

Knockdown of lncRNA PVT1 inhibits the proliferation and accelerates the apoptosis of colorectal cancer cells via the miR-761/MAPK1 axis

YUJING LIU^{1*}, YONGYOU WU^{2*}, ZHU ZHU¹, JIANGBO GONG¹ and WENHUAN DOU^{1,3}

¹Department of General Surgery, The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University; ²Department of General Surgery, The Second Affiliated Hospital of Soochow University,

³Department of Gastroenterology, The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University, Suzhou, Jiangsu 215000, P.R. China

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Abstract. Colorectal cancer (CRC) is associated with high morbidity rates. Long non-coding RNAs (lncRNAs) participate in the development of CRC. However, the potential roles of lncRNA plasmacytoma variant translocation 1 (PVT1) in CRC remain unknown. Therefore, the aim of the present study was to investigate the potential roles of PVT1 in CRC. Reverse transcription-quantitative PCR and western blot analyses were conducted to determine the mRNA and protein expression levels. The cellular behaviors were detected using 5-Ethynyl-2'-deoxyuridine, Cell Counting Kit-8 and flow cytometry assays. The interaction between PVT1 and microRNA (miR)-761 or MAPK1 was confirmed using a dual-luciferase reporter assay. Moreover, the Pearson's method was applied for correlation analysis. The results demonstrated that the expression levels of PVT1 and MAPK1 were upregulated, while miR-761 was downregulated in CRC tissues. The expression of PVT1 was positively correlated with MAPK1 and negatively correlated with miR-761. In addition, PVT1 sponged miR-761 to upregulate MAPK1 expression. It was found that the knockdown of PVT1 expression inhibited the proliferation and promoted the apoptosis of CRC cells, which was more potent in cells transfected with miR-761. The regulatory role of small interfering RNA-PVT1 on the expression of apoptosis-related genes was reduced by MAPK1. Collectively, the present results suggested

that knockdown of PVT1 may inhibit the progression of CRC by regulating the miR-761/MAPK1 axis, which may provide a promising biomarker for the treatment of CRC.

Introduction

Colorectal cancer (CRC) is one of the main causes of cancer-related mortality worldwide (1). In the past decades, treatment strategies for CRC have significantly improved (2,3). However, the fatality and survival rates of patients with CRC remain unsatisfactory (4). In 2018, the incidence of CRC reached 1.8 million new cases and ~861,000 CRC-related mortalities globally (5). Various factors complicate the pathogenesis of CRC, especially gene mutagenesis events induced by inherited and environmental factors, which contribute to the proliferation, apoptosis and differentiation of CRC (6). Thus, numerous studies have focused on molecular therapy in the treatment of CRC (7,8). Collectively, identifying the potential molecular mechanisms involved in the progression of CRC is of great importance.

Long non-coding RNAs (lncRNAs) are non-coding RNAs with a length of ~200 nucleotides (9). lncRNAs, which lack an open reading frame and therefore are unable to encode proteins, are involved in biological processes, including proliferation and apoptosis, by regulating epigenetic changes and gene transcription (10). Accumulating evidence has shown that lncRNAs serve a crucial role in the development of numerous diseases, particularly in the occurrence and progression of cancer (11-14). lncRNA plasmacytoma variant translocation 1 (PVT1) is located near Myc at human chromosome 8q24 (15). Yu *et al* (16) reported that PVT1 functions as an oncogene in human CRC via the microRNA (miRNA/miR)-30d-5p/RUNX family transcription factor 2 axis. Moreover, PVT1 facilitates CRC progression by targeting miR-26b (17). It has also been shown that PVT1 could affect the prognosis of CRC by regulating cyclooxygenase-2 expression (18). The upregulation of PVT1 in cancer predicts poor prognosis (19-22). PVT1 acts as an oncogene in various cancer types, including CRC, and regulates the biological progression of cancer (23,24). In CRC, the

Correspondence to: Dr Wenhuan Dou, Department of General Surgery, The Affiliated Suzhou Science and Technology Town Hospital of Nanjing Medical University, 1 Lijiang Road, Suzhou, Jiangsu 215000, P.R. China
E-mail: 1024929909@qq.com

*Contributed equally

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upregulation of PVT1 predicts advance stage and poor prognosis, as well as induces chemoresistance (15,25). However, the possible molecular mechanisms of PVT1 involved in the progression of CRC are yet to be fully elucidated.

miRNAs are one-stranded short endogenous RNAs (26) that are involved in the modulation of gene transcription by binding to the 3' untranslated region (3'UTR) of target genes (27). Increasing evidence has indicated that miRNAs are abnormally expressed in CRC, which suggests that miRNAs are involved in the progression and development of CRC (28-31). Therefore, verifying the association between lncRNAs and miRNAs in CRC may be beneficial for the prevention, diagnosis and therapy of CRC.

