Aberrant expression of polo-like kinase 4 in renal cell carcinoma: Association with clinicopathological characteristics and long-term survival

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Abstract. Polo-like kinase 4 (PLK4) promotes tumorigenesis and is associated with the prognosis of several solid tumors, while its clinical role in patients with renal cell carcinoma (RCC) remains unidentified. The present study aimed to analyze the association of PLK4 with clinicopathological characteristics and long-term prognosis in patients with RCC. The present study detected PLK4 protein and mRNA expression using immunohistochemical and reverse transcription-quantitative PCR assays in 120 patients with RCC. Disease-free survival (DFS) and overall survival (OS) time were calculated based on a median follow-up duration of 6.9 years (range, 1.2-9.9 years). PLK4 protein expression was elevated in tumor tissues compared with adjacent tissues (P<0.001). Upregulation of PLK4 protein was associated with increased T stage (P=0.023), N stage (P=0.014) and TNM stage (P=0.007). Additionally, elevated tumor PLK4 protein expression exhibited an associating trend (without statistical significance) with reduced DFS rate (P=0.066) and was associated with decreased OS rate (P=0.036). However, univariate Cox's regression analysis indicated that high PLK4 protein expression (compared with low PLK4 protein expression) was associated with reduced OS rate (P=0.040) but not with PFS rate (P=0.070). Following adjustment by multivariate Cox's regression analysis, PLK4 protein expression was associated with neither DFS nor OS rate (both P>0.050). Additionally, PLK4 mRNA expression was further detected in some patients (for which fresh specimens frozen in liquid nitrogen were available) to validate the aforementioned observations, and the expression was elevated in tumor tissues compared with adjacent tissues. Furthermore, increased PLK4 mRNA expression was associated with tumor size ≥7 cm, high TNM stage and reduced DFS rate (all P<0.050). PLK4 possesses a certain clinical utility in monitoring the clinical stage of patients with RCC, while its prognostic value requires further validation.

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Introduction

Renal cell carcinoma (RCC) is a urinary system malignancy originating from tubular epithelial cells, and its pathological subtypes mainly include clear, papillary and chromophobe cell carcinoma (1,2). In RCC epidemiology, it is estimated that RCC accounted for almost 2.2% of all cancer cases worldwide in 2020 and its incidence in China has increased in recent years (3,4). Most patients with RCC are asymptomatic at an early stage, while the presence of the typical symptoms of RCC (including hematuria, abdominal mass and flank pain) is indicative of invasion and metastasis (5). Therefore, nearly 20% of RCC cases are diagnosed at the metastatic stage (1,6). Furthermore, although the overall prognosis of patients with RCC is relatively good, the heterogeneity causes different outcomes among patients (7-9). Therefore, it is meaningful to explore biomarkers that may assist clinicians in monitoring the development and progression of patients with RCC.

Polo-like kinase 4 (PLK4), also referred to as serum-inducible-kinase akin kinase, is a type of serine/threonine-protein kinase with triple polo box architecture, which serves as an indispensable regulator of centriole duplication (10-12). In recent years, several studies have demonstrated that PLK4 promotes tumorigenesis in solid tumors, such as colorectal, prostate and non-small cell lung cancer (13-15). For example, a previous study indicated that PLK4 facilitated cell viability and proliferation in colorectal cancer cells by inhibiting the Wnt/β-catenin signaling pathway (13). An additional study revealed that elevated PLK4 expression is associated with increased tumor size, lymph node metastasis and inferior survival in patients with non-small cell lung cancer (14). In terms of the biological and mechanistic backgrounds, PLK4 upregulation drives centrosome amplification and

the cell cycle via the regulation of several target genes, including epithelial cell transforming sequence 2 (ECT2) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (PIK3CA), which further causes chromosome instability in cancer cells (16-18). In addition, PLK4 has also been reported to modulate epithelial-mesenchymal transition (EMT) in various epithelial cancers, including lung squamous cell carcinoma and epithelial ovarian cancer (19,20). For example, one study revealed that PLK4 dysregulation was a potential indicator of poor prognosis in lung squamous cell carcinoma (19). Concerning the PLK family in RCC, a previous study suggested that PLK1 is upregulated in RCC and facilitates oncogenesis and the progression of renal cancer (21). Subsequently, it was hypothesized that PLK4 may have similar potency in RCC pathogenesis, while its clinical role in patients with RCC has not been previously identified.

The present study explored the association of PLK4 protein expression with clinicopathological characteristics and the long-term prognosis of patients with RCC who underwent surgery, and the results were further validated by PLK4 mRNA expression analysis.

Materials and methods

Patients. The present study retrospectively reviewed a total of 120 patients with RCC who underwent surgical resection at the hospital (Jing'an District Center Hospital of Shanghai, Fudan University, Shanghai, China) between January 2011 and June 2016. The main screening criteria were as follows: i) Pathological confirmation of RCC; ii) age range of 18-80 years; iii) surgical resection for RCC; iv) retrievable carcinoma tissue specimens, which were fixed in formalin and embedded in paraffin (FFEP) for immunohistochemical (IHC) analysis; and v) accessible preoperative clinical characteristics and follow-up data. Patients who had other carcinomas or hematological malignancies (such as lung cancer, colorectal cancer, Hodgkin's lymphoma and leukemia) were excluded. The present study was approved by the Ethics Committee of Jing'an District Center Hospital of Shanghai, Fudan University, Shanghai, China [(2021) ethical approval no 13]. In addition, this was a retrospective study and the collected data were retrieved several years ago; therefore, the requirement for informed consent was waived.

