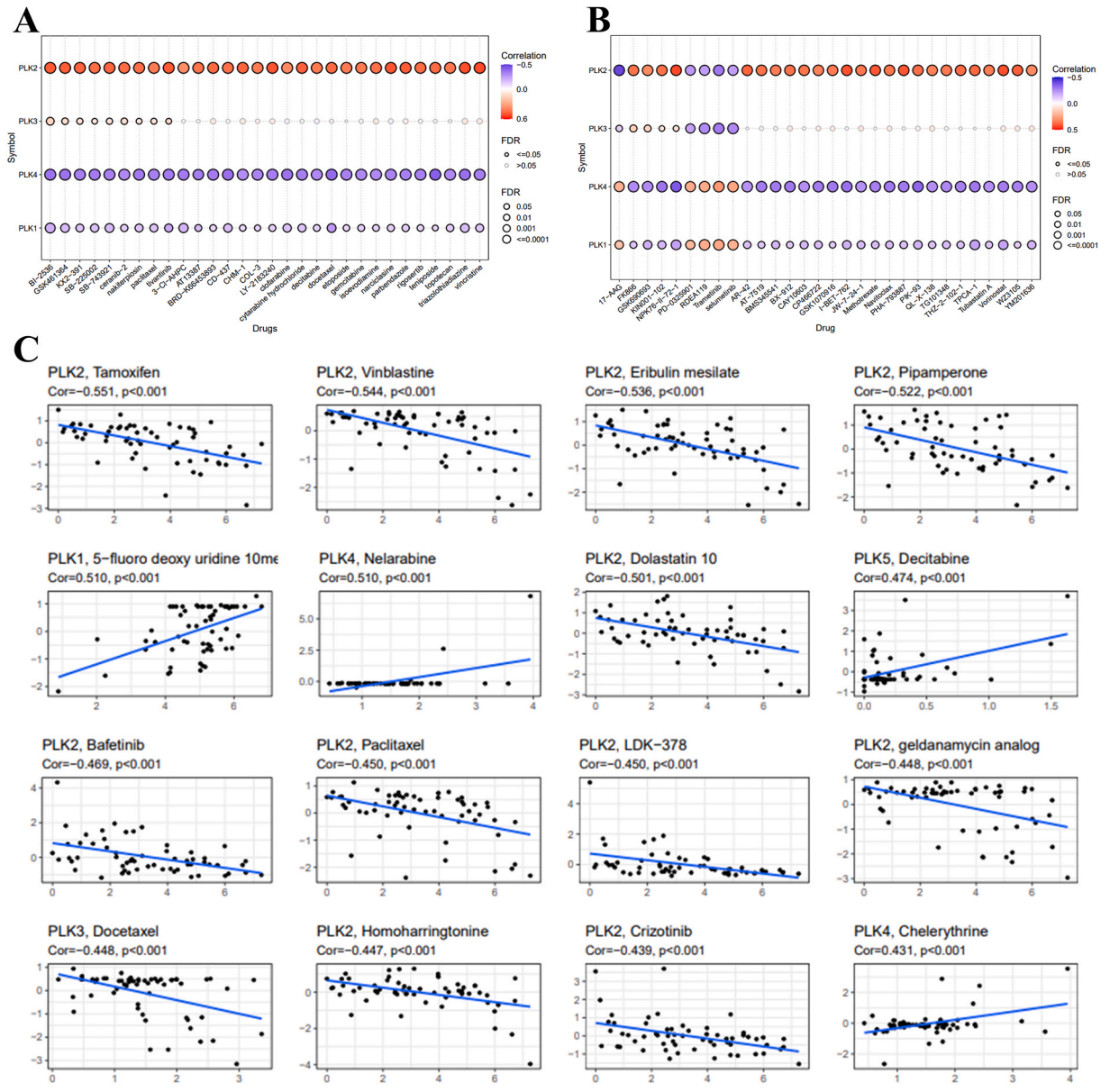

Fig. 8. The drug sensitivity analysis of PLK family genes. A. The correlation between GDSC drug sensitivity and PLK gene expression; **B.** The correlation between CTRP drug sensitivity and PLK gene expression. Red represents the positive correlation, while blue represents negative correlation; **C.** Scatter Plot showing the correlation between PLK genes expression (in horizontal axis) and drug sensitivity from the CellMiner (in vertical axis). Only the 16 with the smallest P values were shown.

