Article

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High Expression of PCBP3 Predicts Poor Prognosis of Colorectal Cancer

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Background: Poly(rC) Binding Protein 3 (PCBP3), a key member of the PCBP family, has been associated with several human malignancies, including pancreatic, prostate, and breast cancer. However, its potential role in colorectal cancer (CRC) requires further investigation.

Methods: We used immunohistochemical assays to measure PCBP3 expression levels in 382 CRC cases and matched adjacent non-tumor tissues. Subsequently, we evaluated the associations among PCBP3 expression, clinicopathological factors, and prognosis in individuals with CRC. Furthermore, Cell Counting Kit-8 (CCK-8) and colony-formation assays, cell-cycle analyses, and a nude mouse xenograft model were used to evaluate the function of PCBP3 in regulating CRC cell proliferation.

Results: PCBP3 levels were much higher in CRC tissues than in adjacent non-tumor tissues ($p < 0.001$) and were strongly correlated with tumor size ($p = 0.010$), invasion depth ($p = 0.006$), and tumor-nodes-metastases (TNM) stage ($p = 0.001$). The Kaplan-Meier curves showed a strong positive association between high PCBP3 expression and worse overall survival (OS) (log-rank: $p < 0.0001$) and disease-free survival (DFS) (log-rank: $p < 0.0001$). Cox regression indicated that upregulation of PCBP3 was independently predictive of worse OS ($p = 0.002$) and DFS ($p = 0.008$). Furthermore, overexpression of PCBP3 markedly enhanced CRC cell proliferation and subcutaneous tumor growth in nude mice, whereas PCBP3 knockdown had the opposite effect.

Conclusion: Taken together, we demonstrate for the first time that elevated PCBP3 expression correlates with poor prognosis in CRC and promotes tumor cell proliferation. These findings position PCBP3 as a potential prognostic biomarker and a promising therapeutic target in CRC.

Keywords: PCBP3; colorectal cancer; immunohistochemistry; prognosis; recurrence

Introduction

Colorectal cancer (CRC) is a prevalent malignancy worldwide and is associated with high incidence and mortality rates, posing a serious threat to human health and a significant burden on society [1]. According to GLOBOCAN 2022 data, CRC ranks third globally in terms of new cancer cases and second in terms of cancer-related deaths [2]. Given the significant advances in diagnostic techniques and treatment modalities over the past few years, the application of various therapeutic strategies, including surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy, has markedly improved survival rates in patients with early-stage CRC [3,4]. However, patients with advanced or recurrent CRC still face a poor prognosis [5]. Tumor recurrence, metastasis, and treatment resistance are critical factors leading to therapeutic failure. Identifying novel, reliable prognostic biomarkers and therapeutic targets is therefore crucial for achieving precision medicine and improving patient outcomes.

Poly(rC) Binding Protein 3 (PCBP3), also known as αCP-3 or hnRNP E3, is a key member of the PCBP family (including PCBP1-4) [6]. As an RNA-binding protein, PCBP3 participates in post-transcriptional gene regulation [7,8]. Research has demonstrated that PCBP3 is essential for regulating a range of biological processes, including development, iron metabolism, and glucose metabolism [9–12]. Further, several human disorders, including type 1 diabetes, Down syndrome, and pulmonary fibrosis, are influenced by PCBP3 [12–14]. It is noteworthy that PCBP3's role in tumors has attracted increasing attention. Studies have shown that PCBP3 is crucial in regulating the

onset and progression of several human malignancies, including pancreatic, cervical, breast, and prostate cancers [8,11,15,16]. In addition, PCBP3 expression in pancreatic cancer is strongly correlated with tumor stage and patient prognosis, suggesting it may serve as a predictive biomarker [17]. However, PCBP3's function in CRC and its prognostic and therapeutic utility remain poorly understood.

Thus, we investigated PCBP3 expression in CRC tissues and its association with clinicopathological characteristics and prognosis. Cellular and animal models were utilized to assess the functions of PCBP3 in CRC. The findings suggested the potential utility of PCBP3 in CRC prognosis and therapy.

Methods

Clinical Data Collection and Cell Culture

We consecutively collected tumor tissues and paired adjacent normal tissues from 382 CRC patients between January 1, 2018, and January 1, 2019. The inclusion criteria were as follows: (1) histologically confirmed primary CRC; (2) underwent curative-intent surgery; (3) age between 18 and 75 years at diagnosis. Patients were excluded if they met any of the following criteria: (1) had a concurrent malignancy at other sites; (2) received preoperative treatments such as chemotherapy, radiotherapy and immunotherapy; (3) the patients or their family members declined to participate in this study. Participants or their family members provided written informed consent. Patients were followed up from February 1, 2018, to August 1, 2025, and all clinical and follow-up data were maintained by dedicated personnel. This study was approved by the Ethics Committee of Tangdu Hospital, Fourth Military Medical University (Approval No. K-HG-202508-08) and was conducted in accordance with the ethical principles of the Declaration of Helsinki. All cell lines were supplied by China Cell Resource Center (Beijing, China), authenticated by STR profiling, and confirmed to be free of mycoplasma contamination. The CRC cell lines COLO320 (Cat# SCSP-5367), DLD1 (Cat# SCSP-5241), and HT29 (Cat# SCSP- 5032) were kept in RPMI 1640 media (Cat# 11875093, Gibco, Waltham, MA, USA); SW480 (Cat# SCSP-5033), LoVo (Cat# SCSP-514) and SW620 (Cat# SCSP-5234) were cultured in DMEM (Cat# 11965092, Gibco, Waltham, MA, USA). All media were kept in a cell culture incubator at 37 °C with 5% CO_2 and supplemented with 10% fetal bovine serum (Cat# A5256701, Gibco, Waltham, MA, USA).