Data documentation and specimen processing. The demographic information and disease characteristics of patients with RCC were collected for analysis. In addition, the follow-up data of patients with RCC were also retrieved from clinical records. These data were collected for the calculation of disease-free survival (DFS) rate and overall survival (OS) rate. The final date of the follow-up period was June 30, 2021. The median follow-up duration was 6.9 years, and the follow-up range was 1.2-9.9 years. The available FFEP specimens (120 tumor tissues and 68 adjacent tissues <2 cm from the tumor tissues) were collected to determine PLK4 protein expression and fresh specimens frozen at -196˚C in liquid nitrogen (60 tumor tissues and 28 adjacent tissues) were collected to examine the mRNA expression levels of PLK4. The pathology department retained core cancer tissues of all patients, while only a small number of adjacent tissues of some patients were retained. Therefore, adjacent tissues were only available in some cases.

Assessment of PLK4 protein expression. The protein expression levels of the PLK4 were assessed by IHC as reported in a previous study (14). Briefly, the FFEP specimens, which were fixed using 10% formalin at room temperature for 24 h, were cut into 4-μm slices. Next, the slides were deparaffinized with xylene. The slides were rehydrated with a descending alcohol series. Subsequently, the slides were heated at 98˚C for 10 min in 0.01 mol/l sodium citrate buffer (pH 6) for antigen retrieval. The slides were treated with fresh 3% hydrogen peroxide to inhibit the activity of endogenous peroxidase. After blocking with 1.5% normal goat serum (cat. no. R37624; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 20 min, the slices were incubated with anti-PLK4 antibody (1:150; cat. no. PA5-29373; Invitrogen; Thermo Fisher Scientific, Inc.) as the primary antibody at 4˚C overnight, and then incubated with goat anti-rabbit IgG H&L (HRP) (1:2,000; cat. no. 31460; Invitrogen; Thermo Fisher Scientific, Inc.) as the secondary antibody at room temperature for 60 min. Finally, 3,3'-diaminobenzidine (room temperature; 3 min; Sangon Biotech Co., Ltd.) and hematoxylin (room temperature; 5 min; Sangon Biotech Co., Ltd.) were used for staining. The antibodies used in the present study were as follows: Primary antibody, anti-PLK4 antibody (1:150; Invitrogen; Thermo Fisher Scientific, Inc.); secondary antibody, goat anti-rabbit IgG H&L (HRP) (1:2,000; Invitrogen; Thermo Fisher Scientific, Inc.).

Following IHC staining, IHC results were scored by two pathologists who were blinded to the patients' information using a light microscope using a semi-quantitative method according to the intensity and density of stained cells. The intensity was scored as: 0 (negative), 1 (weak), 2 (moderate) and 3 (strong). The density was scored as: 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). The final score of the IHC assay was obtained by multiplying the staining intensity and density scores (22). In the analysis, an IHC score of ≤3 in the tumor tissue was considered to indicate low tumor PLK4 protein expression, and an IHC score of >3 was considered to indicate high PLK4 protein expression.

Evaluation of PLK4 mRNA expression. The mRNA expression levels of PLK4 were evaluated by reverse transcription-quantitative PCR (RT-qPCR). Total RNA was obtained using a RNeasy Protect Mini Kit (Qiagen, Inc.). Subsequently, reverse transcription was performed using a QuantiTect Rev. Transcription Kit (Qiagen, Inc.) at 42˚C for 18 min and 95˚C for 3 min. qPCR was initiated using TB Green® Fast qPCR Mix (Takara Bio, Inc.) with the following thermocycling conditions: 95˚C for 30 sec for 1 cycle; followed by 95˚C for 5 sec and 61˚C for 15 sec for 40 cycles. The relative expression levels of PLK4 were assessed using the $2^{-\Delta\Delta Cq}$ method (23), using GAPDH as the internal reference gene. The qPCR primers were designed based on a previous study (22). The following PCR primers were used: PLK4 forward, 5'-CCTTATCACCTCCTCCTTC-3' and reverse, 5'-CCAAGTCCTTCATTTGTAACC-3'; and GAPDH forward, 5'-ACATCATCCCTGCCTCTAC-3' and reverse, 5'-CCTGCTTCACCACCTTCT-3'. The median of the PLK4 mRNA expression noted in the tumor tissues was

used to classify the patients into the high and low tumor PLK4 mRNA expression groups.

Analysis of PLK4, ECT2 and PIK3CA expression using online databases. Additional data were obtained from the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/index.html) to further verify the correlation of PLK4 expression with ECT2 and PIK3CA in patients with RCC. Furthermore, PLK4 expression data of 517 patients with RCC were obtained from GEPIA (http://gepia.cancer-pku.cn/detail.php?gene=PLK4###) to further confirm the association between PLK4 expression and DFS rate in patients with RCC. PLK4 expression data of 877 patients with RCC were obtained from The Human Protein Atlas [derived from The Cancer Genome Atlas (TCGA) analysis; available at https://www.proteinatlas.org/ENSG00000142731-PLK4/pathology/renal+cancer] to further verify the association between PLK4 expression and OS rate in patients with RCC.