(predominantly influenced by TGF-β) [32]. Our analysis has demonstrated a significant correlation between the expression of PLK family genes and immune subtypes across all forms of cancer ($P < 0.001$), as depicted in Fig. 6A. Notably, high expressions of PLK1, PLK2, and PLK3 were found to be specifically associated with subtype C1 (Wound Healing), C2 (IFN-γ dominant), and C6 (TGFβ dominant), suggesting a potential pro-oncogenic role played by these three genes. Furthermore, the TME score results revealed that PLK2 and PLK3 expression was positively associated with the stromal score and immune score in pan-cancer (Table S2). PLK1, PLK4, and PLK5 expression were negatively associated with the stromal score and immune core in most cancer types (Fig. 6B–C). Specifically, PLK3 had an almost consistent positive relationship with these scores across all cancers, whereas PLK5 appeared to have a consistent negative relationship. These results revealed that PLK members vary in their ability to regulate the TME.

These results indicated that PLK family may be the crucial mediators between the immune cells and cancers. So we explore the association between expression of PLKs and ICB responses (one of the most widely used cancer immunotherapies). Fig. S8 showed that all AUC of PLKs are more than 0.4, indicating that PLKs could be potential biomarkers to distinguish the ICB responses. In some datasets, PLKs showed a strong diagnostic efficiency, including the PLK1 in Melanoma-GSE100797, PLK2 in GBM-PRJNA482620, PLK4 in Melanoma-GSE100797, and PLK5 in NSCLC-GSE135222. Fig. S9 revealed that low expression of PLK1 in melanoma and GBM, PLK2 in melanoma and GBM, and PLK4 in melanoma may be the favorable factors for ICB responses, while high expression of PLK4 in STAD and PLK5 in NSCLC predict the better ICB responses (All $P < 0.05$).

Cancer stem cells play crucial roles in malignant behavior, metastasis, therapy resistance, and recurrence [36]. Therefore, we examined the correlation between the expression of the PLK gene and the score of tumor stemness (comprising of RNAss and DNAss). In the analysis conducted across various cancers, we observed both positive and negative associations between the expression of PLK family members and RNAss as well as DNAss (Table S3). Notably, PLK1 and PLK4 showed a positive correlation with RNAss in 32 cancer types, excluding LGG and THCA. In addition, PLK2 and PLK3 were negatively associated with RNAss in many tumor types. The association between PLK5 and RNAss appeared to be weak in pan-cancers (Fig. 6D). We also found that PLK1 was positively associated with DNAss in several cancers, especially in GBM (correlation coefficient = 0.37) and THYM (correlation coefficient = −0.72). In addition, PLK2 had a negative relationship with DNAss in TGCT (correlation coefficient = −0.78) (Fig. 6E). Despite the differences in RNAss and DNAss based on different algorithms, the PLK genes were revealed to be related to the stemness of different tumor types to varying degrees.

3.5. PLK family genes are widely involved in cancer-related pathways and drug sensitivity

In order to gain a comprehensive understanding of the functions of PLKs and their associated proteins, we conducted further investigation into the links between PLK gene expression and ten pathways commonly associated with cancer, namely the PI3K/AKT, TSC/mTOR, RAS/MAPK, ER, RTK, AR, DNA damage response, EMT, cell cycle, and apoptosis pathways. The findings depicted in Fig. 7A demonstrate a clear correlation between PLK expression and the activation or inhibition of these cancer-related pathways. Nevertheless, the precise impact of PLK genes on these pathways may differ depending on the specific type of cancer and the unique characteristics of each pathway. Notably, high expression of PLK1 and PLK4 was significantly correlated with 72 % and 56 % of cell cycle activation in cancers, respectively. Next, we examined the interaction network of PLK family. Visualized networks revealed that PLK genes interact with various other genes to regulate cell biology or pathogenesis through physical or genetic interactions, co-expression or co-localization, pathways, and shared protein domains (Fig. 7B).

We investigated the possible molecular mechanisms of the PLK genes by enrichment analysis. We used GEPIA2.0 and acquired the top 20 genes correlating with PLK1/2/3/4/5 expression by combining 33 TCGA tumor expression data. We combined these PLK-correlated genes (100 genes) to perform GO and KEGG enrichment analyses (Table S4). The results indicated that most of these genes were associated with cell cycle-related biological processes, including centriole and spindle construction, positive cell phases, cyclin-dependent activity, and chromosome segregation (Fig. 7C). In addition, we found that the expression of PLK-correlated genes was closely associated with kinase regulatory activity, especially with protein serine/threonine regulatory activity (Fig. 7D). In terms of cellular components, PLK-associated genes were significantly correlated with many cell cycle-dependent components, such as microtubules, spindles, and cyclin-dependent kinase groups (Fig. 7E). KEGG pathway analysis showed that PLK-correlated genes were mainly involved in the cell cycle, cellular senescence, oocyte meiosis, and Foxo signaling pathways (Fig. 7F). These results indicate that PLKs and PLK-correlated genes may be involved in the cell cycle by regulating essential components. These findings imply that PLK family genes are extensively involved in regulating biological behaviors of the cell cycle and reactions to chemotherapeutic agents. However, there was no heterogeneity in the specific mechanisms across cancers and PLK gene types.