Immunohistochemistry (IHC) Experiment

The IHC experiment was performed according to the previously described procedures [18]. The tissues from 382 cases of CRC, paired normal tissues, and nude mouse xenograft tumors were fixed in paraffin and sectioned (4 μm) before antigen retrieval by boiling in a citrate-sodium solution at pH 6.0 for 2 minutes. Following sequential incubation with 3% hydrogen peroxide solution and goat serum (Cat# L038, Solarbio, Beijing, China), the sections were then incubated overnight at 4 °C with anti-PCBP3 antibody (Cat# PA5-56413, 1:100, Thermo, Waltham, MA, USA) and Ki-67 antibody (Cat# ab15580, 1:1000, Abcam, Cambridge, UK). The secondary antibody labeled with horseradish peroxidase (Cat# RGAR011, neat, Proteintech, Wuhan, China) was added for 20 minutes at 37 °C, followed by DAB (Cat# 8059S, CST, Danvers, MA, USA) staining. Following hematoxylin staining of the cell nuclei, the samples were dehydrated through a graded alcohol series, cleared in xylene, and mounted with neutral balsam. The IHC staining results were independently interpreted by two blinded pathologists. The staining intensity and extent in five randomly chosen high-power fields (×40) were used to assess the IHC results. The extent of staining is scored as follows: 0–9% as 0, 10%–25% as 1, 26%–50% as 2, 51%–75% as 3, and 76%–100% as 4. The staining intensity is scored as follows: 0 = no staining; 1 = mild staining (light yellow); 2 = moderate staining (yellow); 3 = intense staining (darker or brownish yellow). The final score ranges from 0 to 12 and is obtained by multiplying the staining intensity and staining extent scores.

Western Blot Analysis

PCBP3 expression was detected using Western blot analyses. In summary, total proteins were initially extracted from CRC lines using RIPA lysis buffer (Cat# R0020, Solarbio, Beijing, China). The proteins were separated by SDS-PAGE and then transferred onto a PVDF membrane. After 1 hour of blocking with 5% skim milk, the blot membrane was incubated overnight at 4 °C with anti-PCBP3 antibody (Cat# PA5-56413, 1:1000, Thermo, Waltham, MA, USA) and the antibody against β-actin, the internal reference (Cat# ab8227, 1:5000, Abcam, Cambridge, UK). After rewarming, the blot membrane was incubated with an HRP-labeled secondary antibody (Cat# RGAR001, 1:10,000, Proteintech, Wuhan, China) for 1 hour at room temperature. Protein bands were then visualized using ECL Western blot detection solution (Cat# P0018S, Beyotime Biotechnology, Shanghai, China).

Overexpression and Knockdown of PCBP3

All gene modulation reagents were obtained from GenePharma (Shanghai, China). For PCBP3 overexpression, the coding sequence was cloned into the pcDNA3.1(+) vector, with the empty pcDNA3.1(+) vector serving as the negative control (**Supplementary Material**). For transient knockdown, four PCBP3-targeting siRNAs were utilized. Their sequences are: siPCBP3-1, 5′-AGAUGUUGAUCCUUGCACCTT-3′; siPCBP3-2, 5′-UUUCCAUGCAUCAGCAGGCTT-3′; siPCBP3-3, 5′-UCCUUGAUCUUGGAGCCUCTT-3′; and siPCBP3-4, 5′-AGAUCUGCUUGACGCACUGTT-3′. A siC-

trl (5′-ACGUGACACGUUCGGAGAATT-3′) served as the negative control. For stable knockdown, two shRNA sequences targeting PCBP3 were cloned into the LV3 (H1/GFP&Puro) vector. The target sequences are: shPCBP3-1, 5′-GCCTTTGCCATGATCGCATAC-3′ and shPCBP3-2, 5′-GCTGATGCATGGAAAG GAAGT-3′. The shCtrl (5′-TTCTCCGAACGTGTCACGT-3′) was used in parallel. The constructed plasmids were transfected into CRC cells using Lipofectamine 2000 (Cat# GK20005, GLPBio, Montclair, CA, USA), and the cells were selected with puromycin (Cat# HY-B1743, MCE, South Brunswick Township, NJ, USA). The transfection technique was consistent with the previously described method for transfecting CRC cells with plasmids [18].

Cell Counting Kit-8 (CCK-8) Assay

The CCK-8 assays were performed using a kit (Cat# C0038, Beyotime Biotechnology, Shanghai, China) according to the manufacturer's instructions. Briefly, 5000 CRC cells with either PCBP3 overexpression or knockdown, along with control groups, were seeded in 96-well plates. 10 µL of CCK-8 solution was added to each well after 1, 2, 3, and 4 days of culture, and after continued incubation for 2 hours, absorbance at 450 nm was measured using a microplate reader (Infinite 200Pro, Tecan, Männedorf, Zurich, Switzerland). All OD 450 nm values were normalized to the mean value of each group on Day 1 and presented as relative proliferation compared with baseline. Each experiment was independently conducted three times.

Plate Cloning Assay

After being distributed equally into 6-well plates at a concentration of 1000 cells per well, CRC cells in the logarithmic growth phase were cultured for 14 days. Afterwards, the cells were fixed with 4% paraformaldehyde and stained with crystal violet solution. Each group's cell clone count was determined.

Cell Cycle Experiment

Cell cycles were found using the Cell Cycle Detection Kit (Cat# 550825, BD Bioscience, San Jose, CA, USA). The obtained cells were preserved overnight at 4 °C in 70% ethanol. The cells were then resuspended in 500 µL of PI/RNase Staining Buffer and incubated for 30 minutes at 4 °C in the dark. The Flow cytometer (CytoFLEX V2-B5-R3, Beckman Coulter, Brea, CA, USA) was used to examine the results.

