

Original Article

AMP-dependent protein kinase alpha 1 predicts cancer prognosis and immunotherapy response: from pan-cancer analysis to experimental validation

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Abstract: Pancreatic cancer (PC) has poor prognosis. PRKAA1 (AMPK- α 1) is the catalytic subunit of 5'-adenylate-activated protein kinase (AMPK), which plays a critical role in multiple stages of tumorigenesis and development. However, the biological mechanisms of PRKAA1 in the tumor microenvironment have not been well studied. In this study, we performed a combined analysis of data from TCGA and GTEx databases to determine whether PRKAA1 is differentially expressed in a variety of tumors. Kaplan-Meier curve and Cox regression analyses indicated that the differential expression of PRKAA1 affected overall survival in a variety of tumors and was an independent prognostic factor for Brain Lower Grade Glioma (LGG), Brain Lower Grade Glioma (LAML), Liver hepatocellular carcinoma (LIHC), Pancreatic adenocarcinoma (PAAD), and Pancreatic adenocarcinoma (KICH). PRKAA1 was closely associated with various immune profiles, suggesting that PRKAA1 can be used for direct immunotherapy. We investigated the role of PRKAA1 in PC cells. We found that the downregulation of PRKAA1 expression reduced the proliferation, migration, and invasion of PC cells. In addition, we found that PRKAA1 regulated PC progression, possibly through the PI3K/AKT signaling pathway. Treatment of cells with the AKT inhibitors MK2206 and GSK2110183 revealed that the PRKAA1 overexpression group was less sensitive to AKT inhibitors than the negative control group. Taken together, PRKAA1 can be used as a potential prognostic marker and new target for tumor immunotherapy.

Keywords: PRKAA1, pancreatic cancer, pan-cancer, prognosis

Introduction

Pancreatic cancer (PC) is a highly aggressive gastrointestinal cancer with poor prognosis. It is the fourth leading cause of cancer-related death in the United States. Based on the 2022 cancer statistics published by CA (A Cancer Journal for Clinicians), up to 49,830 deaths were projected in the US for 2022 out of 62,210 new cases of PC [1]. According to a study conducted in 28 European countries [2], PC is expected to surpass breast cancer as the third leading cause of cancer-related death by 2025. Most patients with PC are 65-74 years of age (average: 70 years) at the time of diagnosis [3]. Imaging tests, such as ultrasound, computed tomography, and magnetic resonance imaging, are not ideal for the early diagnosis of PC, and the lack of molecular markers to effectively screen and confirm PC is one of the main reasons for the difficulty in early diagnosis [4].

PRKAA1 encodes the catalytic subunit of 5'-adenosine monophosphate (AMP)-activated protein kinase (AMPK) [1]. AMPK is a heterotrimer consisting of a catalytic subunit (α) and two regulatory subunits (β and γ), with phosphorylation of T172 in the α catalytic subunit being a key requirement for the activation of AMPK [2]. AMPK activity is stimulated by increasing the intracellular AMP/adenosine-triphosphate (ATP) ratio or by upstream kinases, such as LKB1/CAMKK β [3]. Currently, there is much debate regarding the role of PRKAA1 in tumors. The specific role of PRKAA1 in tumors is related to their environment. The proliferation and growth of tumor cells are restricted when PRKAA1 is highly expressed in the presence of adequate nutrients. For example, Wu et al. [4] found that metformin inhibited bladder cancer cell proliferation by upregulating phosphorylated AMPK- α to induce cellular G1 cycle arrest, which in turn affected cell proliferation through the P53 axis.

However, when cells are nutritionally restricted, high PRKAA1 expression protects them from death and promotes cellular adaptation to a low-energy status [5]. For example, Jeon et al. [6] showed that under energy stress conditions, where intracellular ATP is reduced to below a certain level, AMPK activation and increased PRKAA1 expression promote the proliferation of lung cancer cells. Thus, PRKAA1 plays a far more complex role in tumors than initially believed.

Ye et al. [7] suggested that PRKAA1 has an inhibitory effect on tumors, while Zhao et al. [8] discovered that PRKAA1 promotes the progression of PC. Consequently, we investigated the role of PRKAA1 in PC. In this study, we comprehensively analyzed the pan-cancer expression of PRKAA1, its prognosis, and its relationship with immunity. We subsequently demonstrated that PRKAA1 was highly expressed in PC and correlated with a poor prognosis. PRKAA1 knockdown may inhibit the malignant behavior of PC cells by downregulating the PI3K/AKT pathway. Thus, PRKAA1 is a potential therapeutic target for PC.

Materials and methods

Acquisition of PRKAA1 expression and prognostic data in pan-cancer

To predict the expression and prognosis of PRKAA1, we downloaded the unified and standardized pan-cancer dataset from the UCSC (<https://xenabrowser.net/>) database, extracted the expression data of PRKAA1 gene in each sample, and obtained the expression data of 33 cancer types and the clinical information of the corresponding samples.

Relationship between PRKAA1 gene expression and immunization, tumor mutational load, microsatellite instability

After extracting PRKAA1 expression data, we calculated the stromal, immune, and ESTIMATE scores for each patient's tumor using gene expression data and the R package ESTIMATE (version 1.0.13). Subsequently, we calculated the B cell, T cell CD4, T cell CD8, neutrophil, macrophage, and dendritic cell (DC) infiltration scores for each patient across the pan-cancer using gene expression data and the R package IOBR (version 0.99.9). The Timer method was

then employed to reassess these infiltration scores based on gene expression. We extracted the expression data of marker genes for the two types of immune checkpoint pathways (Inhibitory, Stimulatory) in each sample and calculated the Pearson's correlation between PRKAA1 and the marker genes of these pathways. Spearman's correlation was used to analyze the correlation between PRKAA1, tumor mutational load (TMB), and microsatellite instability (MSI).

Tissue samples and immunohistochemistry

Eighty patients were selected based on inclusion and exclusion criteria. Postoperatively, PC (n=80) and healthy paracancerous tissues (n=20) were collected and stored in paraffin blocks. This study was approved by the Ethics Committee of the Second Hospital of Lanzhou University, and written informed consent was obtained from all patients.

Inclusion: (1) Radical surgery in the Second Hospital of Lanzhou University. (2) Pathological diagnosis of PC.

Exclusion: (1) Pre-operative chemotherapy or immunotherapy. (2) Patients with positive surgical margins. (3) Severe underlying systemic disease.

The clinicopathological characteristics of the patients are presented in **Table 1**. Paraffin-embedded tissue sections were baked at 65°C for 30 min, dewaxed in xylene, hydrated with graded ethanol, and then macerated in phosphate-buffered saline (PBS) or pure water for several minutes. The sections were boiled in citrate buffer for 20 min for antigen repair before incubation with 10% bovine serum albumin. Samples were incubated with rabbit anti-PRKAA1 primary antibody (Shanghai GeneChem Co., Shanghai, China) overnight. After washing, the sections were incubated with goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, US) for 1 h at room temperature. Tissue sections were stained with 3,3'-diaminobenzidine, counterstained with hematoxylin, dehydrated, and sealed. The sections were examined and scored by two pathologists according to the proportion of positive cells and staining intensity. The proportion of positively stained cells was scored according to the following criteria: 0

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Table 1. Relationships between PRKAA1 expression and the clinicopathological characteristics of patients with PC