The present study aimed to investigate the functional roles and underlying mechanisms of PVT1 in CRC, which may provide a novel molecular mechanism that is associated with the pathology of CRC and offer a new direction for treating CRC.

Materials and methods

Tissue samples. In total, 30 pairs of CRC tissue samples and adjacent normal tissues (13 female patients and 17 male patients; age: 46±8 years) were collected from patients with CRC at The Second Affiliated Hospital of Soochow University (Suzhou, China) between January 2017 and August 2018. Adjacent normal tissues were collected from >2 cm from the tumor margin. The major inclusion criteria were as follows: i) All patients were reviewed by a pathologist and histologically confirmed as CRC, based on histopathological evaluation and without other malignancies; ii) patients who had not received any preoperative chemotherapy or radiotherapy treatment; and iii) patients with complete clinical data, including age, sex, ethnicity, tumor size and local invasion. The exclusion criteria were as follows: i) Patients with other types of malignant tumors; and ii) patients who were diagnosed with malignant lymphoma, and poorly differentiated and anaplastic carcinomas. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (approval no. JS sz-358). All patients provided written informed consent.

Cell treatment. The CRC cell lines, DLD-1, HT-29, SW480 and SW620, and the normal colonic mucosa cell line FHC were purchased from the American Type Culture Collection. Cells were incubated in RPMI-1640 medium (Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (Sigma-Aldrich; Merck KGaA) at 37°C with 5% CO₂.

Cell transfection. For transfection, SW480 cells (1×10⁴ cells/well) were plated in a 6-well plate. After adhering for 24 h, miR-negative control (NC) mimics (50 nM; 5'-UUCUCCGAACGUGUCACGU-3'), miR-761 mimics (50 nM; 5'-UUAUUGCUAAUUGUGAUAGGGGU-3'), miR-NC inhibitor (50 nM; 5'-CAGUACUUUUGUGUAGUACAA-3'), miR-761 inhibitor (50 nM; 5'-ACCCCUAUGACGAUAGCAUUA-3'), MAPK1 overexpression plasmids (pcDNA3.1 MAPK1; 50 nM) and its NC (empty vector; 50 nM), which were provided by Shanghai GenePharma Co., Ltd., were added to the medium for 6 h at 37°C in a CO₂ incubator. The small interfering RNA (siRNA; 50 nM;

5'-GAGCUGCGAGCAAAGAUGU-3') targeting PVT1 was provided by Guangzhou RiboBio Co., Ltd. Non-target scramble controls (50 nM; 5'-UUCUCCGAACGUGUCACGU-3') (Guangzhou RiboBio Co., Ltd.) served as the NC. Cell transfection was performed for 48 h using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. At 24 h post-transfection, subsequent experiments were performed.

Reverse transcription-quantitative PCR (RT-qPCR) analysis. Total RNA were collected from tissues or cell lines (DLD-1, HT-29, SW480, SW620 and FHC) using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using a RevertAid First Strand cDNA Synthesis Kit (Fermentas; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. qPCR was conducted with a SYBR Premix Ex Taq II kit (Takara Bio, Inc.) under the following conditions: Initial denaturation at 95°C for 6 min, followed by 40 cycles at 94°C for 30 sec, 60°C for 30 sec and 73°C for 90 sec. U6 and GAPDH were utilized as the loading controls for miRNA and mRNA, respectively. The relative mRNA expression levels were calculated using the 2^{-ΔΔC_q} method (32). The primer sequences were as follows: PVT1 forward, 5'-TTG GCACATACAGCCATCAT-3' and reverse, 5'-GCAGTAAAA GGGGAACACCA-3'; miR-761 forward, 5'-GCAGCAGGG TGAAACTGAA-3' and reverse, 5'-GAACATGTCTGCGTA TCTC-3'; MAPK1 forward, 5'-CAGTTCTTGACCCCTGGT CC-3' and reverse, 5'-TACATACTGCCGCAGGTAC-3'; GAPDH forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-TGGTGAAGACGCCAGTGGA-3'; and U6 forward, 5'-CTCGCTTCGGCAGCAGCACATATA-3' and reverse, 5'-AAATATGGAACGCTTCACGA-3'.