Nude Mouse Xenograft Experiment

A total of 20 BALB/c nude mice (Cat# 401, female, 6–8 weeks old) from Beijing Vital River Laboratory Animal Technology Co., Ltd. were used in the xenograft model study. All animal experiments were approved by the Ethics Committee of Tangdu Hospital, Fourth Military Medical University (Approval No. 20250295). The mice were randomly divided into five groups (n = 4/per group): the control groups (shCtrl and EV); the PCBP3 knockdown group (shPCBP3-1 and shPCBP3-2), and the PCBP3 overexpression group (PCBP3-OE). A suspension containing Matrigel (Cat# 356234, Corning, Corning, NY, USA) and 1×10^7 SW480 cells was subcutaneously injected into the scapular region of each mouse. Tumor dimensions were measured every four days using vernier calipers, and the volume was calculated as (length $\times$ width^2)/2. Thirty days post-inoculation, all mice were deeply anesthetized with 5% isoflurane (Cat# R510-22, RWD, Shenzhen, China) administered via an anesthesia vaporizer, and then euthanized by cervical dislocation. Fresh tumor tissues were collected for subsequent IHC analysis.

Statistical Analysis

All statistical analyses were performed using SPSS 26.0 (IBM, Armonk, NY, USA). The differences in IHC scores between paired malignant and adjacent non-tumor tissues were examined using the Wilcoxon signed-rank test. Comparisons between two groups of normally distributed data were performed using a Student's *t*-test, while comparisons among multiple groups were analyzed using one-way ANOVA with Dunnett's or Tukey's post-hoc test. Chi-square tests were used to investigate relationships among categorical variables. The Cox proportional hazards model was used to evaluate independent predictors of patient survival, with multicollinearity among all variables assessed by variance inflation factor (VIF) analysis. The Kaplan-Meier method produced survival curves, and the log-rank test was used to assess differences between the two groups.

Results

Detection of PCBP3 Levels in CRC and Adjacent Non-Tumor Tissues

To investigate whether PCBP3 has relevant functions in CRC, we first assessed PCBP3 protein levels in human CRC tissues and adjacent non-cancerous tissues. Fig. 1A,B show that the PCBP3 protein was expressed in the cytoplasm of cells. The level of PCBP3 protein expression in human CRC tissues was substantially higher than that in nearby non-tumor tissues ($p < 0.001$) (Fig. 1C). These findings show that PCBP3 expression is markedly increased in CRC tissues, suggesting a potential association with CRC tumorigenesis.

Relationships Between Clinicopathological Features and PCBP3 Levels

To further assess PCBP3's function in CRC, we next examined whether PCBP3 expression correlated with clinicopathological characteristics in CRC patients. Based on IHC scores of tumor tissues, patients were dichotomized into a PCBP3-high (IHC score >6, n = 191) and a PCBP3-low (IHC score ≤ 6, n = 191) expression group. The results

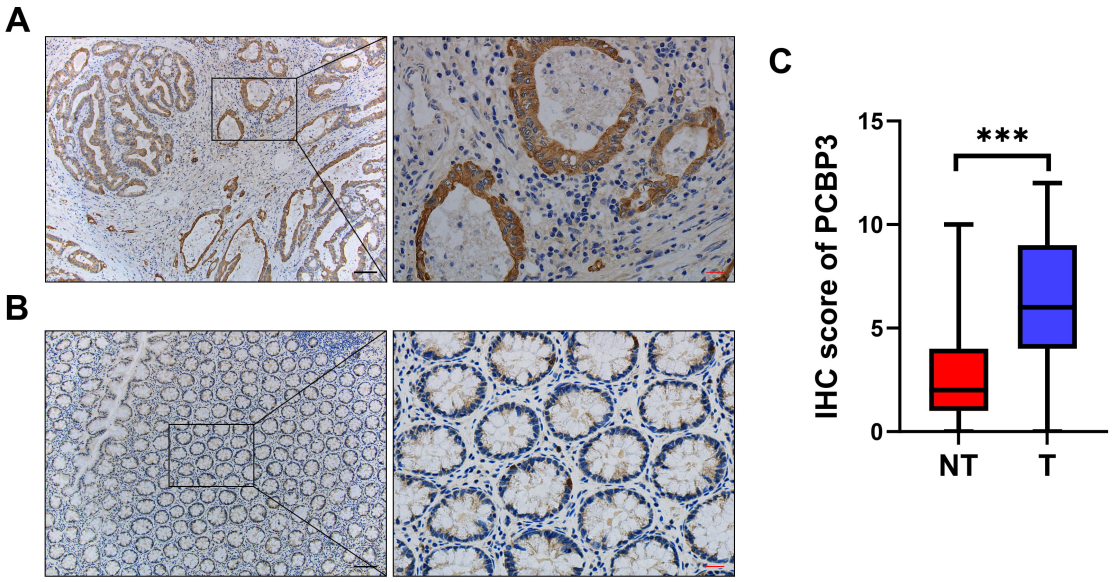


Fig. 1. PCBP3 expression is elevated in CRC tissues. (A,B) To determine PCBP3 levels in CRC and paired non-cancerous tissues, IHC staining was performed. A low-power field can be observed in the left image, while a high-power field can be observed in the right image. Red scale bar: 20 µm, black scale bar: 100 µm. (C) Levels of PCBP3 were evaluated between CRC and neighboring non-tumor tissues. $N = 382$, ***$p < 0.001$. PCBP3, Poly(rC) Binding Protein 3; CRC, colorectal cancer; IHC, immunohistochemistry.

are shown in Table 1, demonstrating that PCBP3 expression significantly correlated with tumor size ($p = 0.010$), tumor-nodes-metastases (TNM) stage ($p = 0.001$), and depth of invasion ($p = 0.006$) in CRC patients. However, no significant correlations were observed with patients' gender, age at onset, lymph node metastasis, distant metastasis, differentiation grade, or tumor location.