Characteristic	Levels	Low expression of PRKAA1	High expression of PRKAA1	P value
Age, n (%)	≥60	24 (32%)	12 (16%)	0.001
	<60	11 (14.67%)	28 (37.33%)	
Sex, n (%)	Male	23 (30.67%)	27 (36%)	0.870
	Female	12 (16%)	13 (17.33%)	
Tumor size (cm), n (%)	≤4	14 (18.67%)	18 (24%)	0.662
	>4	21 (28%)	22 (29.33%)	
Vascular invasion status, n (%)	Yes	15 (20%)	16 (21.33%)	0.802
	No	20 (26.67%)	24 (32%)	
Neural tract invasion status, n (%)	Yes	23 (30.67%)	25 (33.33%)	0.772
	No	12 (16%)	15 (20%)	
Differentiation status, n (%)	Highly differentiated	5 (6.67%)	7 (9.33%)	0.164
	Moderately differentiated	26 (34.67%)	22 (29.33%)	
	Poorly differentiated	4 (5.33%)	11 (14.67%)	
CA19-9 (U/ml), median (IQR)		25.0 (12.8, 57.42)	245.60 (101.01, 745.95)	0.417
AFP (ng/ml), median (IQR)		2.90 (2.01, 4.94)	2.18 (1.73, 3.32)	0.413
T stage, n (%)	T1+T2	15 (20%)	2 (2.67%)	<0.000
	T3+T4	20 (26.67%)	38 (50.67%)	
N stage, n (%)	N0	22 (29.33%)	23 (30.67%)	0.283
	N1	11 (14.67%)	10 (13.33%)	
	N2	2 (2.67%)	7 (9.33%)	
M stage, n (%)	M0	26 (34.67%)	16 (21.33%)	0.003
	M1	9 (12%)	24 (37.33%)	

(<10% positive cells), 1 (10%-25% positive cells), 2 (26%-50% positive cells), 3 (51%-75% positive cells), and 4 (>75% positive cells). Staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The final staining score was calculated as the staining intensity score × scale score, ranging from 0 to 12. A score of ≤4 was classified as low expression (-), and a score >4 was classified as high expression (+).

Cell culture

Human pancreatic ductal epithelial cells hTERT-HPNE, PC cell lines CFPAC-1, and Mia-PaCa-2 were obtained from the Shanghai Cell Culture Bank of the Chinese Academy of Sciences. These cells were identified through short tandem repeat DNA analysis at Shanghai GeneChem Ltd. (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, Carlsbad, CA, US) and 1% penicillin/streptomycin (Beyotime, Shanghai, China). All cells tested in this study were used within 6 months of thawing and stored at 37°C in 5% carbon diox-

ide. Mycoplasma testing was routinely performed.

Cell transfection

Stably transfected cell lines were constructed using short hairpin RNA (shRNA) sequences targeting *PRKAA1* and a negative control shRNA (5'GGGTTCTACCTGCAGCTGAA3', 5'GCGTGTCACCCAGAATGTAG3' and 5'TTCTCCGAACGTGTCACGT3', respectively). The shRNA sequences and *PRKAA1* constructs were subcloned into a lentiviral expression vector containing green fluorescent protein (Shanghai Genechem, Ltd.). Lentiviral transduction was performed according to manufacturer's instructions. The cells were screened with 2 µg/mL puromycin for 7 days, and stable strains were selected for subsequent experiments. Western blotting was performed to measure the protein expression of *PRKAA1*.

Quantitative real-time polymerase chain reaction

Total RNA was extracted utilizing TRIzol reagent (Thermo Fisher Scientific, Shanghai, China)

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according to the manufacturer's protocol. A reverse transcription kit (Agbio, Hunan, China) was used to stably transcribe 1000 ng of RNA into complementary DNA. Quantitative real-time polymerase chain reaction was performed using a SYBR Premix Ex Taq kit (Takara Biomedical Technology, Beijing, China). The Rotor-Gene Q system (QIAGEN, Venlo, Netherlands) was used to measure the expression of *PRKAA1* at the transcriptional level. The specific primers were as follows: *PRKAA1* forward primer: 5'TTGAAACCTGAAAATGTCCTGCT3'; *PRKAA1* reverse primer: 3'GGTGAGCCACAACCTGTTCT-T5'; *GAPDH* forward primer: 5'GAAGGCTGGG-GCTCATTT3'; *GAPDH* reverse primer: 3'CAGGA-GGCATTGCTGATGAT5'. The assay was repeated three times for each sample, and the data were analyzed through relative quantification and normalization to *GAPDH* expression.

Western blot

Western blotting was performed according to conventional methods. The anti-GAPDH antibody (T40004) and goat anti-rabbit immunoglobulin G (TS0001) were purchased from Abmart (Shanghai, China). Antibodies including anti-Akt (4691T), anti-phosphorylated Akt (4060T), anti-PI3K P85 (4257T), and anti-phosphorylated PI3K p85 (Tyr458) (4228T) were purchased from Cell Signaling Technology (Beverly, MA, US). Anti-PRKAA1 antibody (ab-32047) was procured from Abcam (Cambridge, MA, US).

Cell Counting Kit (CCK)-8 cell proliferation assay

The experiments were performed using a Cytotoxicity Assay Kit (Beyotime Biotechnology). Cells grown in the logarithmic phase were prepared in a cell suspension at a density of 1.25×10^4 cells/mL, and 100 μ L/well cell suspension was seeded into 96-well plates. After the cells adhered, 10 μ L of Cell Counting Kit (CCK)-8 reagent was added, and the cells were incubated for another 2 h. The optical density (OD) at 450 nm was then measured using an enzyme marker, and this OD value was recorded as the 0-h data. Thereafter, the OD at 450 nm was measured at 24, 48, and 72 h. The growth curves of the cells were plotted using GraphPad software.

Wound healing assay

Cells were seeded in 6-well plates at a density of 5×10^5 cells/well and cultured overnight. Scratches were created using a 200- μ L gun tip. Serum-free medium was used throughout the incubation period. Images were acquired at various time points using a microscope at 10 \times magnification. Gap distances were assessed using ImageJ software (V1.8.0).

Transwell cell invasion assay

Cell invasion experiments were performed in 24-well Transwell chambers (Corning, NY, US). First, diluted Corning Matrigel was quickly spread into the upper chamber of the Transwell and then placed in a 37°C incubator, where it solidified dynamically. The cell density was adjusted to $1-10 \times 10^5$ /ml and inoculated in the chambers. The next day, the gel and cells in the upper chamber were gently wiped off with a cotton swab, fixed in 4% paraformaldehyde for 10 min, washed briefly with PBS, and incubated for 24 h at 37°C. Crystalline violet (0.1%) was added for 15 min, and the small chamber was washed three times with PBS. Images were captured using an inverted microscope.

Data analysis

Three independent experiments were performed for each analysis. Statistical analysis and visualization were performed employing R software (V4.2.2). Mean values were compared using two-sided t-tests. Count data were compared utilizing Fisher's exact probability method or the χ^2 test, as appropriate. Survival curves were plotted using the Kaplan-Meier method and log-rank test. One-way Cox proportional hazards regression analysis was conducted to assess the hazard ratio (HR) and 95% confidence interval (CI) of each influencing factor for overall survival (OS). Statistical significance was set at $P < 0.05$, and all statistical tests were two-sided.

Results

Expression of PRKAA1 in pan-cancer

By integrating and mining data from TCGA and GTEx databases and performing significance of difference analysis, we observed significant upregulation in 11 tumors, including GBM