Western blot analysis. A total of 30 μg proteins were isolated from tissues and SW480 cells using RIPA lysis buffer containing protease inhibitors (Beyotime Institute of Biotechnology), and the concentration was determined using a BCA kit. Then, equal amounts of protein (20 μg) were isolated via SDS-PAGE on a 10% gel, and the protein was subsequently transferred onto PVDF membranes. Subsequently, the membranes were blocked with TBS with 0.05% Tween-20 buffer containing 5% skimmed milk for 1 h at room temperature. The membranes were incubated with primary antibodies overnight at 4°C and then incubated with HRP-conjugated secondary antibodies (cat. no. ab7090; 1:1,000; Abcam) at 37°C for 2 h. The protein expression levels were determined with an ECL kit (Thermo Fisher Scientific, Inc.) and semi-quantified with ImageJ version 1.6 software (National Institutes of Health). The specific primary antibodies used were as follows: Anti-BAX (cat. no. ab182734; 1:1,000; Abcam), anti-Bcl-2 (cat. no. ab32124; 1:1,000; Abcam), anti-Cleaved caspase-3 (cat. no. ab49822; 1:1,000; Abcam), anti-Caspase-3 (cat. no. ab13847; 1:1,000; Abcam), anti-MAPK1 (cat. no. 4695; 1:1,000; Cell Signaling Technology, Inc.) and anti-β-actin (cat. no. ab8227; 1:1,000; Abcam).

5-Ethynyl-2'-deoxyuridine (EdU) assay. SW480 cells (1×10⁴) were fixed with 4% paraformaldehyde for 30 min at room temperature and permeabilized, and then cell proliferation

was determined with an EdU assay kit (cat. no. C10310; Guangzhou RiboBio Co., Ltd.). After EdU staining, nuclei were stained with DAPI for 20 min at 37°C (Beyotime Institute of Biotechnology). EdU-positive cells were examined by fluorescence microscopy (BX51 microscope; Olympus Corporation) in five randomly chosen fields.

Cell counting kit-8 (CCK-8) analysis. SW480 cells (1×10^4 cells/well) were seeded in 96-well plates and incubated with 10 μ l CCK-8 reagent (cat. no. CA1210; Beijing Solarbio Science & Technology Co., Ltd.), and then incubated in the dark for 2 h at 37°C. Absorbance at 450 nm was measured at 0, 24, 48 and 72 h using a microplate reader. All experiments were performed in triplicate.

Flow cytometry analysis. SW480 cells were collected and centrifuged at $1,000 \times g$ for 6 min at 4°C. The apoptosis (early + late) was detected by an Annexin V-FITC/PI apoptosis detection kit (Nanjing KeyGen Biotech Co., Ltd.). Next, cells were stained with 5 μ l Annexin V-FITC and 10 μ l PI for 20 min in the dark. The apoptotic rates were calculated using a FACScan flow cytometer (BD Biosciences) and Flow cytometry data were analyzed using FlowJo version 10.0 software (FlowJo LLC).

Bioinformatic analyses. Bioinformatics database (<http://starbase.sysu.edu.cn/>; Starbase version 2.0) was used to predict the target of PVT1.

Dual-luciferase reporter assay. The sequences containing the MAPK1 3'untranslated region (UTR) or PVT1 3'UTR wild-type (WT) and mutant (MUT), which was generated by site-directed mutagenesis, binding sites on miR-761 were synthesized and inserted into pmiR-RB-REPORT™ plasmids (Guangzhou RiboBio Co., Ltd.). Then, luciferase reporter plasmids were co-transfected with miR-761 mimics and miR-NC mimics using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h. After 24 h, the relative luciferase activity was calculated using a Dual-Luciferase Reporter Assay System (Promega Corporation). Luciferase activities were normalized to *Renilla* luciferase activities.