The Relationship Between CRC Patients' Overall Survival (OS) and PCBP3 Expression

The median follow-up for the entire cohort, calculated using the reverse Kaplan-Meier method, was 43 months (95% CI: 40.6–45.4). The Kaplan-Meier analysis revealed that patients with high PCBP3 expression had a significantly shorter OS (log-rank: $p < 0.0001$), corresponding to 25 and 67 cancer-related deaths in the PCBP3-low and PCBP3-high groups, respectively (Fig. 2). In addition, univariate analysis showed that poor patient prognosis was significantly positively associated with high expression of PCBP3 ($p < 0.001$), depth of invasion ($p < 0.001$), tumor size ($p = 0.005$), and TNM stage ($p < 0.001$), but not with patient age, gender, or tumor location. Furthermore, multivariate analysis showed significant correlations between shorter patient OS and high PCBP3 expression ($p = 0.002$), tumor invasion depth ($p < 0.001$), tumor size ($p = 0.008$), and TNM stage ($p = 0.009$) (Table 2). Moreover, VIF analysis confirmed no significant multicollinearity among these variables, with all VIF values at 1.02–1.12. These findings suggest that high expression of PCBP3 can be considered as an independent factor affecting poor prognosis in CRC patients.

Relationship Between Disease-Free Survival (DFS) and PCBP3 Expression in CRC

Since high PCBP3 expression was positively correlated with poor CRC prognosis and independently predictive of unfavorable outcome, the following analysis will examine the association between PCBP3 levels and DFS. Patients in the PCBP3-high group had markedly reduced DFS (log-rank: $p < 0.0001$), as shown in Fig. 3. Ninety-seven cases of tumor recurrence were observed in the PCBP3-high group, while 48 cases occurred in the PCBP3-low group. It was also observed that the median DFS time was 36.00 months in the PCBP3-high group. In comparison, the PCBP3-low group did not reach the median survival at the last follow-up. Univariate analysis showed that PCBP3 expression ($p < 0.001$), depth of invasion ($p < 0.001$), tumor size ($p = 0.024$), and TNM stage ($p < 0.001$) were strongly associated with DFS, supporting the OS data. In addition to depth of invasion ($p < 0.001$), tumor size ($p = 0.034$), and TNM stage ($p < 0.001$), multivariate analysis showed that PCBP3 levels were independently predictive of DFS ($p = 0.008$) (Table 3). These findings suggest that individuals with elevated PCBP3 expression face a higher risk of tumor recurrence.

Overexpression of PCBP3 Promotes CRC Cell Proliferation

According to the aforementioned results, larger CRC tumors, deeper tumor infiltration, and higher TNM stages were all positively and significantly associated with increased PCBP3 expression. Therefore, we next investigated whether PCBP3 promotes CRC cell proliferation.

Table 1. Relationship between PCBP3 expression and clinic features of CRC patients.

Variables	No. of cases	PCBP3 expression		χ^2	p value
		High	Low		
All	382	191	191		
Age				3.031	0.082
<60	199	91	108		
≥60	183	100	83		
Gender				0.100	0.752
Female	147	72	75		
Male	235	119	116		
Tumor site				3.029	0.082
Colon	197	90	107		
Rectum	185	101	84		
Tumor size				6.551	0.010
<5.0 cm	197	111	86		
≥5 cm	185	80	105		
Differentiation grade				4.731	0.094
Well	48	28	20		
Moderately	274	140	134		
Poor	60	23	37		
Depth of invasion				7.637	0.006
T1+T2	195	84	111		
T3+T4	187	107	80		
Lymph node metastasis				0.853	0.356
Absent	177	84	93		
Present	205	107	98		
Distant metastasis				2.301	0.129
Absent	332	161	171		
Present	50	30	20		
TNM stage				10.729	0.001
I+II	186	77	109		
III+IV	196	114	82		

PCBP3, Poly(rC) Binding Protein 3; CRC, colorectal cancer; TNM, tumor-nodes-metastases; p value, using the Pearson χ^2 test to evaluate the correlation between PCBP3 expression and clinic features, and p value < 0.05 was considered statistically significant.

Table 2. Association of PCBP3 and clinical factors with OS of CRC patients.

	Unadjusted HR (95% CI)*	p	Unadjusted HR (95% CI)†	p
PCBP3 high expression	2.781 (1.756–4.404)	<0.001	2.149 (1.329–3.477)	0.002
Gender	0.925 (0.610–1.404)	0.715	-	-
Age at diagnosis	0.777 (0.514–1.173)	0.230	-	-
Tumor site	1.227 (0.813–1.851)	0.330	-	-
Tumor size	1.840 (1.205–2.811)	0.005	1.786 (1.161–2.746)	0.008
Depth of invasion	4.821 (2.952–7.873)	<0.001	3.399 (2.038–5.668)	<0.001
TNM stage	3.339 (2.064–5.402)	<0.001	1.968 (1.183–3.275)	0.009

*Hazard ratios in univariate models;

†Hazard ratios in multivariable models.

OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

We selected the CRC cell lines SW480 and SW620 with moderate PCBP3 expression to investigate the effects of PCBP3 overexpression and knockdown on cell proliferation (Fig. 4A). As shown in Fig. 4B, Western blot results demonstrated the successful construction of PCBP3-overexpressing cell lines, which can be used for further studies. PCBP3 overexpression markedly accelerated the proliferation of SW480 ($p < 0.001$) and SW620 ($p < 0.01$) cells, indicated by CCK-8 assays (Fig. 4C). Colony formation assays confirmed that CRC cells overexpressing PCBP3 exhibited substantially enhanced clonogenic ability ($p < 0.001$) (Fig. 4D). Cell cycle analysis revealed that the PCBP3 overexpression group exhibited a marked decrease in the proportion of G1 phase cells ($p < 0.001$), ac-

