

# Polo-like kinase 4 is associated with advanced TNM stages and reduced survival and its inhibition improves chemosensitivity in colorectal cancer

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**Abstract.** High expression of polo-like kinase 4 (PLK4) promotes tumorigenesis and is correlated with poor prognosis in several kinds of cancer. However, the prognostic value of PLK4 in colorectal cancer (CRC) has not been elucidated. The aim of the present study was to investigate the association between PLK4 and the prognosis and effect of PLK4 inhibition on chemosensitivity in CRC. A total of 142 patients with CRC were enrolled, and 142 pairs of CRC and para-carcinoma tissues were used to measure PLK4 protein expression using immunohistochemistry (IHC). Among them, 69 pairs were used to detect PLK4 mRNA expression using reverse transcription-quantitative PCR. In addition, PLK4-small interfering RNA (siRNA) was transfected into CRC cells, followed by 5-fluorouracil (5-FU) treatment for it was a fundamental chemotherapy for CRC. In addition, western blotting was used to detect PLK4 protein expression among human colonic epithelial cell and human CRC cell lines, including HCT-116, LoVo, SW480 and HT-29, as well as nuclear translocation of  $\beta$ -catenin. The IHC score and mRNA expression of PLK4 were higher in CRC tissues compared with para-carcinoma tissues (both  $P < 0.001$ ). Furthermore, the IHC score of tumor PLK4 was not correlated with pathological grade ( $P = 0.585$ ), T stage ( $P = 0.357$ ), N stage ( $P = 0.107225$ ) or tumor-node-metastasis (TNM) stage ( $P = 0.093$ ). The mRNA expression of tumor PLK4 was positively correlated with N stage ( $P = 0.019$ ) and TNM stage ( $P = 0.004$ ), but not with pathological grade ( $P = 0.498$ ) or T stage ( $P = 0.112$ ). Of note, the high protein expression of tumor PLK4 was an independent factor for poor overall survival (OS;  $P = 0.048$ ). In addition, PLK4 was elevated in CRC cell lines; PLK4-siRNA reduced the 50% inhibitory concentration value

of 5-FU in HCT-116 ( $4.4 \pm 0.1 \mu\text{M}$  vs.  $7.6 \pm 1.4 \mu\text{M}$ ) and LoVo cells ( $5.5 \pm 0.6 \mu\text{M}$  vs.  $9.9 \pm 1.8 \mu\text{M}$ ) (both  $P < 0.05$ ). Besides, PLK4-siRNA decreased nuclear translocation of  $\beta$ -catenin. In conclusion, the high expression of tumor PLK4 was associated with advanced TNM stage and shorter OS in patients with CRC. In addition, targeting PLK4 improved chemosensitivity in CRC cells.

## Introduction

Colorectal cancer (CRC), a common digestive tract cancer, has the third highest incidence and second highest mortality rates among malignant carcinomas worldwide (1-3). Although technological advances in surgical resection, chemotherapy/chemoradiotherapy and immunotherapy have led to increases in CRC-related survival rate (4-7), tumor recurrence and metastasis still result in a poor prognosis in patients with CRC (8,9). Traditional clinicopathological parameters (i.e., pathological grade and tumor stage), tumor markers (i.e., carcinoembryonic antigen and defective DNA mismatch repair) and molecular biomarkers (i.e., adenomatous polyposis coli and vascular endothelial growth factor) have been used in the prognostic evaluation of CRC (10-12). To further improve the management of patients with CRC and prediction of the risk for relapse, identifying more potential predictors and therapeutic targets is critical.

Polo-like kinase 4 (PLK4), a serine/threonine kinase, is a regulator of centriole duplication through its autophosphorylation (13,14), while its aberrant expression induces centrosome duplication in cancer cells, and this is potentially associated with tumor progression (15,16). Indeed, PLK4 overexpression increases the proliferative and invasive ability of CRC cells through the Wnt/ $\beta$ -catenin signaling pathway (17). On the other hand, inhibiting PLK4 suppresses cell proliferation, migration and invasion in hepatocellular carcinoma (18). Meanwhile, PLK4 downregulation induces cell cycle arrest at the G1 phase by activating the p38/p53/p21 pathway in bladder cancer (19). In addition, the high PLK4 expression not only reflects an advanced cancer stage, but is also predictive of a poor prognosis in several cancer types, such as epithelial ovarian cancer, lung cancer and neuroblastoma (20-22). However, the association between PLK4 and CRC prognosis is not clear.

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In the present study, the PLK4 levels were compared between CRC tumor and paired adjacent tissues with the objective of investigating its potential in reflecting clinicopathological features, as well as its prognostic value in patients with CRC. The small interfering RNA (siRNA)-mediated downregulation of PLK4 was also induced to explore the effect of PLK4 on chemosensitivity in CRC cells.

## Materials and methods

**Patients.** In the present study, 142 patients with CRC (age ranged 42-80 years) who received surgical resection in our hospitals between July 2013 and June 2017 were retrospectively analyzed. The patient data from the hospital database were screened using the following criteria: i) Diagnosis of CRC based on pathological examination; ii) adult patients aged >18 years; iii) tumor-node-metastasis (TNM) stage I-III; iv) surgical treatment including laparoscopic surgery and radical excision; v) no neoadjuvant therapy; vi) surgically-removed CRC and para-carcinoma tissues were retrievable; vii) clinical data corresponding to surgical specimens of patients were available; and viii) follow-up records were accessible for survival assessment. This study was implemented with the approval of the Institutional Review Board of Xi'an International Medical Center Hospital, and all patient data were analyzed following anonymization; therefore, patient informed consent was waived by the Institutional Review Board.