Statistical analysis. The experiments were performed in triplicate. The data were evaluated using GraphPad version 7.0 software (GraphPad Software, Inc.) and presented as the mean \pm SD. A paired Student's t-test was carried out to evaluate significant differences between two groups. The differences among multiple groups were analyzed using a one-way ANOVA, followed by a Tukey's post hoc test. The Pearson's method was used for correlation analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Downregulated expression levels of PVT1 suppress the progression of CRC. RT-qPCR analysis was performed to examine the expression levels of PVT1 in CRC tissues. As presented in Fig. 1A, the mRNA expression of PVT1 was significantly upregulated in CRC tissues. RT-qPCR analysis was also performed to examine the expression levels of PVT1

in various CRC cell lines. Similarly, it was found that PVT1 expression was upregulated in CRC cell lines compared with the normal colonic mucosa cell line FHC (Fig. 1B). Moreover, the expression of PVT1 in SW480 cells was higher compared with that in DLD-1, HT-29 and SW620. Therefore, SW480 cells were used in the subsequent experiments (Fig. 1B).

It was identified that the expression of PVT1 was significantly decreased after knockdown of PVT1, which was more efficient in the si-PVT1-2 group (Fig. 1C). Thus, si-PVT1-2 was used in the following experiments. CCK-8 assay results indicated that cell viability was significantly decreased in si-PVT1-transfected cells compared with the controls (Fig. 1D). Similarly, the results of the EdU assay demonstrated that the number of EdU-positive cells was significantly decreased following inhibition of PVT1 in SW480 cells (Fig. 1E). Additionally, knockdown of PVT1 significantly increased the apoptotic rate of SW480 CRC cells (Fig. 1F) in comparison with the control group.

PVT1 acts as a competing endogenous (ce)RNA by binding to miR-761. Accumulating evidence has revealed that lncRNAs function as ceRNAs to regulate biological processes by sponging miRNAs (33,34). The online bioinformatics database (Starbase version 2.0) predicted that miR-761 was a target of PVT1 (Fig. 2A). The relative luciferase activity of CRC cells transfected with PVT1 3'UTR WT and miR-761 mimics was significantly decreased (Fig. 2B). Moreover, miR-761 expression was decreased in CRC tissues (Fig. 2C). It was found that knockdown of PVT1 increased the expression of miR-761 (Fig. 2D), and PVT1 was negatively correlated with miR-761 ($r = -0.75$; Fig. 2E).

PVT1 regulates the progression of CRC by binding to miR-761. The significant increase of PVT1 expression in CRC prompted the investigation into the possible biological significance of PVT1 in CRC tumorigenesis. As presented in Fig. 3A, the expression of miR-761 was significantly increased by miR-761 mimics and decreased by miR-761 inhibitor. Furthermore, the CCK-8 results demonstrated that knockdown of PVT1 suppressed the viability of CRC cells, which was more potent in the si-PVT1 + miR-761 mimic group (Fig. 3B). An EdU assay was conducted to further confirm the CCK-8 results, and it was found that the proliferative activity was inhibited by knockdown of PVT1 in the SW480 cells, while miR-761 overexpression partially enhanced the action of PVT1 knockdown (Fig. 3C). By contrast, knockdown of PVT1 increased the apoptosis of SW480 cells, which was more obvious in the si-PVT1 + miR-761 mimic group (Fig. 3D). Additionally, si-PVT1 + miR-761 mimics transfection was more efficient in upregulating BAX and cleaved caspase-3 expression levels and downregulating Bcl-2 expression compared with the si-PVT1 group (Fig. 3E).

PVT1 regulates MAPK1 by sponging miR-761. The binding site of MAPK1 on miR-761 is shown in Fig. 4A. A dual-luciferase assay demonstrated that co-transfection of miR-761 mimics and MAPK1 3'UTR WT significantly decreased cell luciferase activity (Fig. 4B). MAPK1 was highly expressed in CRC tissues (Fig. 4C), and MAPK1 expression was significantly decreased by miR-761 mimics (Fig. 4D).