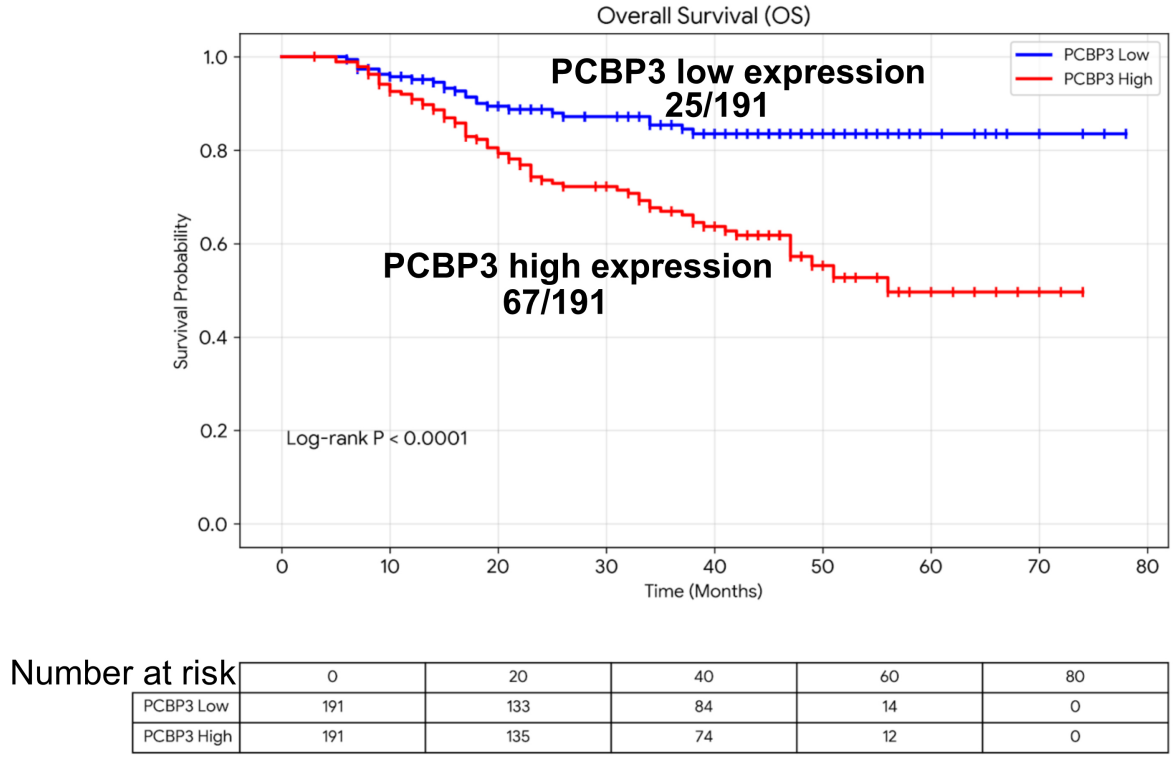


Fig. 2. The impact of PCBP3 expression levels on OS in CRC patients is depicted in the Kaplan-Meier graph. Log-rank tests were applied for comparisons.

Table 3. Association of PCBP3 and clinical factors with DFS of CRC patients.

	Unadjusted HR* (95% CI)	*p*	Unadjusted HR† (95% CI)	*p*
PCBP3 high expression	2.159 (1.527–3.052)	<0.001	1.634 (1.139–2.346)	0.008
Gender	0.890 (0.639–1.240)	0.490	-	-
Age at diagnosis	0.764 (0.550–1.061)	0.108	-	-
Tumor site	1.027 (0.741–1.422)	0.873	-	-
Tumor size	1.460 (1.051–2.026)	0.024	1.434 (1.028–2.001)	0.034
Depth of invasion	4.680 (3.212–6.818)	<0.001	3.666 (2.483–5.412)	<0.001
TNM stage	2.918 (2.029–4.199)	<0.001	1.927 (1.314–2.827)	<0.001

*Hazard ratios in univariate models;

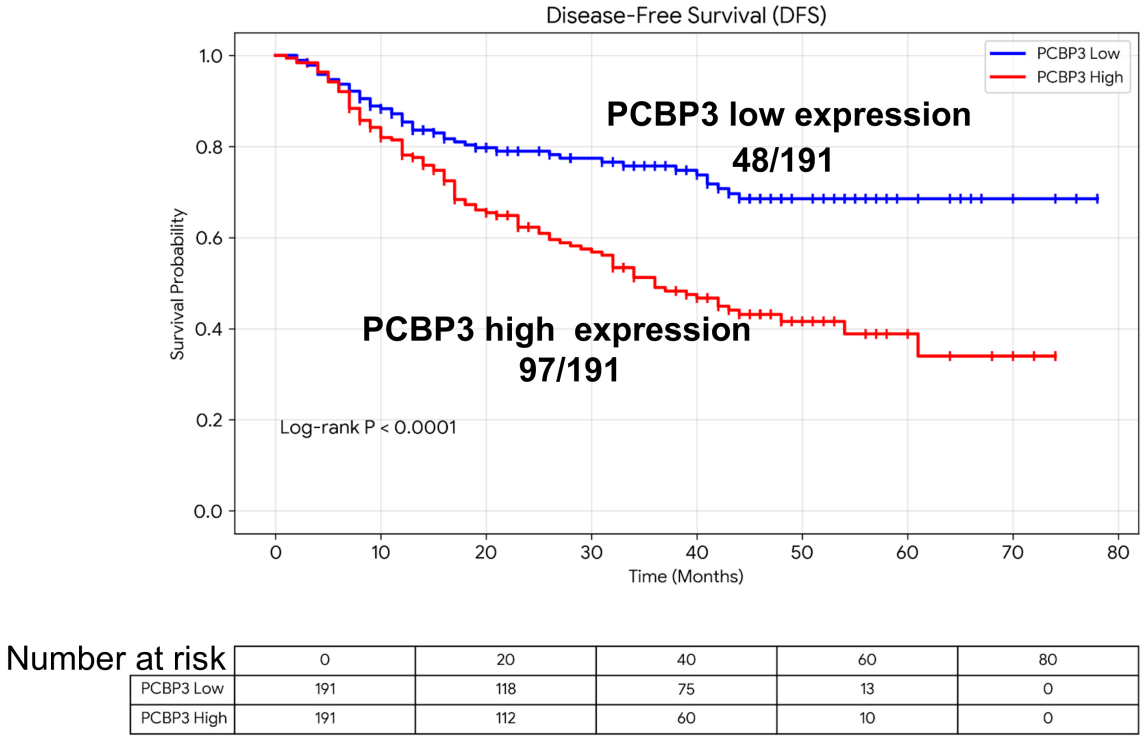
†Hazard ratios in multivariable models.