To determine the potential relationship between PLK gene expression and drug sensitivity, we first analyzed the correlation between PLK gene expression and drug sensitivity in multiple human cancer cell lines using the GDSC and CTRP databases (Table S5). Fig. 8A shows that the expression of PLK2 is positively associated with drug sensitivity to multiple chemotherapeutic drugs, whereas PLK1 and PLK4 are negatively associated with drug sensitivity to these drugs. In addition, PLK1 expression was negatively associated with sensitivity to many chemotherapeutic agents, including YM201636, vorinostat, and TPCA-1. PLK1 expression was also positively associated with sensitivity to many mitogen-activated protein kinase inhibitors (selumetinib, trametinib, RDEA119, PD-0325901, and 17-AAG) (Fig. 8B). We further analyzed the relationship between drug sensitivity and PLK family gene expression using CellMiner. We found that the expression of PLK family genes significantly correlated ($P < 0.05$) with drug sensitivity to multiple natural products and chemical compounds (Table S6). PLK1, PLK2, PLK3, PLK4, and PLK5 correlated with sensitivity to 21, 69, 9, 19, and 4 drugs, respectively. PLK2 expression is likely associated with drug sensitivity. The extent or direction of the correlation was not uniform for every drug and PLK gene. For instance, low expression of PLK2 increased drug resistance to tamoxifen, vinblastine, eribulin mesylate, and pipamperone, whereas low expression of PLK1 decreased drug resistance to 5-fluoro deoxy uridine (Fig. 8C). These comprehensive

analyses indicate that PLK family genes may be involved in chemoresistance, and targeting PLK family genes may overcome cancer drug resistance.

3.6. Validation of PLKs in kidney renal papillary cell carcinoma (KIRP)

We uncovered a relationship between PLK1 expression and genetic alterations, prognosis, and immune cell infiltration in various cancers. Fig. 3B shows that PLK1 expression was most significantly associated with the prognosis of patients with KIRP (HR = 2.31, P = 3.0e-12). Therefore, we aimed to further validate the role of PLK1 in KIRP. We first evaluated the expression of PLK1 in KIRP and found that PLK1 expression was higher in KIRP tissues than in the corresponding and non-corresponding normal tissues (Fig. 9A–B). The ROC curve demonstrated that the predictive power of PLK1 was highly accurate (AUC = 0.903, 95%CI = 0.856–0.951) (Fig. 9C). Further analyses revealed that high PLK1 expression was closely associated with some clinicopathological features, including older patient age, higher cancer stage, and higher nodal metastasis status (Fig. 9D, E, G). In addition, the expression of PLK1 was associated with different histological types of KIRP, and primary tumor tissues were characterized by higher promoter methylation levels of PLK1 compared to normal tissues. (Fig. 9F–H). We also found that high PLK1 expression was a risk factor for OS ($P < 0.001$) in KIRP patients (Fig. 9I).

Based on the expression of PLK1, people with KIRP were divided into a high PLK1 expression group and a low PLK1 expression group. As shown in Fig. 10A, with increased PLK1 expression, individuals with KIRP had a higher death risk. Patients with high PLK1 expression had shorter survival times than those with low PLK1 expression (Fig. 10B). These results suggest that high PLK1 expression