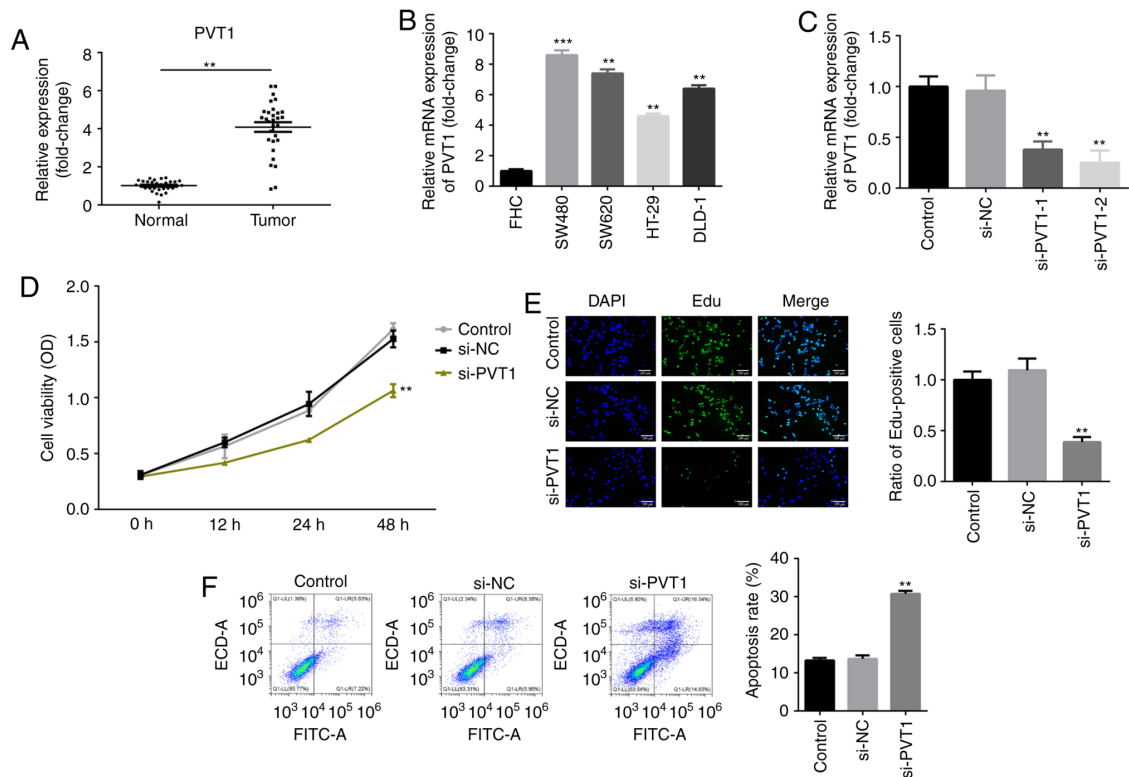


Figure 1. Knockdown of PVT1 inhibits the proliferation and apoptosis of CRC cells. (A) Relative expression of PVT1 in CRC tissues in comparison with the corresponding normal adjacent tissues. ** $P < 0.01$ vs. normal tissues. (B) mRNA expression of PVT1 was analyzed in CRC cells. ** $P < 0.01$, *** $P < 0.001$ vs. FHC cells. (C) mRNA expression of PVT1 was determined via reverse transcription-quantitative PCR. (D) Cell viability was determined using a Cell Counting Kit-8 assay. (E) Cell proliferation was determined using an EdU assay (magnification x200). (F) Cell apoptosis was measured via flow cytometry. ** $P < 0.01$ vs. si-NC. PVT1, plasmacytoma variant translocation 1; CRC, colorectal cancer; EdU, 5-Ethynyl-2'-deoxyuridine; si-, small interfering RNA; NC, negative control.