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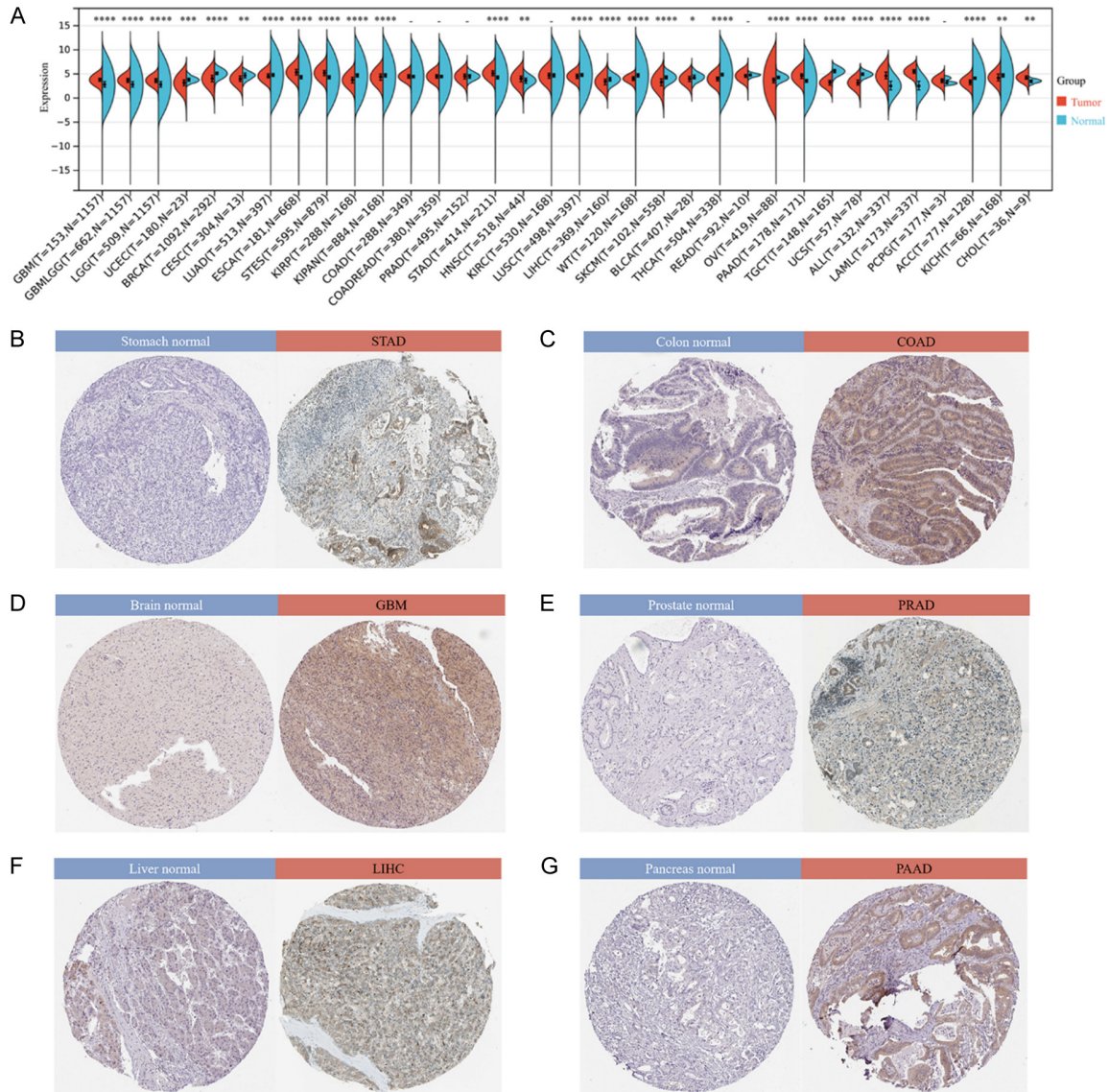


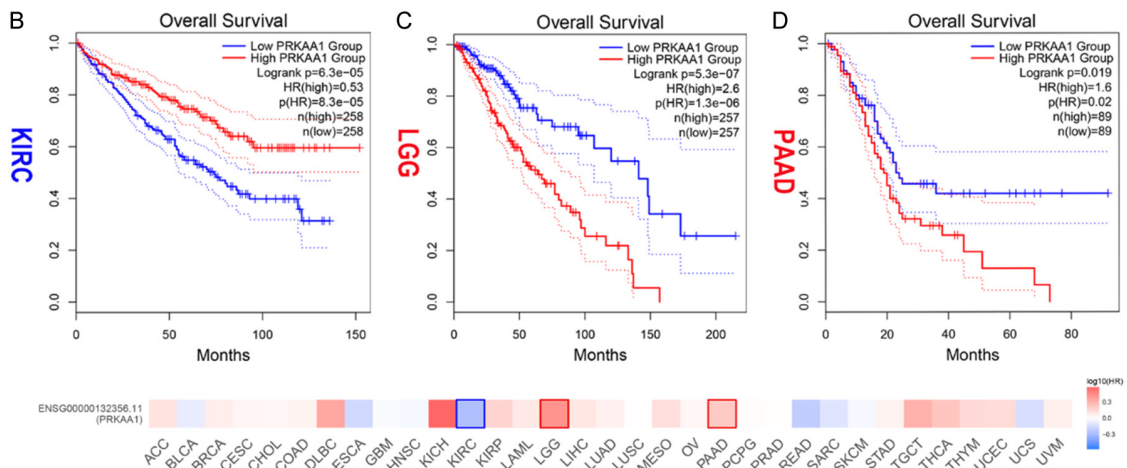
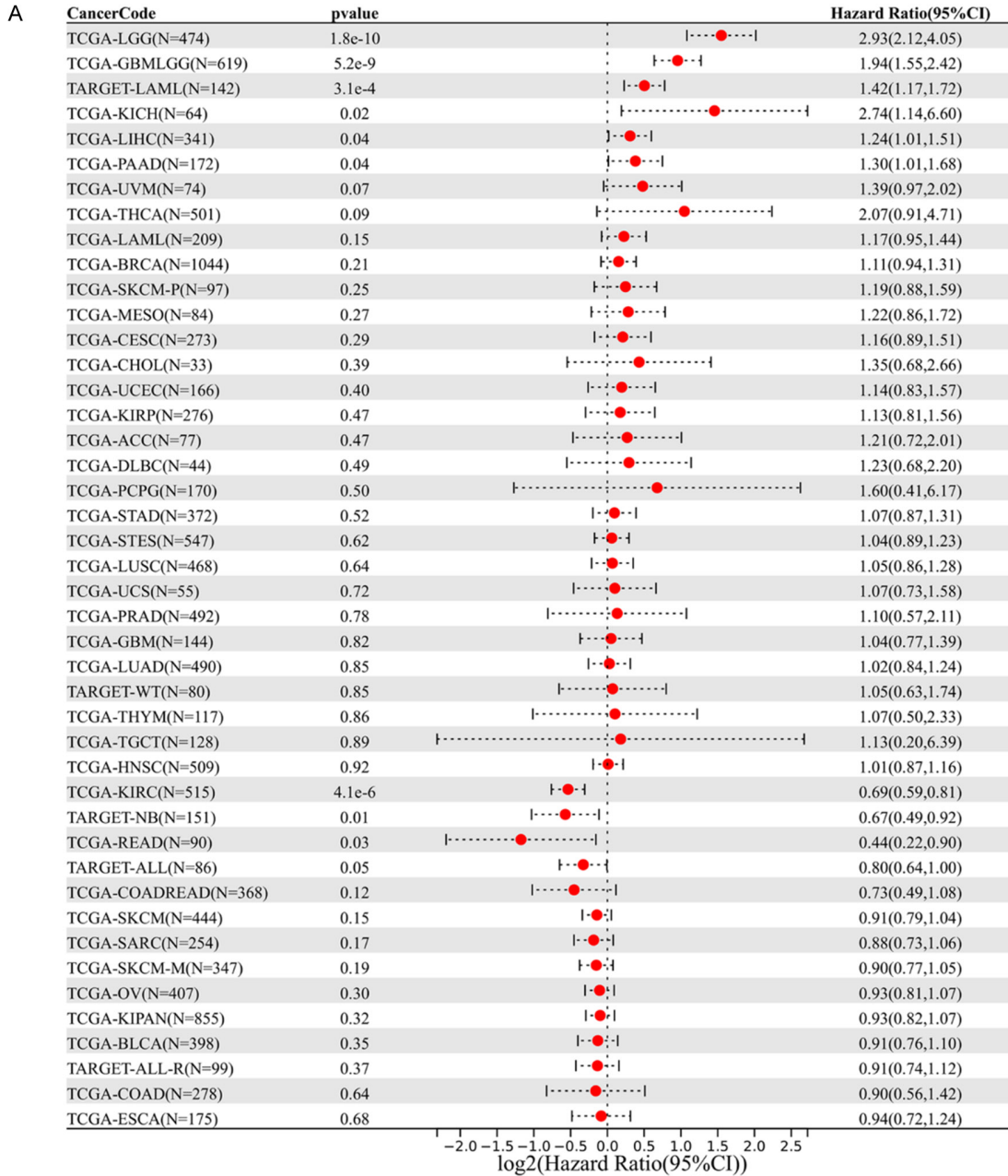
Figure 1. Expression of PRKAA1 in pan-cancer. A. Matched analysis comparing PRKAA1 expression differential in TCGA database and GTEx database. Ns, $P \geq 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; B-G. PRKAA1 expression in STAD, COAD, GBM, PRAD, LIHC and PAAD from the HPA database.