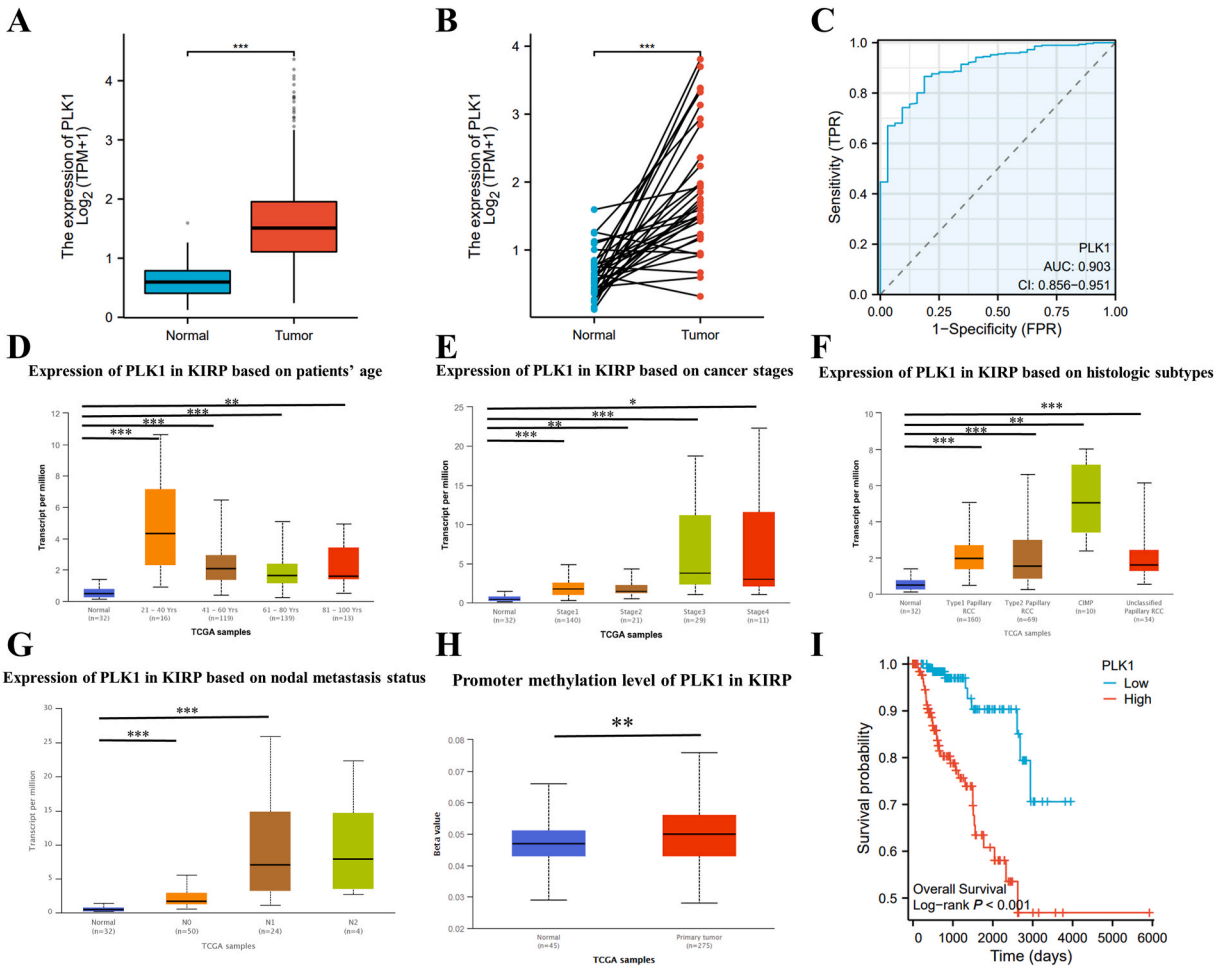

Fig. 9. Further validation of potential value of PLK1 in patients with KIRP. A. Expression of PLK1 in KIRP tissues compared with normal kidney tissues. Log2 (TPM + 1) was applied for log-scale; **B.** Expression of PLK1 in KIRP tissues compared with corresponding normal kidney tissues. Log2 (TPM + 1) was applied for log-scale; **C.** ROC curves of PLK1 for KIRP patients based on TCGA KIRP cohorts; **D-G.** PLK1 expression and clinicopathologic characteristics analysis in KIRP. Association with the PLK1 expression and clinicopathologic characteristics, including patient's age (**D**), cancer stages (**E**), histologic subtypes (**F**), nodal metastasis status (**G**); **H.** correlation of PLK1 expression and promoter methylation level in KIRP; **I.** Kaplan-Meier curves of PLK1 and KIRP patients' overall survival. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