**Acquisition of data and specimens.** Clinicopathological data were collected from the patients' medical records, and survival information, which was used for the evaluation of overall survival (OS), was obtained from the follow-up records. Formalin-fixed and paraffin-embedded (FFPE) specimens (CRC and para-carcinoma tissues) of 142 patients were collected from the specimen library to assess the protein expression of PLK4. Only 69 patients had fresh-frozen specimens stored in liquid nitrogen, which were also collected from the specimen library to evaluate PLK4 mRNA expression.

**Assessment of PLK4 protein expression.** Immunohistochemistry (IHC) was performed to assess the PLK4 protein expression, as previously reported (23). Briefly, the FFPE specimens were cut into slices, followed by deparaffinization and rehydration, followed by H<sub>2</sub>O<sub>2</sub> (Sigma-Aldrich) treatment at room temperature for 10 min to quench endogenous peroxidases. Next, heat-induced antigen retrieval was performed. Following blocking, the slices were incubated with anti-PLK4 antibody (1:5,000; PA5-80907; Thermo Fisher Scientific, Inc.) at 4°C overnight and HRP-conjugated goat anti-rabbit IgG H&L secondary antibody (1:60; 32460; Thermo Fisher Scientific, Inc.) at room temperature at 1 h. Diaminobenzidine (room temperature for 10 sec; Sigma-Aldrich) and hematoxylin (room temperature for 5 min; Sigma-Aldrich) were used for staining and counterstaining, respectively. Following IHC staining, PLK4 protein expression was assessed under a microscope (Eclipse Ti-U; Nikon Corporation). The IHC results were quantified by scoring the staining intensity and density as previously described (24). The staining intensity was scored as 0 (negative), 1 (weak), 2 (moderate) and 3 (strong), and the staining density of positive cells was scored

as 0 (0%), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). The representative images for each score of staining intensity and density in the IHC staining in tumor tissues were shown in Fig. S1. The IHC score was calculated by multiplying the two scores. Two pathologists assessed the IHC score independently. If the two pathologists gave different IHC scores for the same specimen, then the mean IHC score of this specimen was calculated and recorded.

**Evaluation of PLK4 mRNA expression.** Reverse transcription-quantitative PCR (RT-qPCR) was performed in 69 paired CRC and para-carcinoma tissues to determine PLK4 mRNA expression. Total RNA was extracted using RNeasy Protect Mini Kit (Qiagen GmbH) and then converted to cDNA (25°C for 3 min, 45°C for 10 min, 85°C for 5 min) using a QuantiNova Reverse Transcription Kit (Qiagen GmbH). RT-qPCR (95°C for 2 min, then 40 cycles of 95°C for 5 sec and 60°C for 10 sec) was conducted using a QuantiNova SYBR Green PCR Kit (Qiagen GmbH). The primers used in this study were designed as previously described (25) and listed as follows: forward primer for PLK4, 5'-CCTTATCACCTCCTCCTTC-3'; reverse primer for PLK4, 5'-CCAAGTCCTTCATTTGTAACC-3'; forward primer for GAPDH, 5'-ACATCATCCCTGCCTCTA C-3'; reverse primer for GAPDH, 5'-CCTGCTTACCACCT TCT-3'. PLK4 mRNA expression was analyzed using the 2<sup>-ΔΔC<sub>q</sub></sup> method (26) with GAPDH as an internal control.

**Chemosensitivity experiment.** A further *in vitro* experiment was conducted to verify the effect of PLK4 on the chemosensitivity of CRC cells to 5-fluorouracil (5-FU), since all stage III patients and most stage II patients received capecitabine monotherapy or XELOX (capecitabine combined with oxaliplatin) regimen following surgery. Human colonic epithelial cell (HCoEpic) (2950) was purchased from ScienCell Research Laboratories, Inc. Human CRC cell lines including HCT-116 (CBP60028) and LoVo (CBP60032) were purchased from Nanjing Cbioer Biotechnology Co., Ltd., SW480 (CCL-228) and HT-29 (HTB-38) were purchased from ATCC. The HCoEpic and SW480 cells were cultured in Leibovitz's L-15 medium (Gibco; Thermo Fisher Scientific, Inc.) with FBS. The HCT-116 and HT-29 cells were cultured in McCoy's 5a medium (Gibco; Thermo Fisher Scientific, Inc.) with FBS. The LoVo cells were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) with FBS. All cells were maintained in a humidified incubator supplied with 95% air and 5% CO<sub>2</sub> at 37°C. The PLK4-siRNA and corresponding negative control (NC) siRNA were designed by Shanghai GenePharma Co., Ltd. The sequence (5'→3') of PLK4 siRNA was: SS, ACACAUAUUGCUAUCUCAA; AS, ACACAUAUUGCUAUCUCAA. The sequence (5'→3') of NC siRNA was: SS, GAAUAAUUAAGAUGCCCGUUGUACU; AS, UCAUCGAAGUUAUAGGGAUACAUAACGUGAUC. PLK4-siRNA (50 pM) and NC-siRNA (50 pM) were respectively transfected into the HCT-116 and the LoVo cells using Lipofectamine™ 2000 Transfection Reagent (Thermo Fisher Scientific, Inc.) at 37°C for 48 h. Following transfection, the cells in each cell line were categorized as PLK4-siRNA, NC-siRNA and blank control cells (without transfection). The cells were then treated with 5-FU (Merck KGaA) at the following concentrations: 0, 1, 2, 4, 8 and 16 μM for 48 h, which was based on previous studies with

some modification (27,28). Following treatment, Cell Counting Kit-8 reagent (Beyotime Institute of Biotechnology) was added to the cells, followed by incubation at 37°C for 2 h. Finally, absorbance was measured at 450 nm using a microplate reader, and cell viability at different concentrations of 5-FU was calculated. In addition, the 50% inhibitory concentration (IC<sub>50</sub>) was calculated using Probit regression.