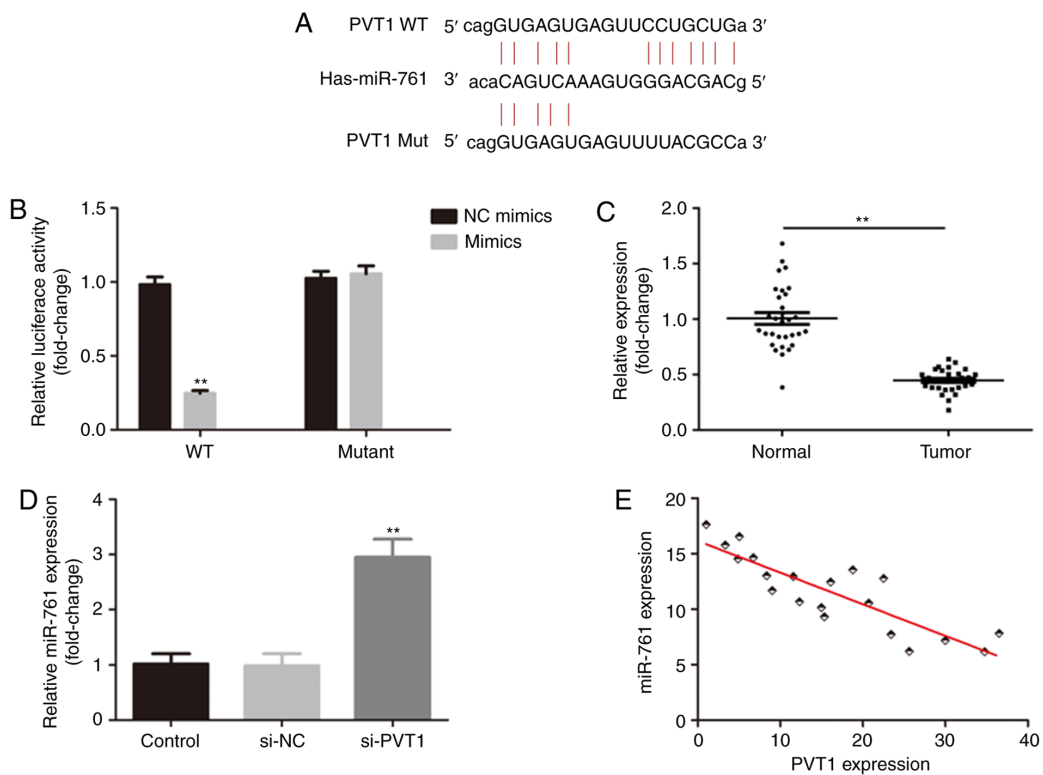


Figure 2. PVT1 acts as competing endogenous RNA by binding to miR-761. (A) Binding site of PVT1 on miR-761. (B) Co-transfection of miR-761 mimics and PVT1 3'untranslated region WT significantly decreased relative luciferase activity. ** $P < 0.01$ vs. NC mimics. (C) Expression of miR-761 was significantly decreased in colorectal cancer tissues. ** $P < 0.01$ vs. normal tissues. (D) miR-761 expression was significantly increased following transfection with si-PVT1. ** $P < 0.01$ vs. control. (E) miR-761 expression was negative correlated with PVT1. NC, negative control; PVT1, plasmacytoma variant translocation 1; miR, microRNA; WT, wild-type; si-, small interfering RNA; MUT, mutant.