Statistical analysis. Statistical analysis was performed using SPSS (version 24.0; IBM Corp.), and the graphs were generated using GraphPad Prism (version 7.01; GraphPad Software, Inc.). Normality determination for continuous variables was performed using the Kolmogorov-Smirnov test. Normally distributed continuous variables are presented as the mean ± SD, continuous variables with a skewed distribution are presented as the median and inter-quartile range (IQR) and categorized variables are presented as the count (percentage). The differences between the expression levels of the tumor and adjacent tissues were compared using the Wilcoxon signed rank test. Receiver operating characteristic (ROC) analysis was performed to evaluate the suitability of PLK4 expression for distinguishing tumor tissues from adjacent tissues. The association between the PLK4 expression levels and the clinical characteristics was analyzed using the Mann-Whitney U test for two groups and Kruskal-Wallis test by ranks followed by Dunn's post hoc test for three groups. The correlation of PLK4 with ECT2 and PIK3CA was analyzed using the Pearson test. DFS and OS rates were assessed using Kaplan-Meier curves and significant differences were determined using the log-rank test. Prognostic factor analysis was carried out using Cox's proportional hazard regression analysis, and all potential factors were included in the multivariate analysis with the forward stepwise method. $P<0.050$ was considered to indicate a statistically significant difference.

Results

Patient characteristics. The data of 120 patients with RCC with a mean age of 58.1±11.7 years were retrospectively reviewed in the present study. The participants included 34 (28.3%) female patients and 86 (71.7%) male patients (Table I). The primary lesions were located in the right kidney for 60 (50.0%) patients and in the left kidney for the remaining 60 (50.0%) patients. In addition, 99 (82.5%) patients were assessed to have an Eastern Cooperative Oncology Group Performance Status (ECOG PS) score of 0, whereas 21 (17.5%) patients were assessed to have an ECOG PS score of 1 (24). Furthermore, 56 (46.7%), 47 (39.1%) and 17 (14.2%) patients were identified as cases with well, moderate and poor tumor differentiation, respectively. The median tumor size of all patients was 5.5 cm (IQR, 4.0-8.0 cm). With regard to the clinical stage, 44 (36.7%), 43 (35.8%), 23 (19.2%), 2 (1.6%) and 8 (6.7%) patients were assessed as T1a, T1b, T2a, T2b and T3, respectively. Furthermore, 109 (90.8%) and 11 (9.2%) patients were diagnosed as N0 and N1, respectively. With regard to their TNM stage, 81 (67.5%) patients were classified as stage I, 23 (19.2%) patients as stage II and 16 (13.3%) as stage III. The patient characteristics are shown in Table I.

Table I. Characteristics of patients with RCC.

Variables	Patients with RCC (N=120)
Age, years (mean ± SD)	58.1±11.7
Sex, n (%)	
Female	34 (28.3)
Male	86 (71.7)
Tumor location, n (%)	
Right	60 (50.0)
Left	60 (50.0)
ECOG PS score, n (%)	
0	99 (82.5)
1	21 (17.5)
Tumor differentiation, n (%)	
Well	56 (46.7)
Moderate	47 (39.1)
Poor	17 (14.2)
Median tumor size, cm (IQR)	5.5 (4.0-8.0)
T stage, n (%)	
T1a	44 (36.7)
T1b	43 (35.8)
T2a	23 (19.2)
T2b	2 (1.6)
T3	8 (6.7)
N stage, n (%)	
N0	109 (90.8)
N1	11 (9.2)
TNM stage, n (%)	
Stage I	81 (67.5)
Stage II	23 (19.2)
Stage III	16 (13.3)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; IQR, interquartile range; RCC, renal cell carcinoma.

PLK4 protein expression. IHC staining was performed in tumor and adjacent tissues of patients with RCC to detect PLK4 expression (Fig. 1A). Notably, PLK4 protein expression was elevated in tumor tissues compared with in adjacent tissues [median (IQR): 4.0 (3.0-8.0) vs. 2.0 (2.0-3.0); $P<0.001$; Fig. 1B]. In addition, PLK4 protein expression could be used to differentiate tumor tissues from adjacent tissues with an area under the curve (AUC) value of 0.757 (95% CI, 0.684-0.830; Fig. 1C).