**Western blot.** The protein level of PLK4 in HCoEpic and CRC cells, as well as nuclear translocation of  $\beta$ -catenin in HCT-116 and LoVo cells after transfection were determined by western blot. Protein of the cells was extracted using a nucleoprotein extraction kit (Sangon Biotech Co., Ltd.) or RIPA reagent (Sangon Biotech Co., Ltd.) and quantified using an enhanced BCA protein assay kit (Beyotime Institute of Biotechnology). Subsequently, the protein (20  $\mu$ g) was separated using 4-20% SDS-PAGE, followed by transferring onto nitrocellulose membranes (Pall Life Sciences). Then, the membranes were blocked with 5% BSA (Sigma-Aldrich; Merck KGaA) at room temperature for 1 h, and incubated with primary antibodies [ $\beta$ -catenin antibody (Cell Signaling Technology, Inc; 8480; 1:1,000), histone H3 antibody (Cell Signaling Technology, Inc; 4499; 1:2,000), PLK4 antibody (Cell Signaling Technology, Inc; 71033, 1:1,000) and GAPDH (Cell Signaling Technology, Inc; 2118; 1:1,000)] at 4°C overnight, followed by an HRP-linked goat anti-rabbit IgG antibody (Cell Signaling Technology, Inc; 7074; 1:3,000) at room temperature for 1 h. Then, the brands were visualized with ECL-PLUS reagents (Thermo Fisher Scientific, Inc.) and analyzed by ImageJ software (v1.5; NIH).

**Statistical analysis.** High PLK4 protein expression was assigned an IHC score of >3, and low PLK4 protein expression an IHC score of  $\leq$ 3. The median PLK4 mRNA expression in CRC tissues was used to classify patients into the high and low PLK4 mRNA expression groups. The IHC score and mRNA expression of PLK4 were compared between CRC and para-carcinoma tissues using a paired t-test or Wilcoxon signed-rank test. The proportion of patients with a high and low PLK4 protein expression were compared between CRC and para-carcinoma tissues using the McNamar's test. The association between the PLK4 expression and tumor characteristics was analyzed using Kruskal-Wallis followed by Dunn's test. The OS was illustrated using a Kaplan-Meier curve and analyzed using a log-rank test. Multivariate Cox's proportional hazard model regression analysis was performed to identify prognostic factors. In the *in vitro* experiment, the PLK4 expression between the between HCoEpic and CRC cells, the PLK4 expression between groups after transfection, cell viability and  $\beta$ -catenin expression were analyzed using one-way ANOVA followed by Dunnett's or Tukey's multiple comparisons test; IC<sub>50</sub> between PLK4-siRNA and NC-siRNA cells were analyzed using a Student's t-test. P<0.05 was considered to indicate a statistically significant difference. Data analysis and graphing were conducted using SPSS 22.0 (IBM Corp.) and GraphPad Prism 7.01 (GraphPad Software Inc.).

## Results

**Baseline characteristics.** A total of 142 patients with CRC were enrolled in the present study [mean age, 65.3 $\pm$ 10.3 years; 55 (38.7%) females and 87 (61.3%) males]. Of those,

Table I. Clinical features of the patients.

Items	CRC patients (n=142)
Age (years), mean $\pm$ SD	65.3 $\pm$ 10.3
Gender, No. (%)	
Female	55 (38.7)
Male	87 (61.3)
Pathological grade, No. (%)	
Grade 1	21 (14.8)
Grade 2	99 (69.7)
Grade 3	22 (15.5)
Tumor size (cm), median (IQR)	4.5 (3.5-5.0)
LYN positive, No. (%)	
No	90 (63.4)
Yes	52 (36.6)
Number of LYN positive, median (IQR)	2.0 (1.0-4.0)
T stage, No. (%)	
T1	3 (2.1)
T2	15 (10.6)
T3	122 (85.9)
T4	2 (1.4)
N stage, No. (%)	
N0	90 (63.4)
N1	35 (24.6)
N2	17 (12.0)
TNM stage, No. (%)	
I	18 (12.7)
II	72 (50.7)
III	52 (36.6)
Adjuvant chemotherapy, No. (%)	
No	40 (28.2)
Yes	102 (71.8)

CRC, colorectal cancer; SD, standard deviation; IQR, interquartile range; LYN, lymph node; TNM, tumor-node-metastasis.

21 (14.8%) had pathological grade 1, 99 (69.7%) patients had grade 2 and 22 (15.5%) patients had grade 3 CRC. In addition, 3 (2.1%) patients had T1 stage, 15 (10.6%) patients had T2 stage, 122 (85.9%) patients had T3 stage and 2 (1.4%) patients had T4 stage CRC. A total of 90 (63.4%) patients had N0 stage, 35 (24.6%) patients had N1 stage and 17 (12.0%) patients had N2 stage CRC. Finally, 18 (12.7%) had TNM stage I, 72 (50.7%) stage II and 52 (36.6%) stage III CRC. Other detailed clinical features are shown in Table I.