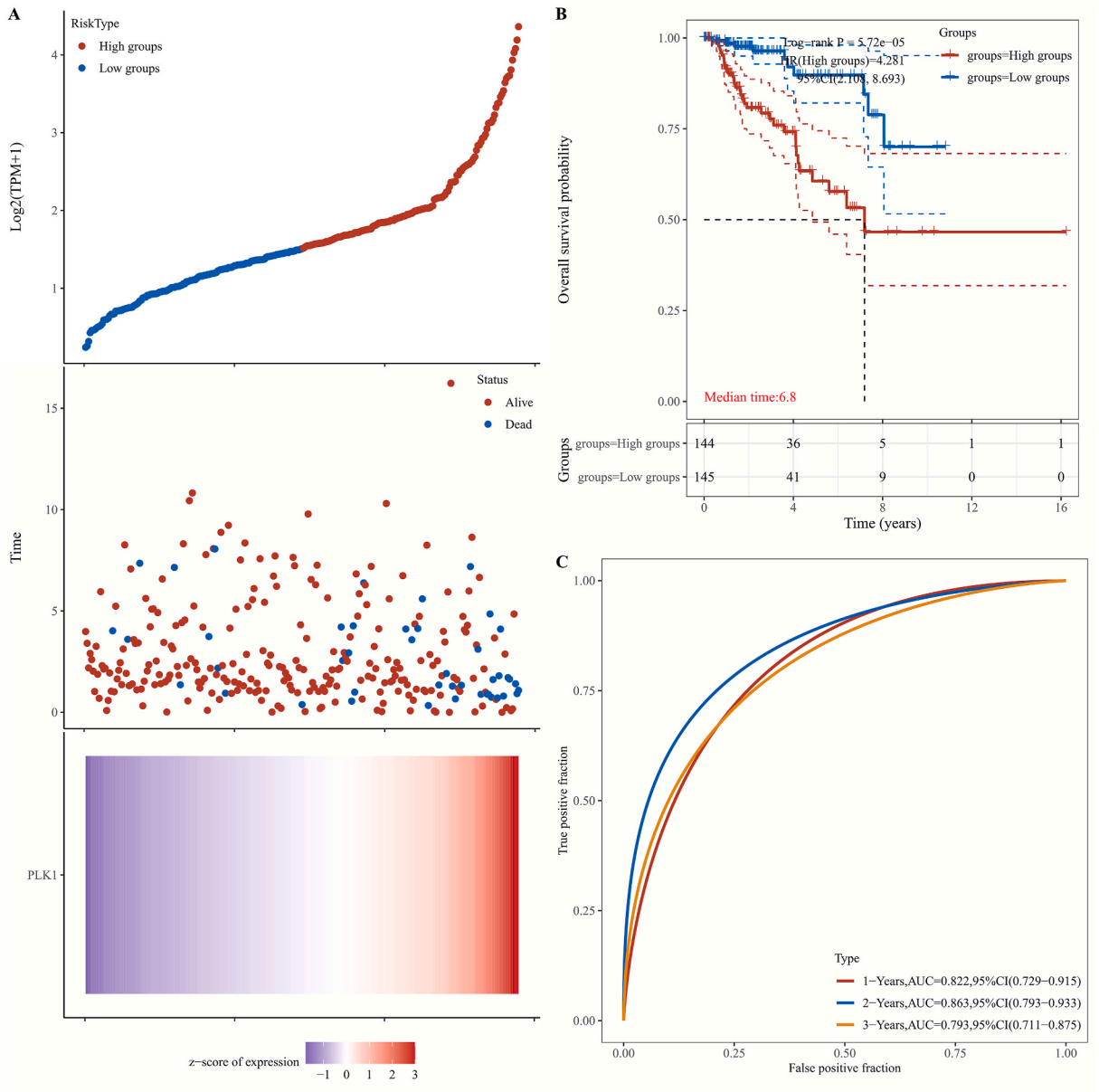

Fig. 10. Further validation of prognostic value of PLK1 in patients with KIRP. A. The relationship between PLK1 expression and patient's survival. The top represents the scatterplot from low to high expression of PLK1, and different colors represent different expression groups, the middle represents the scatter diagram distribution of survival time and survival state corresponding to PLK1 expression in different sample, and the bottom graph represents the heatmap of expression of PLK1; **B.** Kaplan-Meier curves of PLK1 and KIRP patients' overall survival based on the TCGA KIRP cohorts; **C.** Time-depend ROC curves of PLK1 and 1-, 2-, 3-years OS of KIRP patients.

may be a risk factor for KIRP. Time-dependent ROC curve analyses were used to evaluate the potential of PLK1 as a prognostic marker of KIRP. The AUC for 1-, 2- and 3-years OS were 0.822, 0.863, and 0.793, respectively (Fig. 10C). Overall, PLK1 was a strong candidate prognosis biomarker in patients with KIRP.