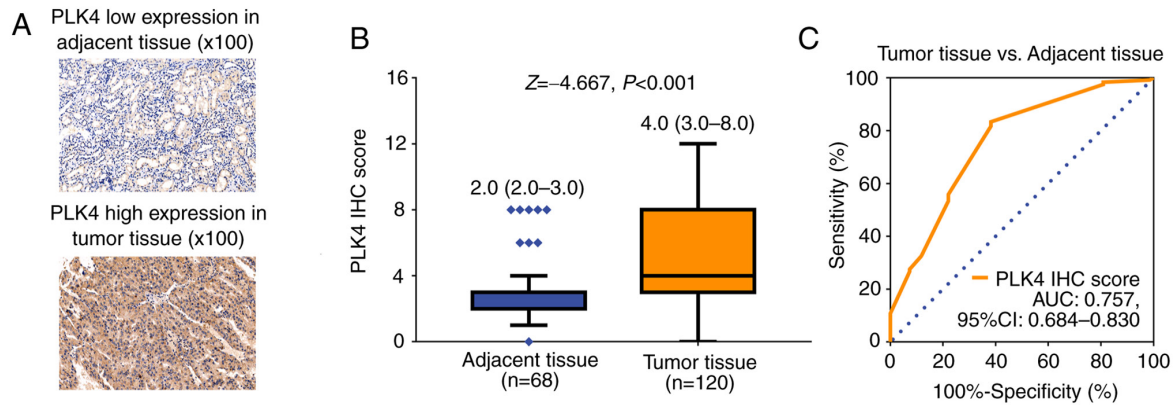

Figure 1. PLK4 protein expression is upregulated in tumor tissues compared with in adjacent tissues of patients with RCC. (A) PLK4 IHC staining examples and (B) PLK4 protein expression reflected by the IHC score analyzed using the Wilcoxon signed rank test in tumor and adjacent tissues of patients with RCC. Magnification, x100. (C) PLK4 protein expression could be used to differentiate tumor tissues from adjacent tissues according to receiver operating characteristic analysis. AUC, area under the curve; IHC, immunohistochemical; PLK4, polo-like kinase; RCC, renal cell carcinoma.

Association of PLK4 protein expression with clinicopathological characteristics. PLK4 protein expression was not associated with age (P=0.224; Fig. 2A), tumor location (P=0.141; Fig. 2B), ECOG PS score (P>0.999; Fig. 2C) or tumor size (P=0.151; Fig. 2D) in patients with RCC. However, upregulation of PLK4 protein expression was related to poor tumor differentiation (P=0.009; Fig. 2E), increased T stage (P=0.023; Fig. 2F), N stage (P=0.014; Fig. 2G) and TNM stage (P=0.007; Fig. 2H).

Association of PLK4 protein expression with survival. The elevated protein expression levels of PLK4 in tumors exhibited an associating trend (without statistical significance) with reduced DFS rate in patients with RCC (P=0.066; Fig. 3A). In addition, high PLK4 expression in tumors was associated with decreased OS rate in patients with RCC (P=0.036; Fig. 3B).

Association between clinicopathological factors and survival. Univariate Cox's regression analysis indicated that PLK4 protein expression was not related to DFS rate in patients with RCC [hazard ratio (HR), 1.707; P=0.070; Table II]. Additionally, tumor differentiation (poor vs. well; P<0.001), tumor size (≥7 vs. <7 cm; P<0.001), T stage (T2 vs. T1; P<0.001), T stage (T3 vs. T1; P<0.001), N stage (N1 vs. N0; P<0.001), TNM stage (stage II vs. I; P<0.001) and TNM stage (stage III vs. I; P<0.001) were linked with shortened DFS. In addition, multivariate Cox's regression analysis demonstrated that the ECOG PS score (1 vs. 0; HR, 2.243; P=0.025), tumor differentiation (poor vs. well; HR, 3.185; P=0.006) and TNM stage (II vs. I, HR, 3.993, P=0.001; III vs. I, HR, 10.488, P<0.001) were independently associated with reduced DFS rate in patients with RCC.

Furthermore, univariate Cox's regression analysis demonstrated that PLK4 protein expression (high vs. low) was associated with reduced OS rate in patients with RCC (HR, 2.049; P=0.040; Table III); however, it was not an independent prognostic factor of OS. Apart from PLK4 protein expression, it was also observed that ECOG PS score (1 vs. 0) (P=0.032), tumor differentiation (poor vs. well) (P<0.001), T stage (T2 vs. T1) (P=0.001), T stage (T3 vs. T1) (P<0.001), TNM stage (stage II vs. stage I) (P=0.001), and TNM stage (stage III vs. stage I) (P<0.001) were related to shortened OS. In addition, multivariate Cox's regression analysis demonstrated that ECOG PS score (1 vs. 0; HR, 2.903; P=0.006), tumor differentiation (poor vs. well; HR, 6.114; P<0.001) and TNM stage (II vs. I, HR, 2.808, P=0.030; III vs. I, HR, 8.010, P<0.001) were independently associated with decreased OS rate in patients with RCC.

Validation of PLK4 mRNA levels. The aforementioned observations were validated by determining the mRNA expression levels of PLK4 in certain patients with RCC, for which fresh specimens frozen in liquid nitrogen were available. The mRNA expression levels of PLK4 were elevated in tumor tissues compared with in adjacent tissues of patients with RCC [2.275 (IQR, 1.430-2.965) vs. 1.025 (IQR, 0.675-1.568); P<0.001; Fig. 4A]. Furthermore, PLK4 mRNA expression possessed a good value to distinguish tumor tissues from adjacent tissues, with an AUC value of 0.849 (95% CI, 0.770-0.927; Fig. 4B).

The analysis of the association of the mRNA expression levels of PLK4 with the clinicopathological characteristics of the patients with RCC indicated that PLK4 mRNA expression was not associated with age (P=0.394), tumor location (P=0.286), ECOG PS score (P=0.406), tumor differentiation (P=0.745), T stage (P=0.064) or N stage (P=0.295); however, increased PLK4 mRNA expression levels were associated with tumor size ≥7 cm (P=0.039) and high TNM stage (P=0.022) (Fig. 4C-J).

In addition, high PLK4 mRNA expression in tumors was associated with reduced DFS rate (P=0.032; Fig. 4K), while it tended to be associated with decreased OS; however, no statistically significant difference was observed (P=0.056; Fig. 4L).