($P=6.8e-36$), ESCA ($P=9.5e-47$), STAD ($P=2.1e-40$), HNSC ($P=1.3e-3$), PAAD ($P=3.9e-32$), and CHOL ($P=1.0e-3$). Significant downregulation was observed in 17 tumors such as UCEC ($P=1.4e-4$), BRCA ($P=2.6e-66$), CESC ($P=6.8e-3$), LUAD ($P=1.2e-7$), KIRP ($P=1.0e-24$), LIHC ($P=2.2e-11$), THCA ($P=1.7e-80$), OV ($P=2.0e-14$), ACC ($P=1.4e-12$), and KICH ($P=3.6e-3$) (Figure 1A). In addition, we determined the expression of PRKAA1 in tissues using the HPA database. The results showed that PRKAA1 was highly expressed in STAD, COAD, GBM, PRAD, LIHC, and PAAD (Figure 1B-G).

The prognostic value of PRKAA1 in pan-cancer

We used the survival package of R software to analyze the relationship between PRKAA1 gene expression and the prognosis of each tumor, and prognostic significance was obtained with the log-rank test for statistical testing. High PRKAA1 expression was a risk factor for OS in patients with six tumor types: GBMLGG, LGG, LAML, LIHC, PAAD, and KICH. Low PRKAA1 expression suggested a lower OS in patients with KIRC, TARGET-NB, READ, and TARGET-ALL (Figure 2A). Kaplan-Meier survival curves were

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Figure 2. The prognostic value of PRKAA1 in pan-cancer. A. Univariate Cox regression analysis of PRKAA1 with OS; B-D. Kaplan-Meier survival analysis for OS in KIPAC, LGG and PAAD. $P < 0.05$ was considered statistically significant (Red for high PRKAA1 expression and blue for low PRKAA1 expression).

plotted, which further confirmed that patients with low PRKAA1 expression had worse prognosis in KIRC, and patients with high PRKAA1 expression had worse prognosis in LGG and PAAD (**Figure 2B-D**).

Immunological relevance of PRKAA1 in pan-cancer

The tumor microenvironment plays a crucial role in tumor diagnosis, treatment, and prognosis. To explore the pan-cancer role of PRKAA1 in the tumor microenvironment, we comprehensively analyzed the relationship between PRKAA1 and immune infiltration scores using the *psych* package of R software, and the results are presented in the form of three scores (StromalScore, ImmuneScore, and ESTIMATEScore). We found that PRKAA1 expression was significantly correlated with immune infiltration in six cancer types, with four significant positive correlations (GBMLGG, KIPAN, COAD, and COADREAD) and two significant negative correlations (CESC and BLCA) (**Figure 3A-F**), suggesting that the PRKAA1 gene plays a role in regulating the tumor immune microenvironment in these tumors. We analyzed the relationship between PRKAA1 and immune cells and showed that PRKAA1 interacted with immune cells (B cell, T cell CD4, T cell CD8, neutrophil, macrophages, and DC) in most tumors (**Figure 3G**). In addition, we found that PRKAA1 was significantly and positively associated with immune checkpoint genes (e.g., EDNRB, VEGFA, HMGB1, and TNFSF4) in many cancers (**Figure 3H**).

Relationship between PRKAA1 expression and TMB/MSI in pan-cancer

The TMB, which is the total number of mutations present in a tumor sample, was assessed via whole exome sequencing. TMB levels correlate with PD1/PD-L1 antibody efficacy, and tumor cells with higher TMB levels are more likely to be recognized by the immune system; hence, the probability that immunotherapy will be effective in these patients is higher [9, 10]. MSI is a phenomenon in which the length of a microsatellite sequence is altered by insertion or deletion mutations during DNA replication.

Some clinical studies have confirmed that patients with MSI-H colorectal cancer have a significant survival advantage, with poorer clinical performance but better prognosis [11, 12]. Correlation analysis showed that PRKAA1 expression positively correlated with TMB in LAML, LGG, PAAD, SARC, STAD, and UCEC tumors, and PRKAA1 expression positively correlated with TMB in LIHC (**Figure 4A**). Regarding the correlation analysis between PRKAA1 and MSI, the results showed that PRKAA1 was positively correlated with MSI in ACC, UCEC, READ, MESO, and LGG and negatively correlated with MSI in PRAD, PAAD, and DLBC (**Figure 4B**).

PRKAA1 expression is significantly increased in PC

We first examined the expression of PRKAA1 in PC and healthy pancreatic tissues in paraffin-embedded tissue sections. Immunohistochemical staining showed increased PRKAA1 expression in PC tissues. **Figure 5A** shows representative images of PRKAA1 staining in tumors and healthy tissues at different magnifications. We also found that PRKAA1 protein expression was significantly higher in PC cell lines than in healthy pancreatic cells (hTERT-HPNE) (**Figure 5B, 5C**). Quantitative real-time polymerase chain reaction revealed similar results (**Figure 5D**).

High PRKAA1 expression in PC correlates with age, metastasis, and poor survival

To investigate the relationship between PRKAA1 expression and clinical characteristics, the clinical data of 75 patients (five patients lost to follow-up) with PC were analyzed (**Table 1**). Patients were divided into two groups based on the median level of PRKAA1 expression (high vs. low) according to immunohistochemical staining. Pearson's χ^2 test was used to analyze the correlations between PRKAA1 expression and the clinicopathological variables. PRKAA1 expression significantly correlated with age ($P=0.001$), M stage ($P=0.003$), and T stage ($P<0.000$). No significant correlation was observed between PRKAA1 expression and other clinical features, including histological grade, sex, tumor size, serum alpha-fetoprotein