**PLK4 is highly expressed in CRC tissues.** The PLK4 expression in the NC, para-carcinoma and CRC tissues was detected using IHC staining (Fig. 1A). In addition, the IHC score of PLK4 in CRC tissues was increased compared with that in para-carcinoma tissues (mean value: 5.2 $\pm$ 2.7 vs. 3.1 $\pm$ 2.2, P<0.001; Fig. 1B). In addition, a PLK4 IHC score of 3 was used as the cut-off value for determining high and low PLK4

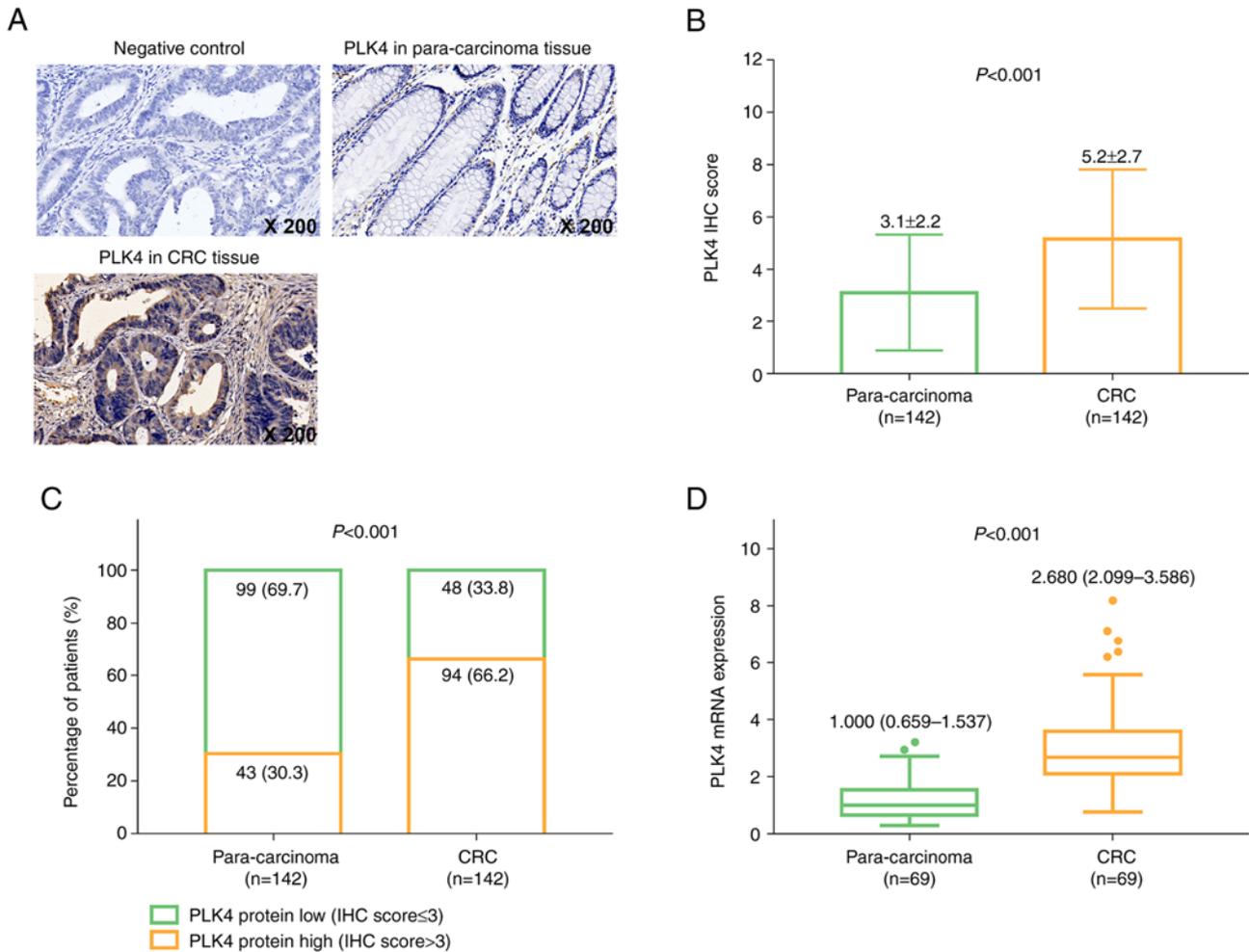


Figure 1. Comparison of PLK4 between para-carcinoma and CRC tissues. (A) IHC staining for PLK4 in the negative control, para-carcinoma and CRC tissues. (B) PLK4 IHC score in para-carcinoma and CRC tissues. Paired-samples t-test was applied. (C) Distribution of CRC patients with a different PLK4 expression in para-carcinoma and CRC tissues. P-value representing the difference of the proportion of PLK4 protein high and low expressions between para-carcinoma and CRC tissues. McNemar's test was applied. (D) PLK4 mRNA expression in para-carcinoma and CRC tissues. Wilcoxon signed-rank test was applied. PLK4, polo-like kinase 4; CRC, colorectal cancer; IHC, immunohistochemistry.

protein expression. Further analysis revealed that PLK4 protein expression was increased in CRC compared with para-carcinoma tissues ( $P < 0.001$ ; Fig. 1C). In addition, PLK4 mRNA expression in CRC tissues was elevated compared with that in para-carcinoma tissues [median, 2.680 (2.099-3.586) vs. 1.000 (0.659-1.537);  $P < 0.001$ ; Fig. 1D).

#### Tumor PLK4 is associated with advanced tumor properties.

Tumor PLK4 protein expression was not associated with pathological grade, T stage, N stage, or TNM stage (all  $P > 0.05$ ; Fig. 2A-D). Meanwhile, the high tumor PLK4 mRNA expression was associated with more advanced N stage ( $P = 0.019$ ) and TNM stage ( $P = 0.004$ ), but not with pathological grade or T stage (both  $P > 0.050$ ; Fig. 2E-H).

**Tumor PLK4 is correlated with poor prognosis.** A high tumor PLK4 protein expression was correlated with a short OS ( $P = 0.022$ ). The 1-, 3- and 5-year OS rates were 93.6, 75.5 and 61.7%, respectively, in patients with a high PLK4 protein expression, and 97.9, 87.5 and 77.1% in patients with a low PLK4 protein expression (Fig. 3A). Tumor PLK4 mRNA expression was not correlated with OS ( $P = 0.056$ ). The 1-,

3- and 5-year OS rate was 94.3, 77.1 and 62.9% in patients with a high PLK4 mRNA expression, and 97.1, 88.2 and 79.4% in patients with a low PLK4 mRNA expression (Fig. 3B).