We performed univariate and multivariate Cox regression analyses to explore whether PLK1 is an independent prognostic biomarker for patients with KIRP. The univariate analysis indicated that the pTNM-stage ($P < 0.0001$), PLK1 ($P < 0.001$), and PLK4 expression ($P < 0.001$) were related to OS based on the TCGA KIRP cohort (Fig. 11A). Further, multivariate Cox regression analysis revealed that PLK1 expression ($P = 0.00017$) and pTNM-stage ($P = 0.0045$) were independent prognostic factors for KIRP patients (Fig. 11B). Finally, to study the potential role of PLK1 in the prognosis of patients with KIRP, we constructed a nomogram to predict the OS of patients with KIRP based on TCGA cohorts. PLK1 expression and the pTNM stage were included as prognostic factors in the nomogram (Fig. 11C). The calibration curve showed that the nomogram was reliable in predicting 1-, 2-, and 3-year OS in patients with

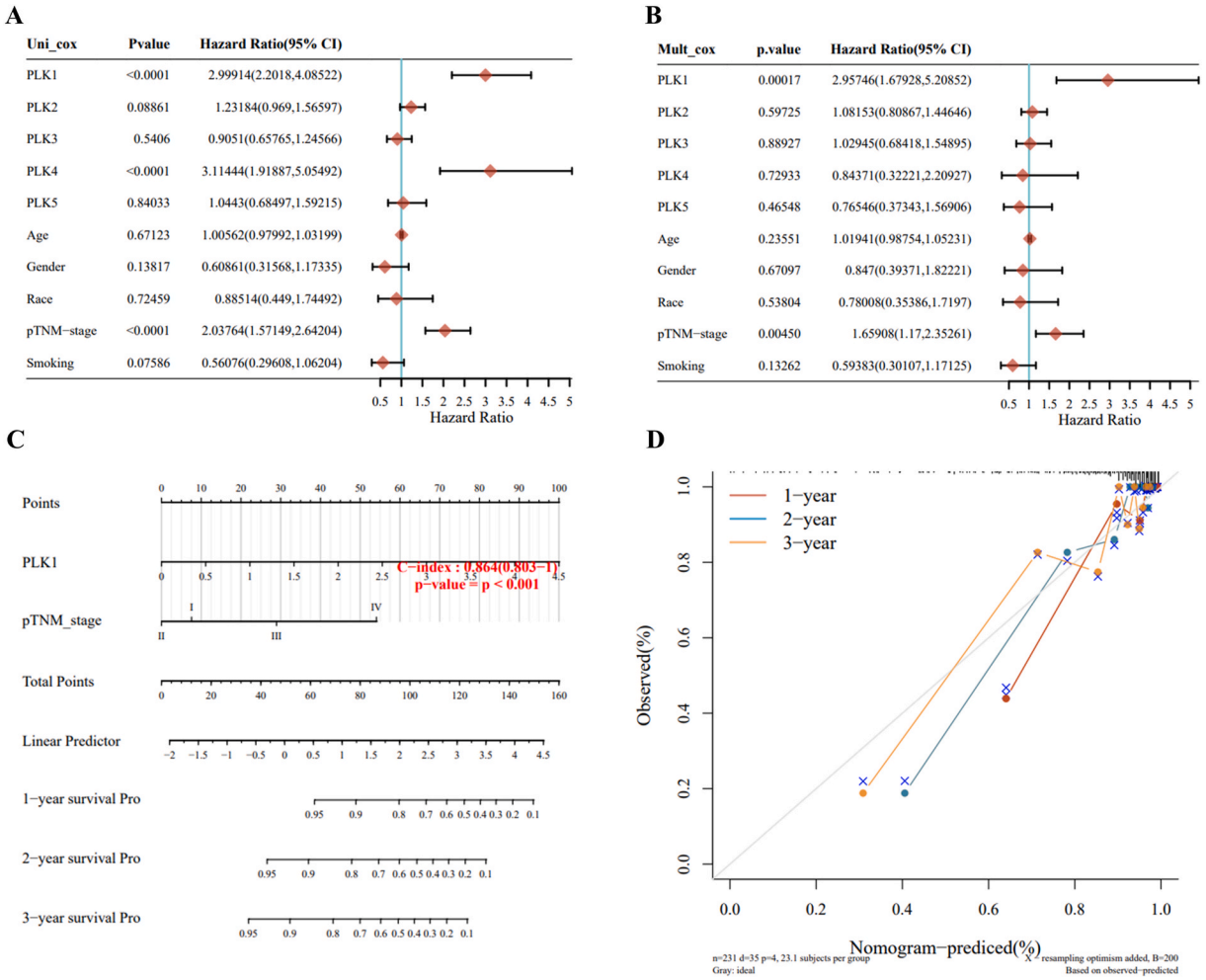

Fig. 11. Cox regression and construction of nomogram. A-B. Univariate (**A**) and multivariate (**B**) analysis of PLK family genes and some clinical information in OS based on TCGA KIRP cohort. (**C**) The nomogram is applied by adding up the PLK1 expression level and p_TNM stage. The total points projected on the bottom scales suggested the probability 1-, 2-, 3-years. (**C**) Calibration curves of the nomogram for the prediction of survival rates at 1-, 2-, 3-year. The nomogram-predicted probability of survival is plotted on the x-axis; and the actual survival is plotted on the y-axis.