DFS, disease-free survival; HR, hazard ratio; 95% CI, 95% confidence interval.

companied by significant increase in the proportion of S phase cells ($p < 0.001$) (Fig. 4E). The mouse xenograft models showed that PCBP3 overexpression substantially enhanced subcutaneous tumor growth ($p < 0.001$) (Fig. 4F). Tumor tissues from the PCBP3 overexpression group exhibited a considerably higher percentage of Ki67-positive cells ($p < 0.001$), an indication of cell proliferation, than those from the control group (Fig. 4G). The findings suggest that PCBP3 promotes CRC cell proliferation in both cellular and animal models.

Knockdown of PCBP3 Blocks CRC Cell Proliferation

To explore the effects of PCBP3 knockdown, we employed both transient siRNA transfection to investigate immediate phenotypic consequences and stable shRNA-mediated silencing to facilitate long-term functional assays. As illustrated in Fig. 5A, Western blotting revealed that siPCBP3-2 and siPCBP3-4 exhibited the strongest silencing effects among the four siRNA sequences targeting PCBP3 in SW480 and SW620 cells ($p < 0.001$). Western blotting also validated the successful construction of shPCBP3-1 and shPCBP3-2 in both SW480 and SW620 cell lines ($p < 0.001$) (Fig. 5B). As shown in Fig. 5C, CCK-8 assay revealed that in SW480, proliferation was significantly inhibited in shPCBP3-1 and shPCBP3-2 ($p < 0.001$); in SW620, shPCBP3-1 ($p < 0.0001$) and shPCBP3-2 ($p < 0.001$) also showed significant inhibition. Similarly, colony formation was significantly reduced in the shPCBP3



Number at risk

	0	20	40	60	80
PCBP3 Low	191	118	75	13	0
PCBP3 High	191	112	60	10	0

Fig. 3. Kaplan-Meier curves indicating the influence of PCBP3 levels on DFS in CRC patients. Log-rank tests were applied for comparisons.

knockdown group ($p < 0.01$) (Fig. 5D). Cell cycle analysis revealed that the siPCBP3 knockdown increased the proportion of cells in the G1 phase compared with controls ($p < 0.001$). In contrast, the proportion in S phase was markedly decreased ($p < 0.001$) (Fig. 5E). This phenotype was also confirmed in HCT116, another CRC subtype cell line ($p < 0.001$) (**Supplementary Fig. 1**). This indicates that PCBP3 knockdown causes cell cycle arrest, thereby inhibiting cell division. Moreover, the shPCBP3 knockdown group showed a markedly reduced capacity to generate xenograft tumors ($p < 0.001$) (Fig. 5F). In contrast to the control group, tumor tissues from the shPCBP3 knockdown group exhibited a significantly lower percentage of Ki67-positive cells ($p < 0.001$) (Fig. 5G). These findings demonstrate that PCBP3 knockdown reduces CRC cell proliferation in both cellular and animal models.

Discussion

Previous research suggests that PCBP3 plays a critical role in the onset and progression of numerous cancers, including pancreatic, breast, prostate, and cervical cancers [8,11,15,16]. Moreover, PCBP3 expression in pancreatic cancer is strongly correlated with tumor stage and patient outcome, suggesting it may be useful as a predictive biomarker [17]. Unfortunately, the relevance of PCBP3 in CRC remains unknown. This investigation is the first to examine PCBP3 expression in CRC tissues and its association with clinicopathological features and patient prognosis. Functional tests conducted *in vitro* and *in vivo* provided preliminary evidence of PCBP3's biological significance in CRC development.

According to our findings, the CRC tissues showed significantly higher PCBP3 expression than the adjacent non-tumor tissues. This suggests that PCBP3 may be involved in CRC tumorigenesis. In individuals with CRC, high PCBP3 expression was linked with tumor size, invasion depth, and TNM stage. Meanwhile, patients in the PCBP3-high group showed significantly poorer OS and DFS rates than those in the PCBP3-low group. High PCBP3 levels were independently predictive of prognosis, as shown by multivariate Cox regression. These results suggest that PCBP3 may be involved in CRC tumorigenesis and be useful for predicting prognosis.

Our additional investigation into the biological roles of PCBP3 in CRC cells showed that overexpression of PCBP3 greatly increases CRC cell proliferation. Furthermore, the *in vivo* xenograft tumor model demonstrated that PCBP3 can significantly accelerate CRC cell growth. On the other hand, PCBP3 knockdown inhibited CRC cell proliferation and suppressed tumor growth in nude mice. The aforementioned findings suggest that PCBP3 may contribute to CRC cell proliferation, thereby playing a biological role in accelerating the disease's progression, and that targeting PCBP3 expression or function may represent a novel therapeutic strategy. This outcome aligns with Nguyen *et al.*'s findings [15] that PCBP3 can function as