In addition, multivariate Cox's proportional hazards regression analysis revealed that tumor PLK4 protein expression (high vs. low, HR=1.862,  $P = 0.048$ ), age ( $\geq 65$  years vs.  $< 65$  years; HR=2.226,  $P = 0.006$ ), higher pathological grade (HR=2.568,  $P < 0.001$ ), tumor size ( $\geq 5$  cm vs.  $< 5$  cm; HR=2.943,  $P < 0.001$ ), LYN positivity (yes vs. no; HR=2.473,  $P = 0.001$ ) were identified as independent factors for a poor OS (Fig. 4).

**PLK4-siRNA improves 5-FU sensitivity in CRC cells.** The protein level of PLK4 was increased in HCT-116, LoVo and SW480 cells compared with HCoEpic (all  $P < 0.05$ ; Fig. 5A and B); since it was increased more predominantly, the HCT-116 and LoVo cells were chosen for further experiments. In the HCT-116 cell line, PLK4-siRNA reduced mRNA level of PLK4 ( $P < 0.001$ ; Fig. 6A) as well as its protein level ( $P < 0.001$ , Fig. S2A and B); and it markedly reduced relative cell viability in cells treated with 2-16  $\mu\text{M}$  5-FU (all  $P < 0.05$ ). Meanwhile, the  $\text{IC}_{50}$  value of 5-FU in PLK4-siRNA cells was decreased compared with that in NC-siRNA cells ( $4.4 \pm 0.1 \mu\text{M}$  vs.  $7.6 \pm 1.4 \mu\text{M}$ ;  $P = 0.019$ ;

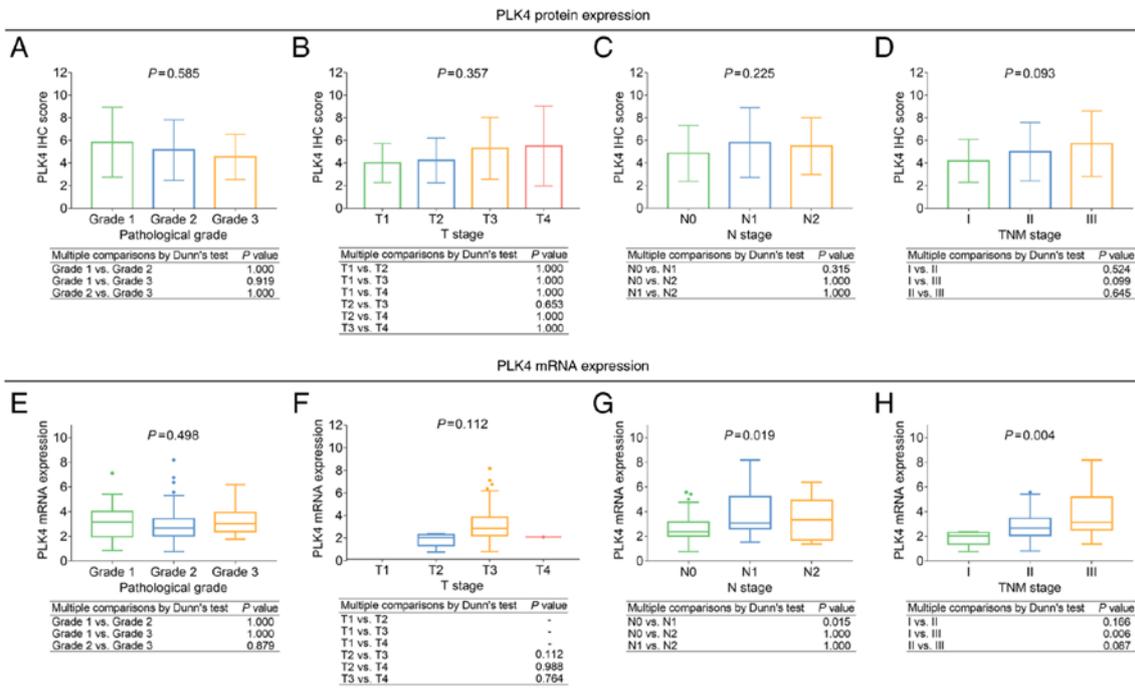


Figure 2. Correlation between tumor PLK4 expression and tumor features. Association between tumor PLK4 protein expression and (A) pathological grade, (B) T stage, (C) N stage or (D) TNM stage; the relationship of tumor PLK4 mRNA expression with (E) pathological grade, (F) T stage, (G) N stage or (H) TNM stage. Kruskal-Wallis followed by Dunn's test was applied. PLK4, polo-like kinase 4; IHC, immunohistochemistry; TNM, tumor-node metastases.