KIRP (Fig. 11D). These results show that PLK1 expression may serve as a cancer biomarker for the diagnosis and prognosis of patients with KIRP.

4. Discussion

In this study, we conducted a comprehensive bioinformatics analysis by integrating multiple databases and datasets to explore the potential roles of PLK family genes in various cancer types. First, our analyses depicted a spectrum of expression of the five PLK genes, revealing that PLKs were enriched in multiple genomic alterations and were differentially expressed in most cancers, where no intrinsic unified mode was shown. The CNV profile indicated that the heterozygous type was the most frequent functional mode, and PLK1 heterozygous amplification seems to play an important role in KIRP. PLK1 and PLK2 were the most commonly mutated PLK genes in the 32 human malignancies, according to the SNV profile. The most common PLK mutations were missense mutations. Notably, 62 % of all cancer types show genomic alterations in the structural domain of PLK1. The expression profiles of PLKs have demonstrated that PLK family genes tend to be upregulated in multiple cancers. PLK2 had the highest expression level, whereas PLK5 had the lowest in all cancers. In addition, PLK genes are widely associated with cancer stages but vary with changes in specific cancer types. This means that in some selected cancer types, with an increase in cancer stage, the expression level of PLK is higher, indicating its crucial role as a biomarker for specific cancer types. Overall, the PLK family may play an important role in developing pan-cancers and provide new insights into antitumor therapy.

The prognostic analysis suggested that the survival of patients with cancer correlated with the differential expression of PLK family genes. In our analysis, the altered PLK gene group tended to correlate with a poorer prognosis, including various survival indicators

such as OS, PFS, DSS, and DFI. Their correlation with the survival risk was further confirmed using a univariate Cox proportional hazards model. But notably, in some cancers, PLK genes served as protective factors accompanied by lower survival risk and longer survival time, such as PLK1 in THYM and READ, PLK2 in NB and ALL-R, PLK4 in COADREAD, THYM, and READ, PLK5 in GBMLGG, LGG, and NB. Elucidating the specific mechanism of this phenomenon requires further investigation.

The TME is widely thought to play a critical role in carcinogenesis and tumor progression, involving a range of immunological and metabolic components [37]. Therefore, targeting TME is a promising therapeutic approach [38]. However, there are few studies on the relationship between PLK family genes and the TME or immune cell infiltration/immune subtypes. In our analysis, the expression of PLK family genes was significantly correlated with six immune subtypes (C1–C6) reported previously in pan-cancers [39]. PLK1, PLK2, and PLK3 tended to be highly expressed in subtypes C1 (Wound Healing), C2 (IFN-γ dominant), and C6 (TGFβ dominant), respectively, indicating their enrichment and cancer-promoting roles. Our study also presented the TME scores of PLK genes across pan-cancers, where PLK2 and PLK3 expression was significantly positively associated with stromal and immune scores, whereas the expression of PLK1, PLK4, and PLK5 was negatively associated with stromal and immune scores in most cancer types. This highlights the differences in their abilities to regulate the TME, and the associations needs further clarification. In addition, the expression of PLK genes correlated positively or negatively with RNAss and DNAss in pan-cancers, based on stemness analyses. These results suggest that PLK genes can be targeted to reprogram TME and inhibit tumorigenesis, progression, and metastasis by affecting stemness. Recent research suggests that PLK1 inhibition could upregulate PD-L1 by activating the NF-κB pathway; thus, targeting PLK1 could sensitize pancreatic cancer to immune checkpoint therapy [13]. Another study also revealed that PLK1 inhibition and immunotherapy combination may achieve a synergistic antitumor efficacy [40]. Inhibition of PLK1 selectively kills cancer cells and upregulates PD-L1 expression in surviving cancer cells thereby providing opportunity for combined immunotherapy in a feedforward manner [41]. Meanwhile, our results also showed that PLKs may be the biomarkers for ICB responses. Therefore, the combination strategy of PLK targeting and immunotherapy may be an appealing therapy for cancer treatment.