PRKAA1 as a potential biomarker for pancreatic cancer

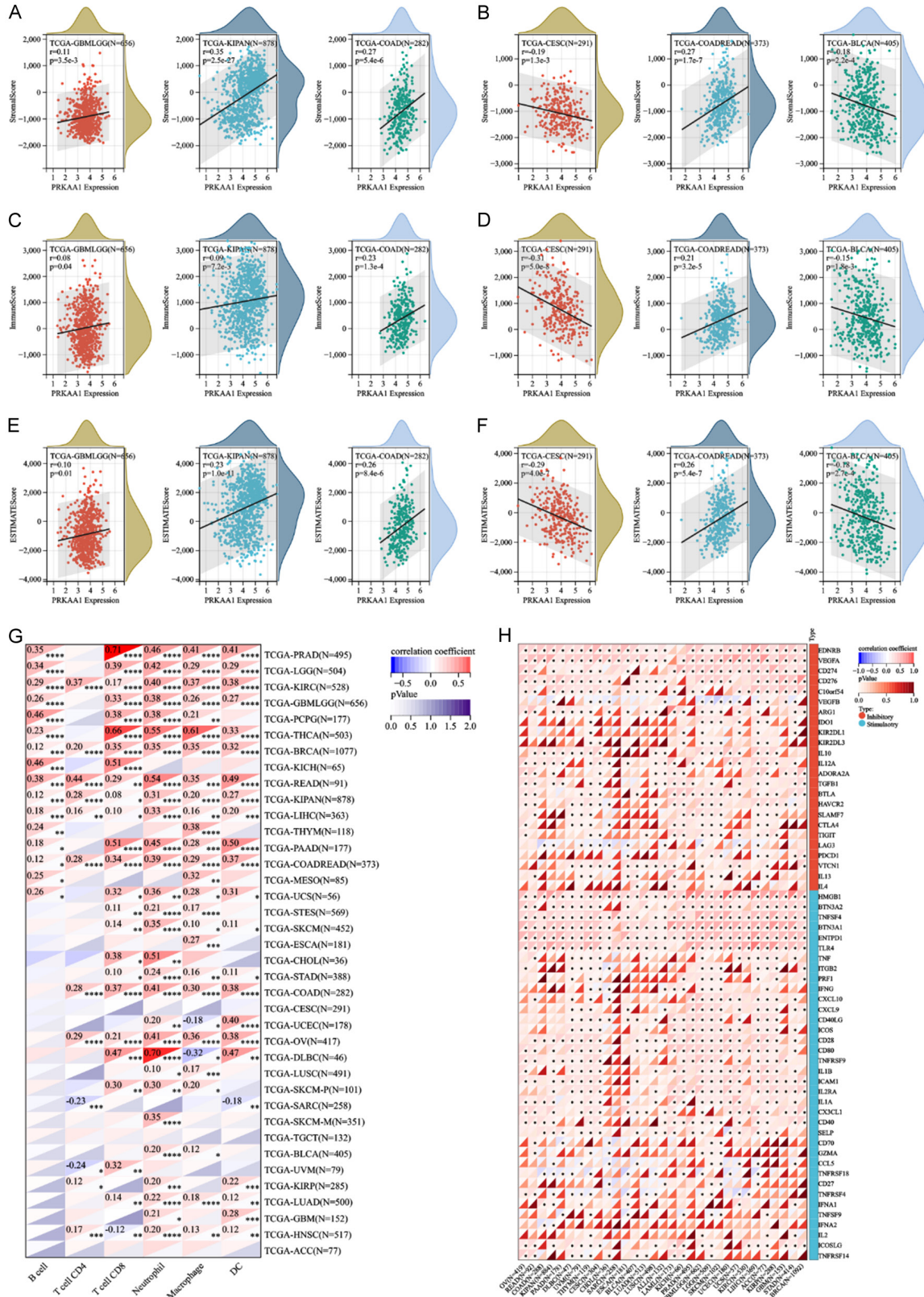


Figure 3. The correlation between PRKAA1 expression and immune infiltration and immune checkpoints. A, B. Correlation of PRKAA1 expression with StromaScore; C, D. Correlation of PRKAA1 expression with ImmuneScore; E, F. Correlation of PRKAA1 expression with EstimateScore; G. Relationship between PRKAA1 and immune cells in pan-cancer; H. Relationship between PRKAA1 and immune checkpoint genes in pan-cancer.

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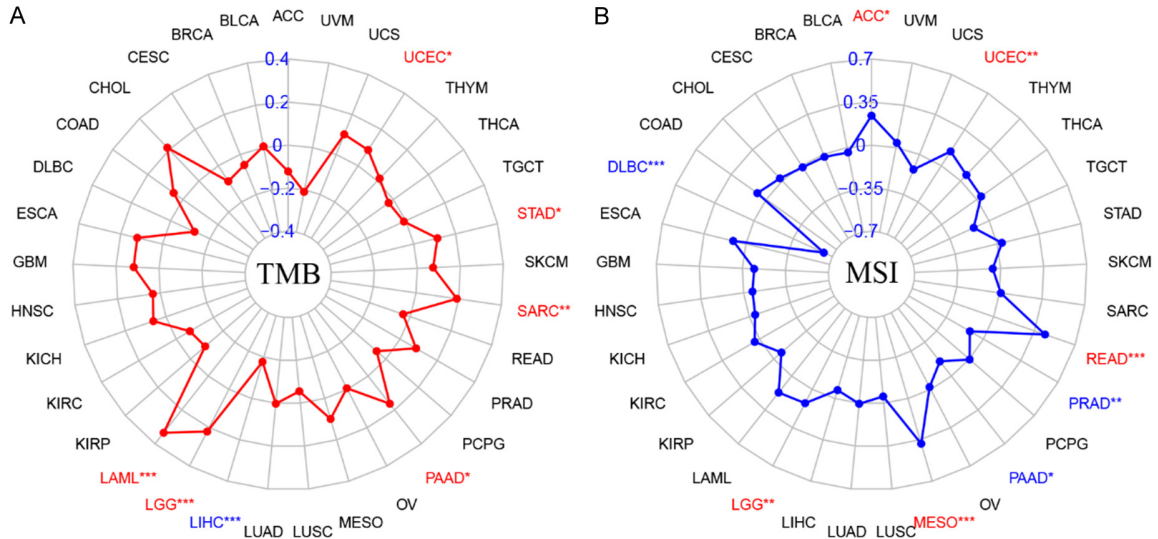


Figure 4. A. Relationship between TMB and PRKAA1 expression in pan-cancer; B. Relationship between MSI and PRKAA1 expression in pan-cancer.

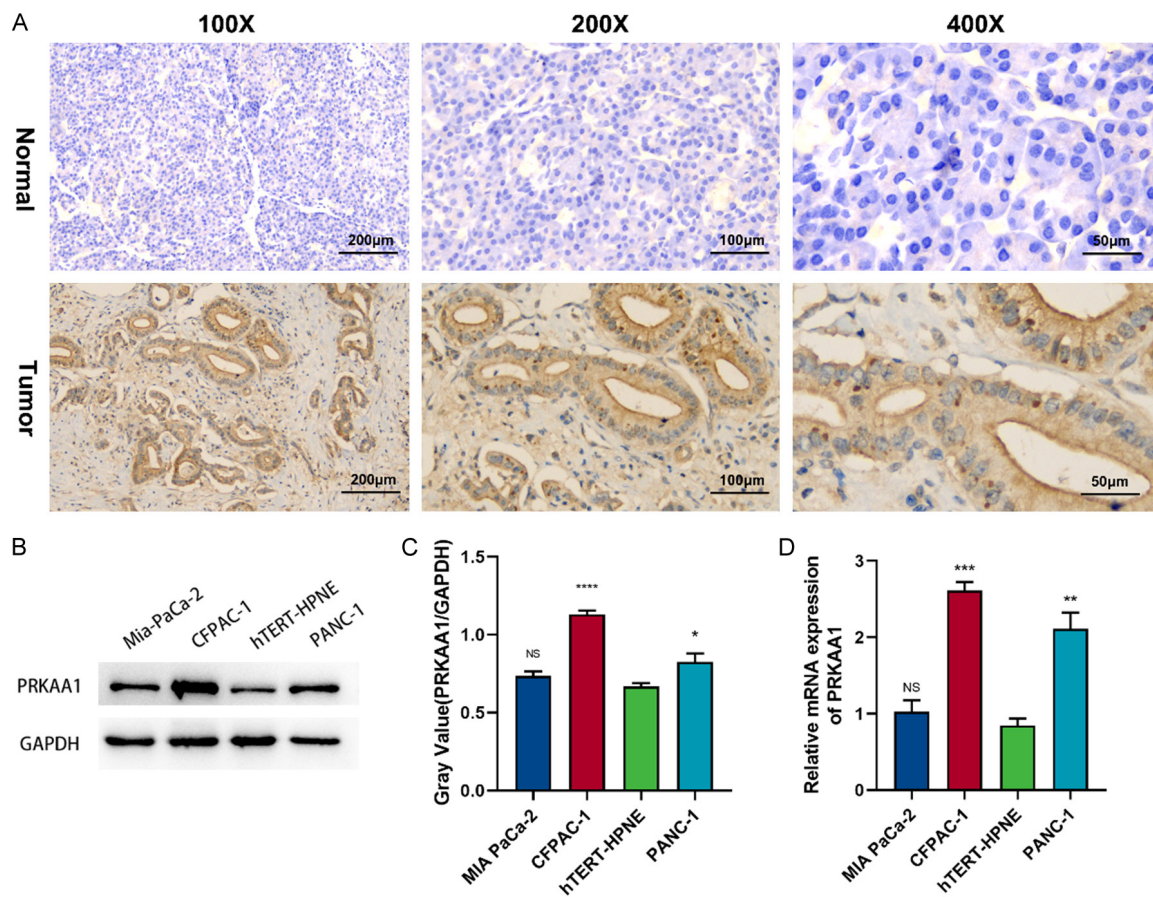


Figure 5. (A) Expression of PRKAA1 in pancreatic cancer and normal tissues detected by immunohistochemical staining; PRKAA1 expression in PC cells was determined by Weston blotting (B, C) and qRT-PCR (D).