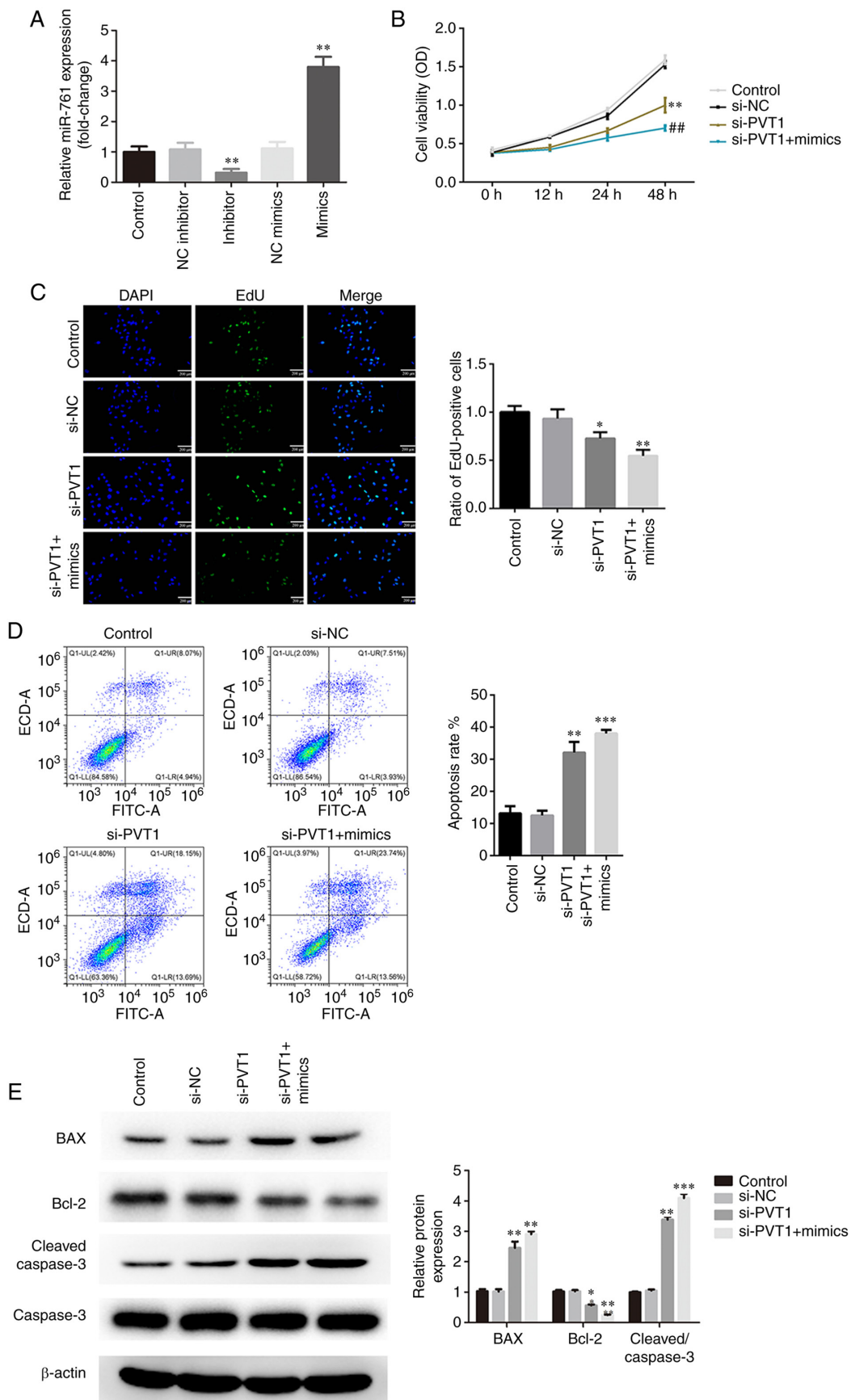


Figure 3. PVT1 regulates the proliferation and apoptosis of CRC cells by binding to miR-761. (A) Expression levels of miR-761 were evaluated after cells were transfected with inhibitor or mimics. si-PVT1 + miR-761 mimics had a greater effect on inhibiting the viability and proliferation of CRC cells, as determined via (B) Cell Counting Kit-8 and (C) EdU assays (magnification x200). ** $P < 0.01$ vs. NC inhibitor or NC mimics. (D) si-PVT1 + miR-761 mimics had a greater effect in promoting the apoptosis of CRC cells, as measured via flow cytometry. (E) si-PVT1 + miR-761 mimics were more efficient in regulating the expression levels of Bcl-2/BAX and caspase-3, as detected via western blotting. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. si-NC; ## $P < 0.01$ vs. si-PVT1. PVT1, plasmacytoma variant translocation 1; miR, microRNA; CRC, colorectal cancer; si-, small interfering RNA; EdU, 5-Ethynyl-2'-deoxyuridine; NC, negative control.