PLK4 potential target genes. Additional data were obtained from the GEPIA database to further verify the correlation of PLK4 expression with its potential target genes in patients with RCC. PLK4 mRNA was positively correlated with ECT2 mRNA expression (P<0.001; Fig. S1A) and PIK3CA mRNA expression (P<0.001; Fig. S1B) in patients with RCC.

Further validation of the prognostic value of PLK4. Further survival analysis using data obtained from GEPIA revealed

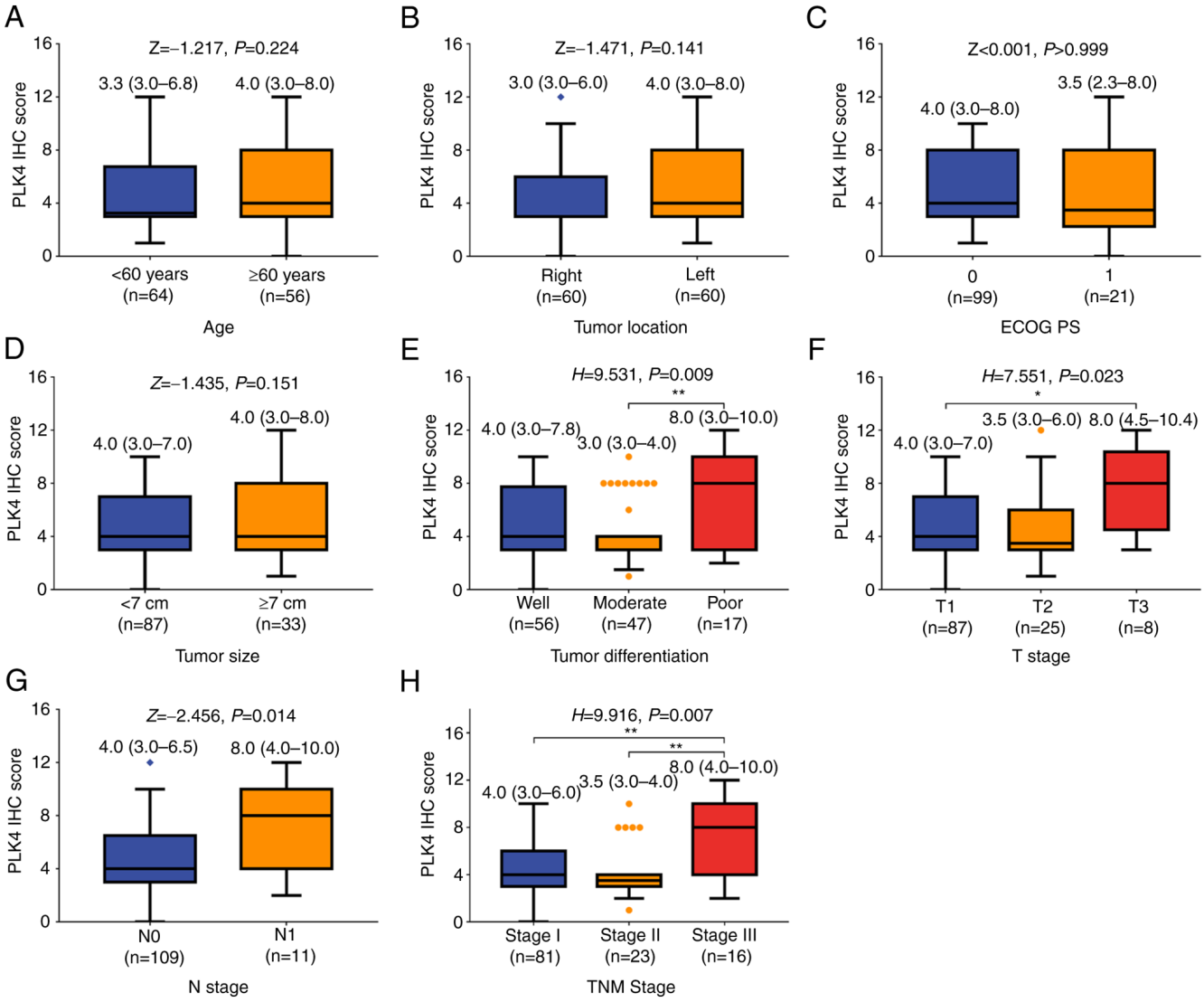

Figure 2. Elevated PLK4 protein expression is associated with poor tumor differentiation, and increased T, N and TNM stages in patients with RCC. Comparison of PLK4 protein expression in patients with RCC with different (A) age (Mann-Whitney U test), (B) tumor location (Mann-Whitney U test), (C) ECOG PS score (Mann-Whitney U test), (D) tumor size (Mann-Whitney U test), (E) tumor differentiation (Kruskal-Wallis test by ranks followed by Dunn's post hoc test), (F) T stage (Kruskal-Wallis test by ranks followed by Dunn's post hoc test), (G) N stage (Mann-Whitney U test) and (H) TNM stage (Kruskal-Wallis test by ranks followed by Dunn's post hoc test). *P<0.05, **P<0.01. ECOG PS, Eastern Cooperative Oncology Group Performance Status; IHC, immunohistochemistry; PLK4, polo-like kinase; RCC, renal cell carcinoma.