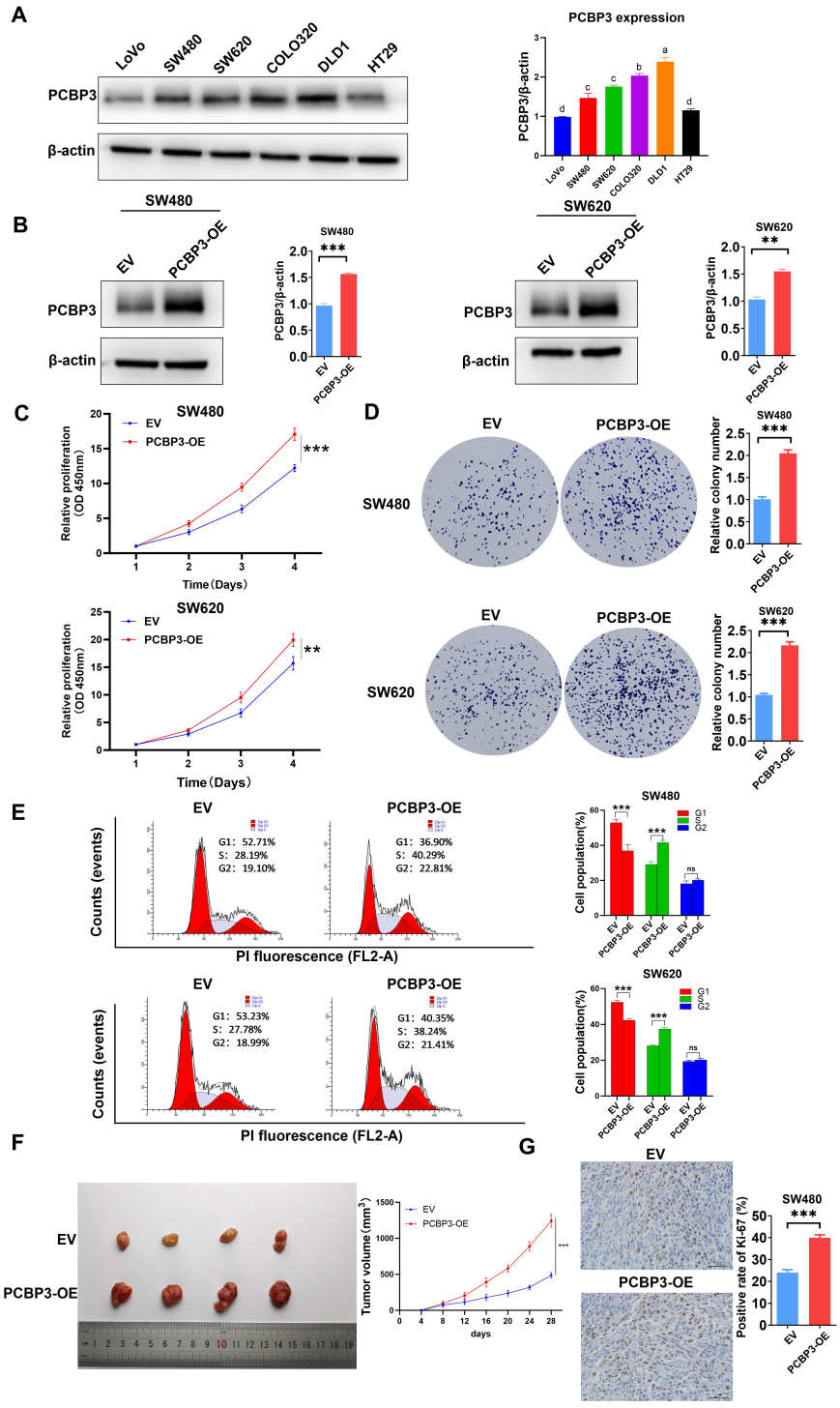


Fig. 4. PCBP3 overexpression stimulates tumorigenic behavior in both cellular and animal models. (A) PCBP3 protein expression levels in several CRC cell lines. Groups labeled with different letters indicate statistically significant differences, $p < 0.05$. (B) Western blot analysis was performed to detect PCBP3 protein expression in CRC cell lines SW480 and SW620 after transfection with PCBP3-OE. (C) The proliferation of CRC cells after PCBP3 overexpression was assessed using CCK-8 assays. (D) Colony formation assays showing the colony-forming ability after PCBP3 overexpression. (E) The impact of PCBP3 overexpression on cell cycle progression. (F) Comparison of tumor sizes and proportions of Ki-67-positive cells (G) in CRC tissues between the PCBP3 overexpression and control groups (n = 4). Scale bar, 50 μm. PCBP3-OE, Poly(rC) Binding Protein 3 overexpression vector; EV, empty vector; CCK-8, Cell Counting Kit-8. $^{**}p < 0.01$, $^{***}p < 0.001$, and "ns" means not significant.