(AFP) concentration, serum cancer antigen (CA)19-9 levels, vascular invasion, nerve bun-

dle invasion, or N stage. Therefore, high PRKAA1 expression may be a potential risk factor

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Table 2. Univariate and multivariate Cox proportional-hazards regression analyses for patients with PC

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.284 (0.787-2.095)	0.316		
Sex	1.385 (0.812-2.363)	0.232		
Tumor size (cm)	1.364 (0.829-2.244)	0.222		
Diferentiation status	1.042 (0.705-1.538)	0.837		
Vascular invasion status	1.018 (0.621-1.670)	0.943		
Neural tract invasion status	1.160 (0.699-1.925)	0.565		
CA199 (U/ml)	1.001 (1.000-1.002)	0.014	1.000 (0.999-1.001)	0.847
AFP (ng/ml)	0.256 (0.760-1.076)	0.256		
T stage	1.959 (1.241-3.094)	0.004	0.982 (0.584-1.651)	0.944
N stage	1.307 (0.911-1.876)	0.146		
M stage	4.498 (2.548-7.939)	0.001	4.453 (2.244-8.837)	0.001
PRKAA1	1.991 (1.206-3.288)	0.007	1.879 (1.067-3.310)	0.029

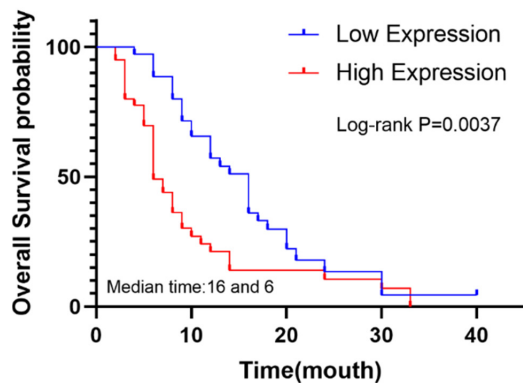


Figure 6. The overall survival curve of 75 pancreatic cancer patients with high and low expression of PRKAA1 was analyzed.

for PC development. However, its predictive accuracy for prognosis needs to be further investigated.

High PRKAA1 expression is an independent risk factor for poor prognosis in PC patients

To examine whether PRKAA1 expression was an independent prognostic factor for PC, we performed univariate and multivariate Cox proportional hazards regression analyses using data on clinicopathological features and PRKAA1 expression levels in 75 patients with PC (**Table 2**). The univariate analysis revealed that serum CA19-9 levels (HR=1.001 [95% CI 1.000-1.002]; P=0.014), M-stage (HR=4.498 [95% CI 2.548-7.939]; P=0.001), T-stage (HR=1.959 [95% CI 1.241-3.094]; P=0.004), and PRKAA1 expression (HR=1.991 [95% CI 1.206-

3.288]; P=0.007) were associated with shorter survival. In contrast, age, sex, tumor size, degree of differentiation, vascular invasion, nerve bundle invasion, serum AFP concentration, and N stage were not significantly associated with PRKAA1 expression.

We then included the variables with P<0.1 from the univariate analysis - serum CA19-9 concentration, M-stage, T-stage, and PRKAA1 expression - in the multivariate Cox proportional-hazards regression analysis. The results showed that M stage (HR=4.453 [95% CI 2.244-8.837]; P=0.001) and PRKAA1 expression (HR=1.879 [95% CI 1.067-3.310]; P=0.029) were independent prognostic predictors of PC.

We followed up the survival data of the patients, and a combined analysis of PRKAA1 expression in conjunction with immunohistochemistry revealed that high PRKAA1 expression was associated with poorer OS (HR=2.667; 95% confidence interval 1.637-4.343; P=0.037) (**Figure 6**).

Inhibition of PRKAA1 expression suppresses PC cell proliferation, migration and invasion

The observation that PRKAA1 was upregulated in PC was immediately followed by investigation of its biological role in PC progression. We used a lentivirus encoding PRKAA1 to construct PRKAA1-silenced cell lines (CFPAC-1 and PANC-1) that were stably transfected with PRKAA1. PRKAA1 mRNA and protein expression were knocked down in both cell lines (**Figure 7A, 7B**).

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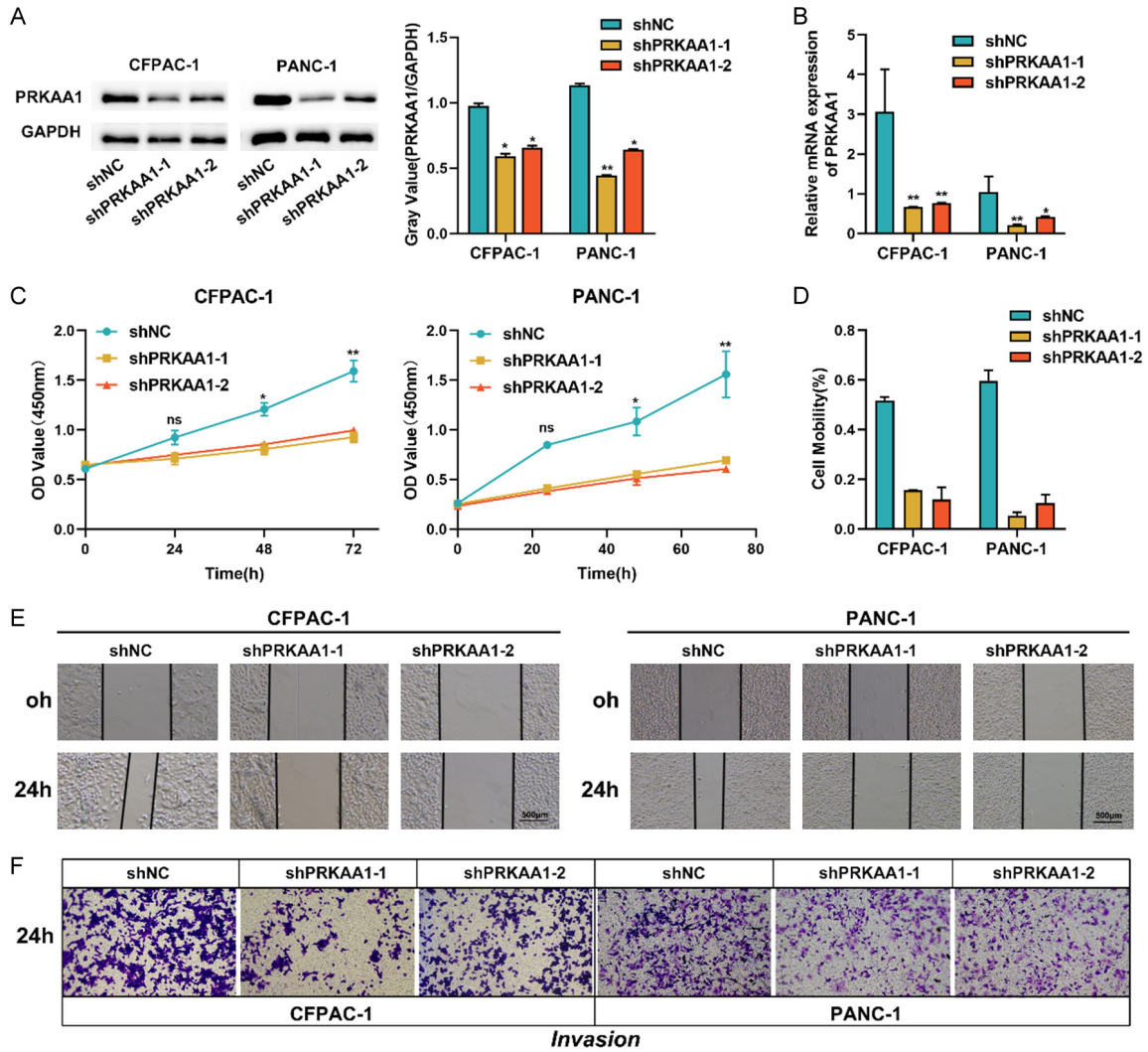


Figure 7. The knockdown efficiency of PRKAA1 in PC cells was determined by Weston blotting (A) and qRT-PCR (B); (C) Cell proliferation was detected by CCK8 assay; (D, E) Wound healing assay was used to detect cell migration; (F) Transwell assay was used to detect cell invasion.