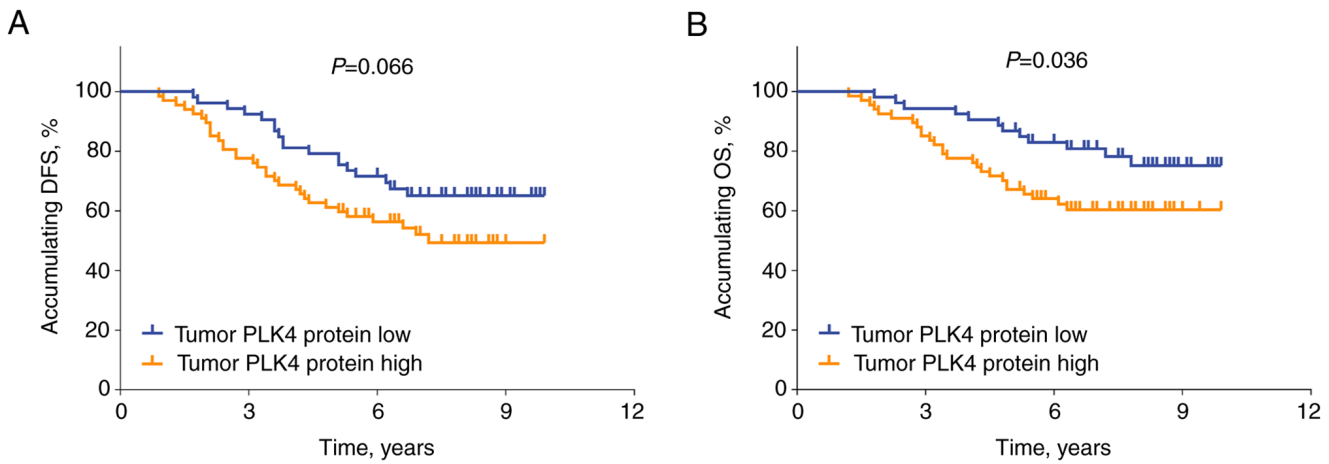

Figure 3. Elevated PLK4 expression in tumors is associated with reduced OS rate in patients with RCC. Association of PLK4 protein expression with (A) DFS rate and (B) OS rate in patients with RCC. DFS and OS rates were assessed using Kaplan-Meier curves and the significant differences were determined using the log-rank test. DFS, disease-free survival; OS, overall survival; PLK4, polo-like kinase; RCC, renal cell carcinoma.

Table II. Univariate and multivariate Cox proportional hazards regression model analyses of factors predicting disease-free survival.

Items	P-value	HR	95% CI	
			Lower	Upper
Univariate Cox's regression analysis				
PLK4 protein (high vs. low)	0.070	1.707	0.958	3.044
Age (≥60 vs. <60 years)	0.347	1.306	0.749	2.278
Sex (male vs. female)	0.626	1.170	0.622	2.201
Tumor location (left vs. right)	0.401	1.269	0.728	2.215
ECOG PS score (1 vs. 0)	0.094	1.774	0.908	3.466
Tumor differentiation				
Well	Ref.			
Moderate	0.251	1.476	0.759	2.870
Poor	<0.001	7.224	3.520	14.828
Tumor size (≥7 vs. <7 cm)	<0.001	4.596	2.624	8.050
T stage				
T1	Ref.			
T2	<0.001	3.772	2.041	6.973
T3	<0.001	9.714	4.261	22.145
N stage (N1 vs. N0)	<0.001	6.985	3.448	14.150
TNM stage				
Stage I	Ref.			
Stage II	<0.001	4.483	2.277	8.825
Stage III	<0.001	12.849	6.273	26.320
Multivariate Cox's regression analysis				
ECOG PS score (1 vs. 0)	0.025	2.243	1.109	4.539
Tumor differentiation				
Well	Ref.			
Moderate	0.739	0.874	0.395	1.933
Poor	0.006	3.185	1.384	7.330
TNM stage				
Stage I	Ref.			
Stage II	0.001	3.993	1.765	9.034
Stage III	<0.001	10.488	4.784	22.995

Variables without statistical significance are not shown in the multivariate Cox's regression model. ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; PLK4, polo-like kinase 4.

that high PLK4 expression (vs. low PLK4 expression) was associated with reduced accumulating DFS rate in patients with RCC (P=0.029; Fig. S2A). Further survival analysis using data from The Human Protein Atlas (derived from TCGA analysis) revealed that high PLK4 expression (vs. low PLK4 expression) was associated with shortened accumulating OS rate in patients with RCC (P<0.001; Fig. S2B).

Discussion

PLK4 is located on human chromosome 4q27-28 and has been reported to modulate centriole duplication, which affects cancer invasion and metastasis (25). Previous studies have detected its expression in various solid tumors, including those of the bladder and prostate (15,18). For example, a previous study has demonstrated elevated PLK4 expression in human prostate cancer cell lines and tumor tissues derived from patients with prostate cancer (15). It has also been reported that PLK4 expression is upregulated in bladder cancer tissues compared with in normal bladder tissues (18). The present study indicated that both PLK4 protein and mRNA expression were upregulated in tumor tissues compared with in adjacent tissues of patients with RCC. The possible explanations are as follows: i) PLK4 expression was positively associated with the number of centrioles that were excessively amplified in cancer cells, and thus, its expression levels were upregulated in tumor tissues compared with in adjacent tissues of patients with RCC (26). ii) Although PLK4 could autoregulate its stability to prevent centrosome amplification, under the cancerous condition, it has been observed that some specific genes, such

Table III. Univariate and multivariate Cox proportional hazards regression model analyses of factors predicting overall survival.