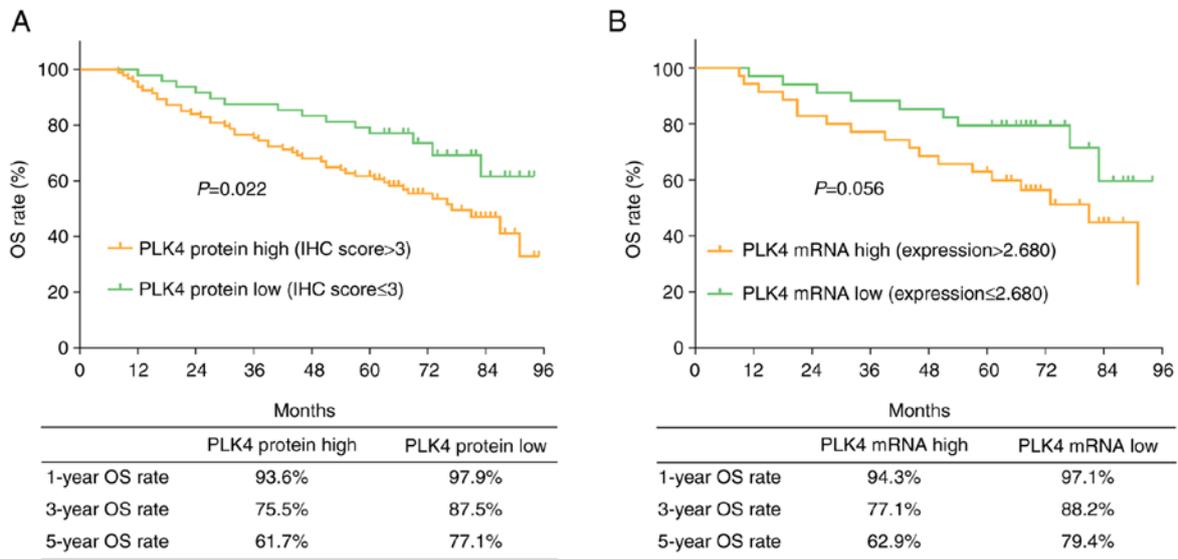


Figure 3. Correlation between tumor PLK4 and OS. Association between tumor (A) protein and (B) mRNA PLK4 expression and OS. Kaplan-Meier method by log-rank test was applied. PLK4, polo-like kinase 4; OS, overall survival.

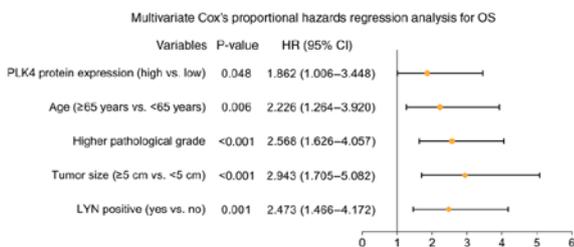


Figure 4. Multivariate Cox-regression analysis for OS. OS, overall survival; PLK4, polo-like kinase 4; HR, hazard ratio; CI, confidence interval; LYN, lymph node.

Fig. 6B and C). In the LoVo cell line, PLK4-siRNA also decreased mRNA level of PLK4 ( $P < 0.001$ ; Fig. 6D) as well as its protein level ( $P < 0.001$ , Fig. S2A and B). Besides, a relative decrease in cell viability was observed in PLK4-siRNA cells compared with NC-siRNA cells treated with 4-16  $\mu\text{M}$  5-FU (all  $P < 0.05$ ), and the  $\text{IC}_{50}$  value of 5-FU in PLK4-siRNA-transfected cells was reduced compared with that in NC-siRNA cells ( $5.5 \pm 0.6 \mu\text{M}$  vs.  $9.9 \pm 1.8 \mu\text{M}$ ;  $P = 0.014$ ; Fig. 6E and F). In addition, the nuclear translocation level of  $\beta$ -catenin was reduced in PLK4-siRNA-transfected cells was reduced compared with that in NC-siRNA cells (both  $P < 0.01$ ; Fig. 7A and B).

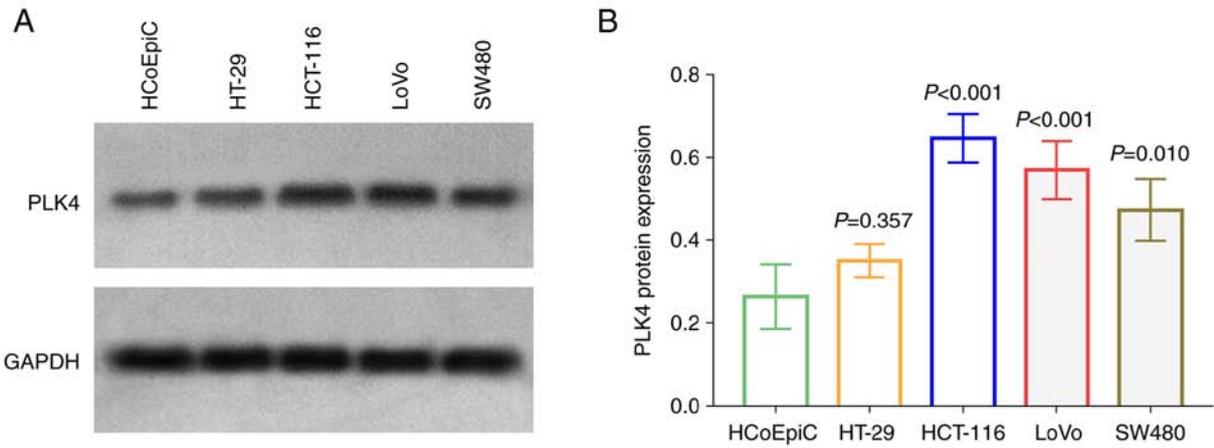


Figure 5. PLK4 expression in HCoEpic and CRC cells. (A) Representative images of PLK4 detection by western blot. (B) Comparison of PLK4 expression between HCoEpic and CRC cells. One-way ANOVA followed by Dunnett's multiple comparisons test was applied among HCoEpic, HT-29, HCT-116, LoVo and SW480 cells. PLK4, polo-like kinase 4; CRC, colorectal cancer.