First, we investigated the effect of PRKAA1 expression on PC cell proliferation. **Figure 7C** shows that the knockdown of PRKAA1 significantly reduced the proliferation of PANC-1 and CFPAC-1 cells in the CCK-8 assay. In addition, the scratch assay (**Figure 7D, 7E**) confirmed that PRKAA1 knockdown reduced the motility of PANC-1 and CFPAC-1 cells. The Transwell cell invasion assay (**Figure 7F**) confirmed that PRKAA1 silencing inhibited the invasive ability of PC cells.

PRKAA1 overexpression promotes the proliferation, migration, and invasion of PC cell lines

The effects of PRKAA1 overexpression are shown in **Figure 8A**. The proliferation of cell

lines overexpressing PRKAA1 was significantly enhanced at 48, 72, and 96 h compared to that of the negative control (**Figure 8B**). The results of the scratch migration assay (**Figure 8C**) showed that the migration of cells overexpressing PRKAA1 was significantly enhanced after 24 h. In the subsequent cell invasion assay, we found that cell lines overexpressing PRKAA1 had significantly enhanced invasion ability after 24 h (**Figure 8D**).

PRKAA1 promotes phosphorylation activation of the PI3K/ATK pathway

The PI3K/AKT signaling pathway is a key signaling pathway in tumor research. We investigated the changes in this signaling pathway in

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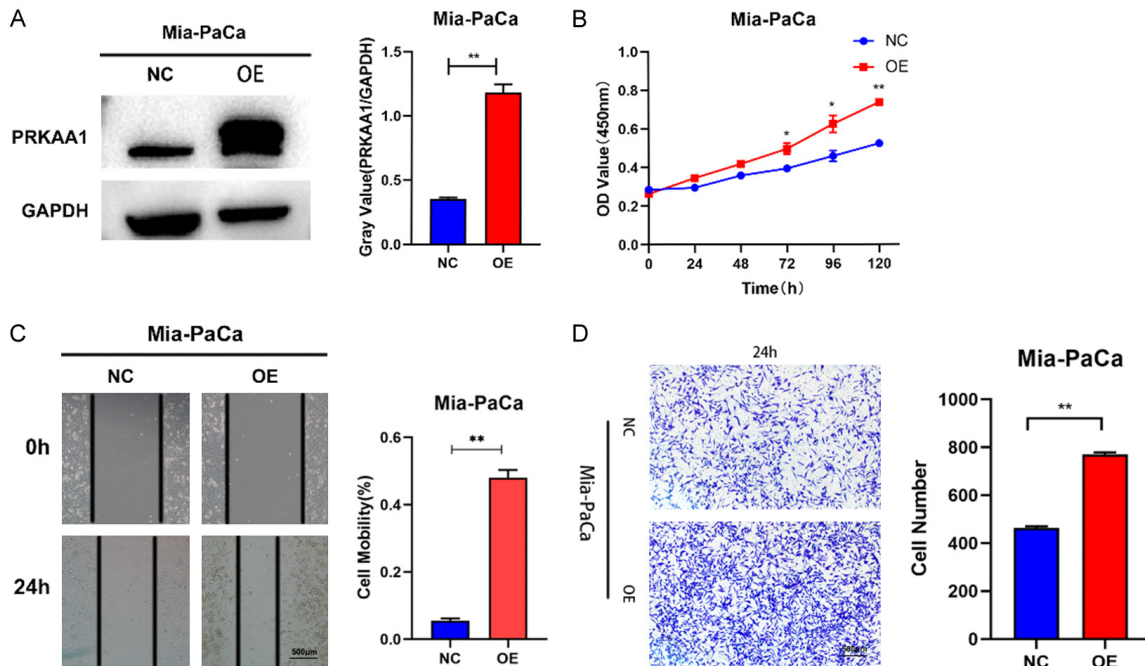


Figure 8. Western blotting (A) Detection of PRKAA1 overexpression efficiency in PC cells; (B) CCK8 assay for cell proliferation; (C) Scratch assay for cell migration; (D) Transwell assay for cell invasion.

PRKAA1 overexpressing cell lines and knock-down cell lines. We found that (Figure 9A, 9B) compared to the control group, P-PI3K and P-AKT were significantly reduced in the PRKAA1 knockdown group, as were the ratios of P-PI3K/PI3K and P-AKT/AKT. Conversely, PRKAA1 overexpression led to increased levels of P-PI3K and P-AKT, as well as higher P-PI3K/PI3K and P-AKT/AKT ratios.

We determined the IC₅₀ values of the AKT inhibitors, MK2206 and GSK2110183 (Figure 9C). Drug survival experiments revealed that the PRKAA1 overexpression group was less sensitive to AKT inhibitors than the NC group (Figure 9D).

Discussion

Despite significant research efforts, PC remains highly aggressive and has a poor prognosis; therefore, new therapeutic targets are urgently required. PRKAA1 has been found to be involved in glycolipid metabolism, vasodilation, and autophagy [13-16]; however, research on its role in cancer is incomplete. We integrated data from the TCGA and GTEx databases and performed pan-cancer expression analysis, which showed that PRKAA1 was significantly

and abnormally highly expressed in many tumors. In addition, we performed COX regression and Kaplan-Meier survival curve analyses, confirming that PRKAA1 has the potential to be a reliable biomarker. PRKAA1 has been shown to have a unique role in cancer evolution. For example, Zhang et al. [17] explored the role of PRKAA1 in gastric cancer and found that PRKAA1 promotes the proliferation of gastric cancer cells and inhibits apoptosis by activating the JNK1 and Akt pathways. Zhang et al. [18] also found that PRKAA1 promotes cell proliferation and glycolysis in gastric cancer by regulating redox homeostasis. Obba et al. [19] explored the role of PRKAA1 in CMML and found that PRKAA1 triggers autophagy during the CSF1-induced differentiation of human monocytes. Yi et al. [20] found that high expression of AMPK α 1 in advanced breast cancer prevents tumor metastasis.