Items	P-value	HR	95% CI	
			Lower	Upper
Univariate Cox's regression analysis				
PLK4 protein (high vs. low)	0.040	2.049	1.032	4.065
Age (≥60 vs. <60 years)	0.093	1.740	0.911	3.324
Sex (male vs. female)	0.454	1.331	0.630	2.812
Tumor location (left vs. right)	0.124	1.667	0.869	3.196
ECOG PS score (1 vs. 0)	0.032	2.202	1.069	4.537
Tumor differentiation				
Well	Ref.			
Moderate	0.487	1.336	0.590	3.029
Poor	<0.001	10.424	4.677	23.232
Tumor size (≥7 vs. <7 cm)	<0.001	4.315	2.274	8.188
T stage				
T1	Ref.			
T2	0.001	3.296	1.612	6.740
T3	<0.001	10.886	4.447	26.646
N stage (N1 vs. N0)	<0.001	6.245	2.987	13.058
TNM stage				
Stage I	Ref.			
Stage II	0.001	3.737	1.672	8.355
Stage III	<0.001	11.158	5.152	24.167
Multivariate Cox's regression analysis				
ECOG PS score (1 vs. 0)	0.006	2.903	1.354	6.226
Tumor differentiation				
Well	Ref.			
Moderate	0.985	0.991	0.385	2.549
Poor	<0.001	6.114	2.402	15.560
TNM stage				
Stage I	Ref.			
Stage II	0.030	2.808	1.104	7.138
Stage III	<0.001	8.010	3.385	18.954

Variables without statistical significance are not shown in the multivariate Cox's regression model. ECOG PS, Eastern Cooperative Oncology Group Performance Status; HR, hazard ratio; PLK4, polo-like kinase 4.

as centrosomal protein 131, could regulate PLK4 stability and further promote centrosome amplification (27,28). iii) The stress-activated protein kinase pathway and P53 cooperatively suppress PLK4 activity, and the two pathways are frequently inactive in malignant tumors (29-31). Combining the two aforementioned aspects (ii and iii), PLK4 is elevated in the cancerous condition of RCC.

The association of PLK4 expression with clinicopathological features has been previously investigated (13,32). For example, a previous study indicated that high PLK4 expression was associated with elevated T and TNM stages in patients with gastric cancer (32). However, the detailed association of PLK4 with the clinicopathological characteristics of patients with RCC is still unclear. In the present study, upregulation of PLK4 protein expression was associated with poor tumor differentiation, and elevated T, N and TNM stages. The possible reasons for these findings are as follows: i) PLK4 overexpression results in genomic instability, aberrant cell cycle and tumorigenesis (9,33). Therefore, upregulation of PLK4 expression was associated with a higher T stage in patients with RCC. ii) As discussed previously, PLK4 induces EMT, which accelerates epithelial cancer migration and invasion (19,20). Consequently, elevated PLK4 expression was associated with a higher N stage in patients with RCC. iii) The TNM stage was determined using the T and N stages in patients with RCC. It was hypothesized that the association of PLK4 expression with TNM stage was due to the association of PLK4 expression with T and N stage.

Considering that PLK4 can regulate malignant behavior, such as tumor migration and invasion by regulating the actin related protein 2/3 complex, it was hypothesized that it may be associated with the survival of patients with cancer (25).