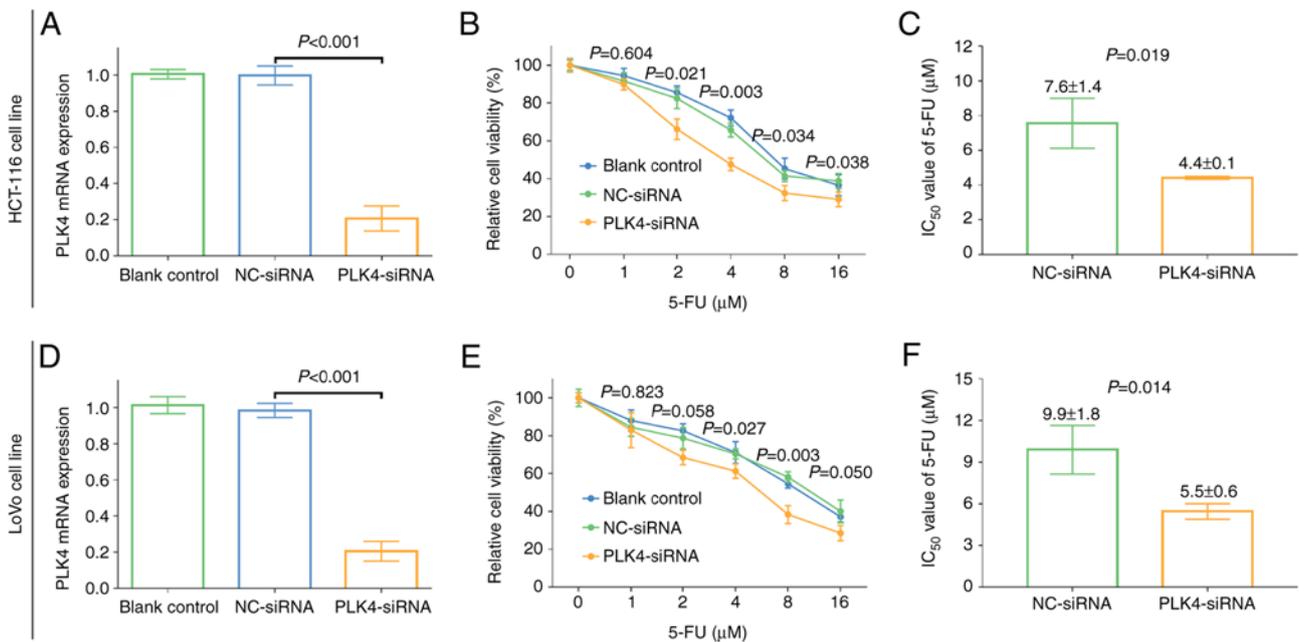


Figure 6. Effect of PLK4-siRNA on 5-FU sensitivity in CRC cell lines. (A) Comparison of the mRNA expression of PLK4 among blank control, NC-siRNA and PLK4-siRNA-treated HCT-116 cells. One-way ANOVA followed by Tukey's multiple comparisons test was applied. (B) Comparison of cell viability among blank control, NC-siRNA and PLK4-siRNA-treated HCT-116 cells groups. One-way ANOVA followed by Dunnett's multiple comparisons test was applied. (C) Changes in the  $\text{IC}_{50}$  value of 5-FU between NC-siRNA and PLK4-siRNA-treated HCT-116 cells. Student's t-test was applied. (D) Comparison of the mRNA expression of PLK4 among blank control, NC-siRNA and PLK4-siRNA-treated LoVo cells. One-way ANOVA followed by Tukey's multiple comparisons test was applied. (E) Comparison of cell viability among blank control, NC-siRNA and PLK4-siRNA-treated LoVo cell groups. One-way ANOVA followed by Dunnett's multiple comparisons test was applied. (F) Changes in the  $\text{IC}_{50}$  value of 5-FU between NC-siRNA and PLK4-siRNA-treated LoVo cells. Student's t-test was applied. PLK4, polo-like kinase 4; 5-FU, 5-fluorouracil; CRC, colorectal cancer;  $\text{IC}_{50}$ , 50% inhibitory concentration; siRNA, short interfering RNA; NC, negative control.

## Discussion

The PLK family members show distinct effect on cancer progression, among which PLK1 critically regulates cell cycles in various malignancies, while PLK4 may have less effect on this (29). Previous studies have implied that PLK4 may serve as a potential treatment target in cancers (30-32). Meanwhile, high PLK4 expression is associated with poor clinical and pathological features in cancer patients (23,25,33). For instance, high PLK4 is associated with LYN metastasis,

distant metastasis or surrounding recurrence in patients with breast cancer (25). Furthermore, high PLK4 expression is associated with a large tumor size, LYN metastasis and advanced TNM stage in patients with non-small cell lung cancer (23). In hepatocellular carcinoma patients, high PLK4 expression was associated with a more advanced TNM stage (34). However, the role of PLK4 in CRC has not been elucidated. In the present study, PLK4 protein and mRNA expression levels were higher in CRC compared with para-carcinoma tissues, while tumor PLK4 was positively