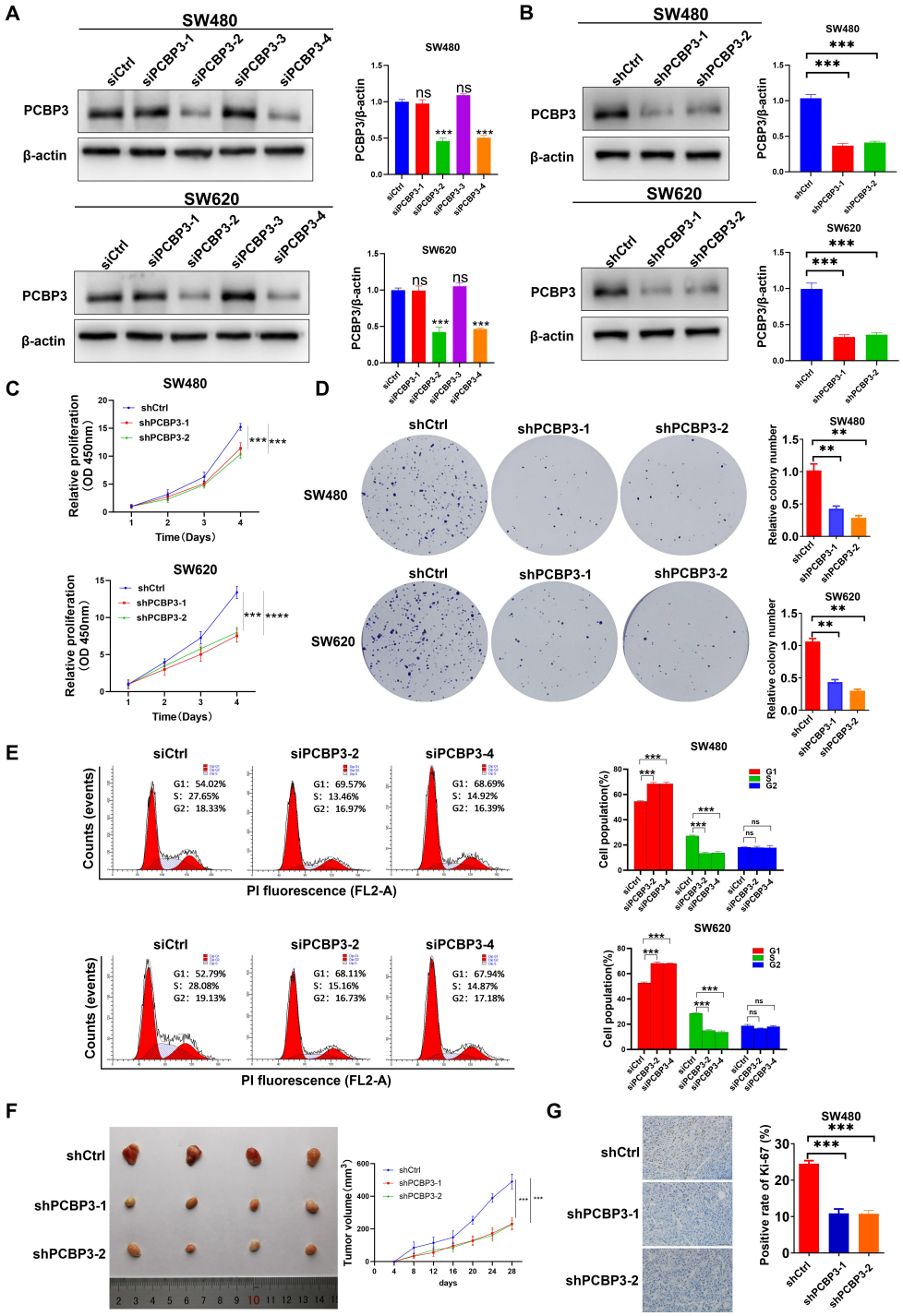


Fig. 5. Inhibitory effects of PCBP3 knockdown on CRC tumorigenic behavior in cellular and animal models. (A) The best siRNA sequence targeting PCBP3 in CRC cell lines SW480 and SW620 was identified using Western blotting. (B) The shRNA sequence targeting PCBP3 in CRC cell lines SW480 and SW620 was identified using Western blotting. (C) CCK-8 assays were used to assess CRC cell proliferation following PCBP3 knockdown. (D) The ability to form colonies after PCBP3 knockdown was assessed using colony-forming assays. (E) Effects of PCBP3 knockdown on cell cycle progression. (F) Comparison of tumor sizes and proportions of Ki-67-positive cells (G) in CRC tissues between the PCBP3 knockdown group and the control group of nude mice (n = 4). Scale bar, 50 µm. shPCBP3, CRC cells transfected with shRNA against PCBP3; shCtrl, CRC cells transfected with empty vector. siPCBP3, CRC cells transfected with small interfering RNA targeting PCBP3; siCtrl, CRC cells transfected with scrambled siRNA as a control. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, and "ns" means not significant.

a predictive biomarker for fostering prostate cancer resistance after HSP90 inhibitor therapy, indicating a bad prognosis for prostate cancer. However, in pancreatic cancer, PCBP3 expression is positively correlated with better patient prognosis [17]. This may be attributed to the distinct biological functions of PCBP3 in different tumor types, which require further exploration in subsequent studies.

According to earlier research, PCBP3 is involved in several biological processes, including iron metabolism, translational control, and mRNA stability [9–12]. PCBP3 may regulate CRC progression through the aforementioned mechanisms. Due to the distinct biological characteristics of different tumors, whether other regulatory mechanisms play a role in CRC requires further investigation.

Despite a marked association being observed between PCBP3 upregulation and poor patient prognosis, this study has several limitations. First, the study is retrospective, single-center, and has a limited sample size. To confirm PCBP3's clinical utility, further extensive, multicenter prospective cohort studies are required. Second, this study focused on the role of PCBP3 in regulating the proliferative phenotype, its potential functions in tumor cell metastasis or stemness maintenance remain unexplored. Third, the specific biological mechanisms underlying PCBP3 regulation in CRC progression remain poorly understood and warrant further investigation.

Conclusion

This study is the first to demonstrate that PCBP3 is substantially expressed in CRC tissues, and its high expression is positively correlated with poor prognosis. PCBP3 may participate in the occurrence and progression of CRC by regulating the proliferation of CRC cells. Thus, PCBP3 can not only serve as a potential biomarker for predicting the prognosis of CRC patients, but also hold promise as a novel therapeutic target for CRC treatment.

Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding authors upon reasonable request.

Author Contributions

Conceived and designed the analysis: NW, GW. Collected the clinical data: XF, PG, TS, LN, XH. Performed the clinical data analysis: XL, LT. Conducted the experiments and performed statistical analysis: XF, PG, TS, LN, XH. Wrote the paper: XF, PG, NW, GW. All authors participated in critical revision of the manuscript. All authors have agreed to publish the final version of the manuscript, take responsibility for all aspects of the work, and ensure the accuracy or completeness of any part of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of Tangdu Hospital, Fourth Military Medical University (Approval No. K-HG-202508-08) and was conducted in accordance with the ethical principles of the Declaration of Helsinki. All animal experiments were approved by the Ethics Committee of Tangdu Hospital, Fourth Military Medical University (Approval No. 20250295). All procedures complied with the ARRIVE Guidelines 2.0.

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202638209.144.

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