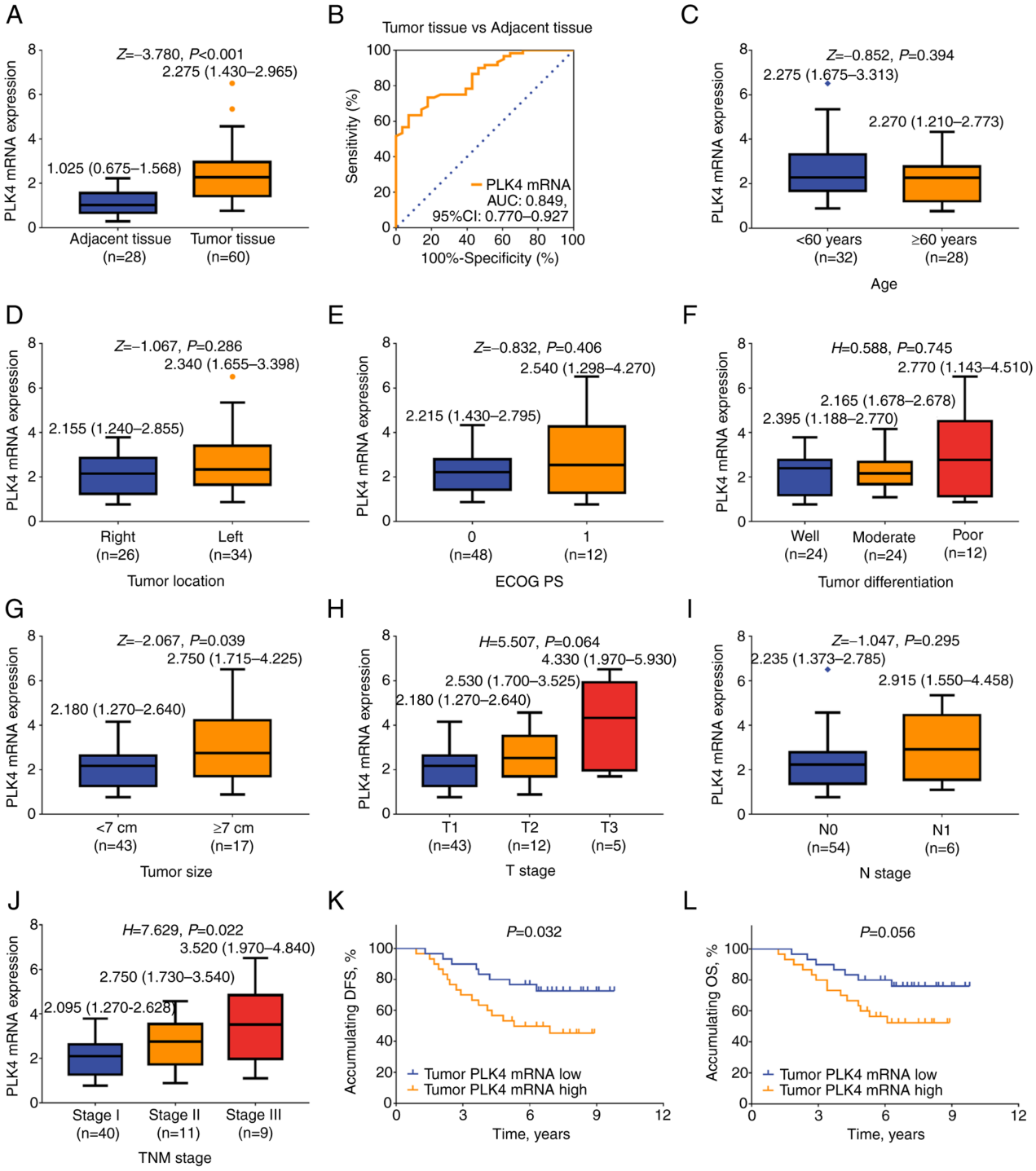

Figure 4. Association of PLK4 mRNA expression with clinicopathological characteristics and survival of patients with RCC. (A) mRNA expression levels of PLK4 were assessed in tumor and adjacent tissues of patients with RCC (paired-samples Wilcoxon signed ranks test). (B) Diagnostic value of PLK4 in distinguishing tumor tissues from adjacent tissues (receiver operating characteristic analysis). Association of PLK4 mRNA expression with (C) age (Mann-Whitney U test), (D) tumor location (Mann-Whitney U test), (E) ECOG PS score (Mann-Whitney U test), (F) tumor differentiation (Kruskal-Wallis test by ranks), (G) tumor size (Mann-Whitney U test), (H) T stage (Kruskal-Wallis test by ranks), (I) N stage (Mann-Whitney U test), (J) TNM stage (Kruskal-Wallis test by ranks followed by Dunn's post hoc test); post hoc test results lacked statistical significance, (K) DFS rate and (L) OS rate in patients with RCC. DFS and OS rates were assessed using Kaplan-Meier curves and the significant differences were determined using the log-rank test. AUC, area under the curve; DFS, disease-free survival; ECOG PS, Eastern Cooperative Oncology Group Performance Status; OS, overall survival; PLK4, polo-like kinase; RCC, renal cell carcinoma.

A previous study indicated that both DFS and OS rates were reduced in patients with non-small cell lung cancer with high PLK4 expression compared with patients with low PLK4 expression (14). Similarly, the present study revealed that high PLK4 expression was partially associated with poor survival in patients with RCC, which could be explained by PLK4 facilitating excessive centrosome amplification (15). The latter could induce the metastatic potential and invasion of tumor cells, which in turn may result in poor survival of patients with RCC (34). Nevertheless, the multivariate Cox's

regression analysis demonstrated that PLK4 was not independently associated with DFS and OS rates in patients with RCC, which could be explained by the following: The upregulation of PLK4 protein expression was related to increased T stage, N stage and TNM stage, and the latter factors would weaken the association of PLK4 with survival in the multivariate Cox's regression analysis. Therefore, the independent prognostic value of PLK4 in patients with RCC requires further study.

The present study demonstrated two main findings. Firstly, a long-term follow-up duration was designed (median 6.9 years; range, 1.2-9.9 years) to increase the reliability of the prognostic value of PLK4. Secondly, in addition to PLK4 protein expression, the present study analyzed the mRNA expression levels of PLK4 to further validate the clinical role of this enzyme in patients with RCC. However, the present study has certain limitations. Firstly, only surgically resectable patients were enrolled, and thus, the association of PLK4 expression with the incidence of advanced RCC requires further investigation. Secondly, this was a retrospective study, which may cause selection bias. Thirdly, the underlying mechanism of action of PLK4 in the malignant behavior of RCC cells requires further exploration, which was not included in the present study.

In conclusion, PLK4 possesses a certain clinical utility in reflecting the clinical stage of patients with RCC, while its prognostic value requires further validation.

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Availability of data and materials

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

Authors' contributions

WJ and JM conceived and designed the study. YZh, SZ and YZe were involved in performing the experiments, and collected and analyzed the data. WJ and JM confirm the authenticity of all the raw data. All authors wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Jing'an District Center Hospital of Shanghai, Fudan University, Shanghai, China [(2021) ethical approval no 13]. In addition, this was a retrospective study and the collected data were retrieved several years ago; therefore, the requirement for informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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