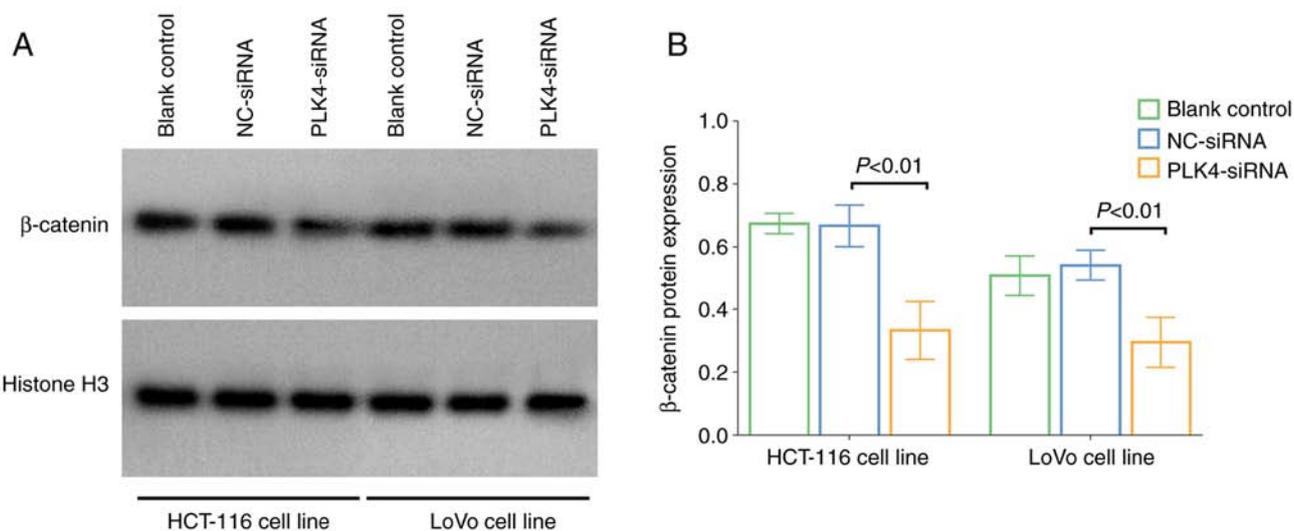


Figure 7. Effect of PLK4-siRNA on  $\beta$ -catenin nuclear translocation in CRC cell lines. (A) Detection of  $\beta$ -catenin in the nuclei of CRC cells. (B) Comparison of  $\beta$ -catenin in the nuclei of CRC cells. One-way ANOVA followed by Tukey's multiple comparisons test was applied. PLK4, polo-like kinase 4; CRC, colorectal cancer; siRNA, short interfering RNA; NC, negative control.

correlated with TNM stage, which was consistent with the results of previous studies on other tumors (23,34). There are several potential reasons for these findings. i) PLK4 reflected the increased proliferation rate of cells, which is a common characteristic of CRC cells but not of para-carcinoma cells; thus, PLK4 expression was upregulated in CRC tissues compared with para-carcinoma tissue. ii) PLK4 upregulation may cause centrosome amplification, which facilitates tumor progression (16). iii) PLK4 may promote CRC invasion and metastasis by regulating actin-related protein 2/3-mediated actin cytoskeleton or Tec kinase phosphorylation (35,36), and suppress CRC apoptosis through the Ataxia telangiectasia and Rad3-related-checkpoint kinase 1 signaling pathway (34,37). Meanwhile, CRC progression, invasion and metastasis may cause larger tumor size and lymph node metastasis, thus PLK4 was positively correlated with TNM stage in patients with CRC.

PLK4 is a potential predictor of poor outcomes in cancer patients (38,39). For instance, upregulated expression of PLK4 is correlated with worse progression-free survival and OS in breast cancer patients (25). Meanwhile, the high PLK4 mRNA expression was associated with a shorter OS in patients with high-grade glioma (35). Bladder cancer patients with high PLK4 expression have a lower OS than those with a low PLK4 expression (19). In the present study, tumor PLK4 protein expression was negatively correlated with OS, but tumor PLK4 mRNA expression was not. Furthermore, high tumor PLK4 protein expression was an independent predictive factor for a shorter OS. The reasons for this may be: i) the high expression of tumor PLK4 was correlated with a higher TNM stage, indirectly leading to a poor prognosis in patients with CRC; ii) PLK4 reduced chemosensitivity through inhibitor of NF- $\kappa$ B kinase subunit  $\epsilon$  (IKBKE) signaling to influence the efficacy of adjuvant chemotherapy, which can reduce the OS of patients with CRC (40); iii) PLK4 may promote CRC stemness and induce epithelial-mesenchymal transition by regulating the Wnt/ $\beta$ -catenin signaling pathway, increasing the risk of CRC recurrence (17,41).

It has been confirmed that suppressing PLK4 not only decreases viability of CRC cells, but also increases chemosensitivity in several types of cancer (17,39,40). For instance, PLK4 affects temozolomide (TMZ) sensitivity, while PLK4 inhibitor could enhance TMZ sensitivity through the phosphorylation of IKBKE in glioblastoma (40). PLK4 inhibitor increases conventional chemotherapeutic DNA-damaging agents (doxorubicin or etoposide) sensitivity in rhabdoid tumors and medulloblastomas (39). Considering that PLK4 is associated with a poor prognosis in patients with CRC, the effect of PLK4-siRNA on 5-FU sensitivity was evaluated. Consistent with previous studies, PLK4-siRNA enhanced the 5-FU susceptibility in the HCT-116 and LoVo cell lines. This may have been due to the fact that the suppression of PLK4 may increase the anti-tumor effect of 5-FU by inhibiting the Wnt/ $\beta$ -catenin signaling pathway, which is a key pathway causing chemoresistance in several cancer types (17,42). Furthermore, it was observed that PLK4 knockdown suppressed the nuclear translocation of  $\beta$ -catenin in CRC cells, which could explain the effect of PLK4 on chemosensitivity in CRC cells.

The present study had certain limitations: i) Only CRC patients with TNM stage I-III were recruited; therefore, our conclusion is not suitable for TNM stage IV patients. ii) Further proof on the potential mechanism of PLK4 in CRC progression is required. iii) The samples size of this study was somewhat small.

In conclusion, the high expression of tumor PLK4 is associated with an advanced TNM stage and shorter OS in patients with CRC. Therefore, targeting PLK4 improves chemosensitivity in CRC cells.

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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

WW and ZD contributed substantially to the conception and design of the study. LC and WW contributed substantially to the acquisition, analysis and interpretation of the data, and was involved in the drafting of the manuscript. ZD and WW confirm the authenticity of all the raw data. LC and JC contributed substantially to the interpretation of the data and was involved in the critical revisions of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

### Ethics approval and consent to participate

This study was conducted with approval from the Institutional Review Board of Xi'an International Medical Center Hospital (approval no. 2021013). All patients' data were analyzed following desensitization and therefore patient' informed consent was waved by the Institutional Review Board.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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