The tumor microenvironment in PC is closely associated with chemotherapy resistance. It is worth noting that PRKAA1 is involved in tumor immunotherapy. Gao et al. [21] found that PRKAA1 deactivation can lead to lung cancer via CD8⁺/CD4⁺ T-cell infiltration and antigen presentation reduction, which can lead to immune escape by lung cancer cells. Chen et

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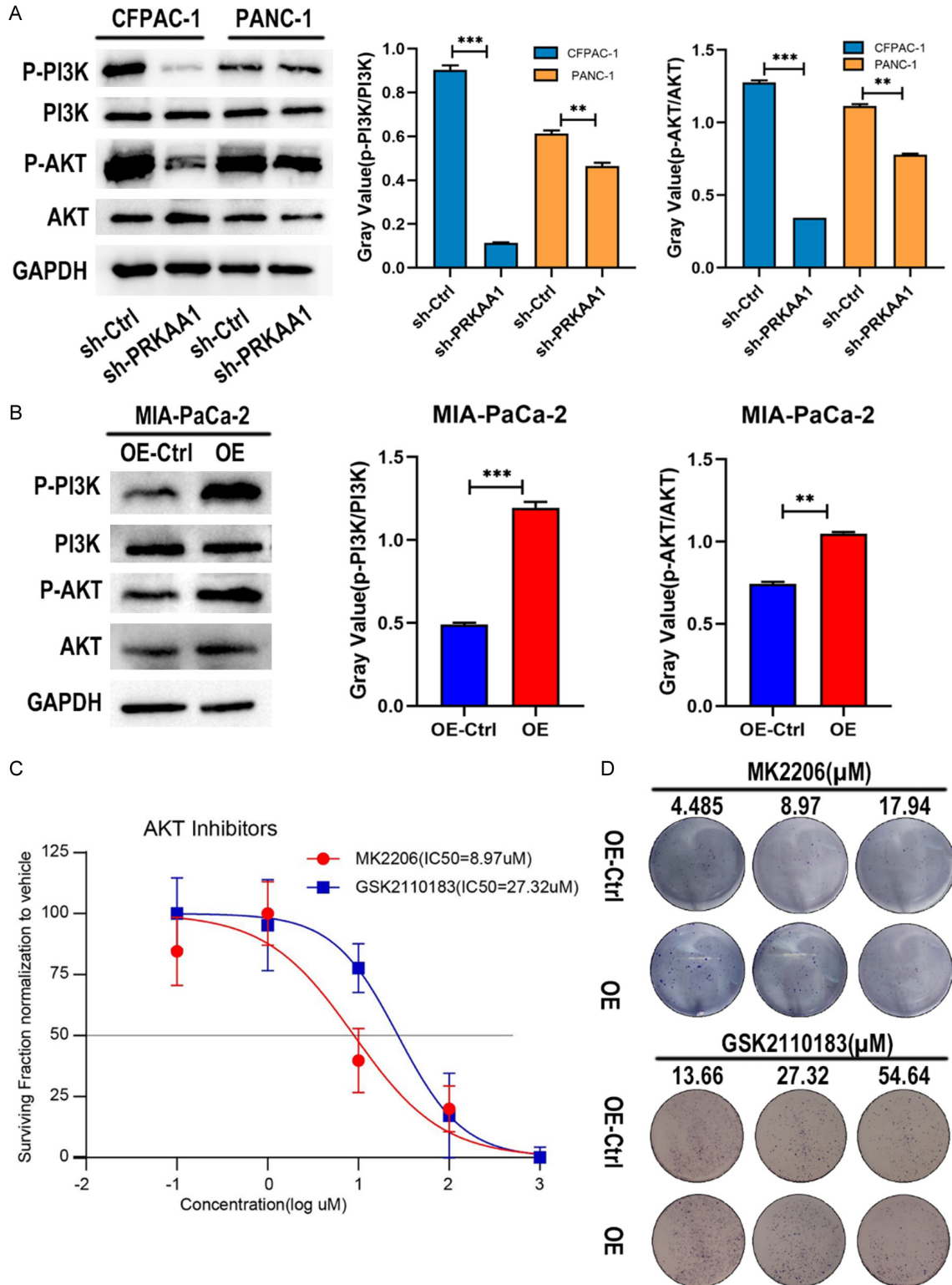


Figure 9. A. Western blotting was performed to detect changes in p-PI3K/PI3K and p-AKT/AKT ratios in CFPAC-1 and PANC-1 cell knockdown models; B. Western blotting was performed to detect changes in p-PI3K/PI3K and p-AKT/AKT ratios in the Mia-PaCa cell overexpression model; C. IC50 values of MK2206 and GSK2110183 in Mia-PaCa cells determined by CCK8 assay; D. Drug survival assay.

al. [22] also noted that PRKAA1 expression in gastric cancer is associated with immune infiltration. However, none of these studies have examined the relationship between PRKAA1 and other immune features. Therefore, we first investigated the relationship between PRKAA1 and immune infiltration scores and found that PRKAA1 expression was negatively correlated with stromal and immune scores in the four swellings. This suggests that high PRKAA1 expression suppresses the immune response in patients and that a reduction in immune infiltration hinders the effectiveness of immunotherapy. PRKAA1 expression significantly correlated with B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and DC in 33 tumors. Targeting immune checkpoint genes reverses the immunosuppressive tumor microenvironment, which in turn activates immune cells to fight tumors [23]. We found that PRKAA1 positively correlated with a variety of immune checkpoint genes, such as EDNRB, VEGF, and CD274. These results suggest that PRKAA1 may be involved in tumor immunotherapy and that PRKAA1 may be involved in tumor immunotherapy, providing insights for the development of immune checkpoint inhibitors.

Biomarkers, such as TMB, MSI, and PD-L1, predict the efficacy of tumor immunotherapy. Patients with both TMB-H and MSI-H can benefit from immunotherapy [24]. We explored the relationship between PRKAA1, TMB, and MSI in pan-cancer and showed that PRKAA1 expression positively correlated with TMB in six tumors and with MSI in five tumors. Notably, PRKAA1 expression positively correlated with both TMB and MSI in LGG and UCEC. We speculate that patients with high PRKAA1 expression in LGG and UCEC will better benefit from immunotherapy.

Subsequently, we used PC as an entry point to explore the effect of PRKAA1 on the proliferation, invasion, and migration ability of PC cells, as well as its potential mechanisms of action. We compared the clinicopathologic information of patients with varying PRKAA1 levels and found that the high expression of PRKAA1 was correlated with the patients' M stage. We analyzed the relationship between PRKAA1 expression and patient prognosis and determined that patients with high PRKAA1 expression had a poor prognosis. The above analyses and the

univariate and multivariate COX regression analyses showed that PRKAA1 was an independent prognostic factor for PC. Knockdown and overexpression of PC cells showed that PRKAA1 overexpression promoted the proliferation, invasion, and migration of PC cells, whereas PRKAA1 knockdown inhibited their malignant biological behaviors. These results suggest that PRKAA1 can promote the biological progression of PC. Aberrant activation of the PI3K/Akt signaling pathway promotes the survival and proliferation of various human tumor cells [25]. Interestingly, our analysis showed that the PRKAA1 knockdown significantly reduced the levels of p-PI3K/PI3K, p-Akt/Akt. Using Akt inhibitors to treat cells, we found that low PRKAA1 expression enhanced the antitumor effects of Akt inhibitors. These results further indicate that PRKAA1 may promote PC progression through the PI3K/Akt signaling pathway.

This study had certain limitations that should be acknowledged. Although we propose that PRKAA1 promotes the malignant biological process of PC through the PI3K/Akt signaling pathway, its direct target in PC was not identified in the present study. It is also noteworthy that the number of patients included was limited, especially because fresh specimens were not collected for protein expression assays. Furthermore, our study lacked a direct *in vivo* validation of the role of PRKAA1 in PC. Studying the specific mechanisms of action of PRKAA1 in PC will expand our understanding of its role. In future studies, we will continue to explore the detailed mechanisms through which PRKAA1 regulates the PI3K-Akt pathway in PC.

In summary, PRKAA1, a pan-oncogene that is differentially expressed in different malignancies, could reflect the survival prognosis of patients with diverse tumors and could act as a therapeutic and prognostic marker for a variety of malignancies. PRKAA1 exhibited a remarkable correlation with both immune cell infiltration and immune checkpoint genes, suggesting its potential as a target for tumor immunotherapy. In addition, the present study revealed that PRKAA1 is aberrantly overexpressed in PC and ultimately promotes PC cell proliferation, migration, and invasion, in part through the activation of the PI3K/Akt signaling pathway. Therefore, PRKAA1 is a potentially useful therapeutic target for PC.

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Disclosure of conflict of interest

None.

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