The PLK family has been widely observed in cancer-related pathways, particularly the cell cycle-related pathway, consistent with previous studies [42]. Notably, high expression of PLK1 and PLK4 was significantly correlated with 72 % and 56 % cell cycle activation in cancers, respectively. The PLK4 inhibitor CFI-400945 suppresses liver cancer through cell cycle perturbation and elicits antitumor immunity [43]. In addition, functional enrichment analysis revealed that PLK-related genes were mostly associated with cell cycle-related biological processes and kinase regulatory activity. RPL27, through PLK1 signaling, contributes to colorectal cancer proliferation and stemness. RPL27 silencing decreases the levels of PLK1 protein and G2/M-associated regulators, such as phosphorylated cell division cycle 25C, CDK1, and cyclin B1 [44]. Drug sensitivity analysis implied that PLK family genes may be involved in chemotherapy resistance and that targeting PLK family genes may overcome drug resistance. In addition, CCNE1 and PLK1 mediate palbociclib resistance in $HR^{+}/HER2^{-}$ metastatic breast cancer. Patients with high PLK1 mRNA levels respond poorly to palbociclib, indicating that PLK1 may also play a critical role in resistance to CDK4/6i [45].

Prognostic analysis in pan-cancers implied that the PLK1 expression level was most significantly associated with the survival of patients with KIRP. Based on different databases, we further confirmed the higher expression of PLK1 in KIRP tissues than in corresponding and non-corresponding normal tissues. We also confirmed that a high expression level of PLK1 was correlated with some clinicopathological features and could effectively predict the survival of patients with strong predictive power, serving as an independent prognostic factor for KIRP patients. These results indicated that PLK1 expression may serve as a potential cancer biomarker for the diagnosis and prognosis of patients with KIRP.

Currently, no evidence exhibits that which one kind of PLK family gene is more important for cancer therapy. Most of them are being evaluated in pre-clinical studies. The PLK1 has been widely studied in cancer research, which mainly serves as an oncogene [46]. Other PLK family genes, including PLK2-5, have not get enough attention, because of the strong ontogenetic effect of PLK1 [15,47–49]. Many targeted drugs of PLK family are mainly based on PLK1 targeting strategy [50]. To date, a minimum of 10 PLK1 inhibitors have been introduced into clinical trials, with the kinase domain inhibitor, Volasertib, being awarded "breakthrough therapy designation" by the US Food and Drug Administration in 2013 [51]. Other PLKs inhibitors are main evaluated in pre-clinical studies, such as PLK4 inhibitor CFI-400945 [43,52]. So Further studies of PLK family should focus more on the role of PLK2-4 in cancers and unveil whether a potential link among PLK genes.

Although we have analyzed and consolidated pan-cancer analysis from public databases, our research still had some limitations. First, bioinformatics analysis only provided us a preliminary finding of the biological role of PLK family in various malignant biological behaviors and drug resistance, so experiments in vivo and in vitro are still needed to verify these results and promote further clinical application. Mechanism studies will help us determine the exact molecular function of PLK family in tumor biology, especially PLK1 in KIRP. Second, our findings lack direct evidence that PLK family are involved in TME and the mechanisms by which PLK family are involved in affecting cancer immunity remain unknown. Third, the biological role of PLK family in various cancers is heterogeneous, so the mechanism of heterogeneity still should be further explored, which can accelerate the development of PLK-based drugs for cancer personalized treatment.

5. Conclusion

In brief, our study depicted a mutant landscape of PLK family genes across pan-cancers and uncovered a significant association between the expression of PLK family genes and patient survival, indicating their prognostic value in pan-cancers, especially in KIRP. PLK family genes are associated with immune subtype, TME, and stemness score. Notably, enrichment analyses revealed that the PLK family and related genes were closely related to cell cycle-related pathways, and drug sensitivity analysis implied that PLK family genes might be involved in chemoresistance. Furthermore, the integrative analysis identified PLK1 as a novel oncogene in KIRP, which

requires further in vitro and in vivo verification. These findings may provide deep insights for further research on PLK family genes as potential targets in pan-cancers, thereby promoting their translation from the bench to the bedside.

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Data availability

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article. Further inquiry can contact with corresponding authors.

Ethics approval and inform consent

Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Guo Zhao: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation. **Yuning Wang:** Writing – review & editing, Writing – original draft. **Jiawei Zhou:** Writing – review & editing. **Peiwen Ma:** Writing – review & editing. **Shuhang Wang:** Writing – review & editing, Investigation, Conceptualization. **Ning Li:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29373.

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