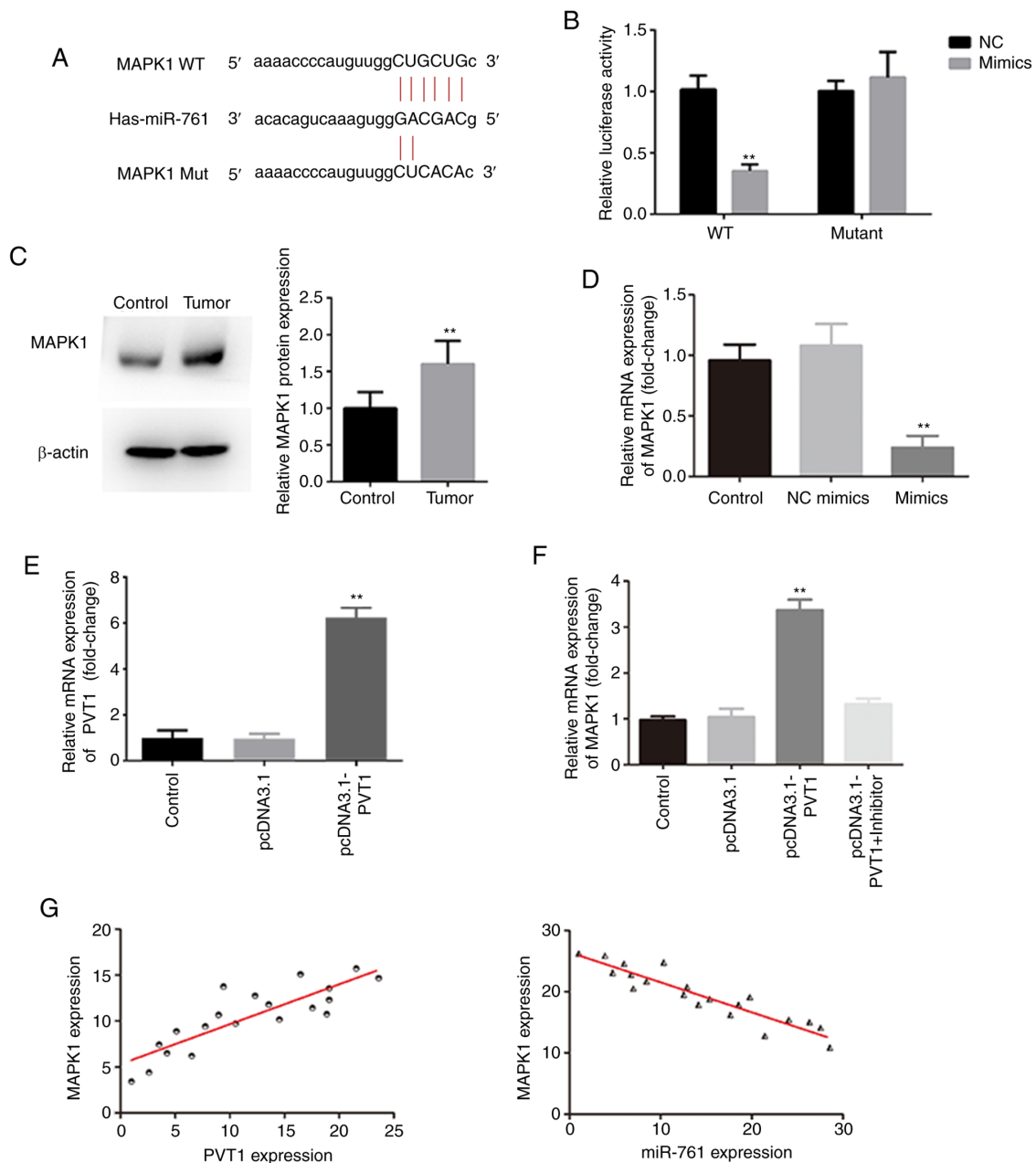


Figure 4. MAPK1 is a target of miR-761. (A) The binding site of MAPK1 on miR-761. (B) Luciferase activity was determined via a dual-luciferase reporter assay. ** $P < 0.01$ vs. NC mimics. (C) Protein expression of MAPK1 in colorectal cancer tissues was determined via western blotting. ** $P < 0.01$ vs. normal tissues. (D) mRNA expression levels of MAPK1 were determined following transfection with miR-761 mimics via RT-qPCR. (E) mRNA expression levels of PVT1 following transfection with pcDNA3.1-PVT1 were determined via RT-qPCR. (F) mRNA expression levels of MAPK1 were determined following transfection with pcDNA3.1-PVT1 and miR-761 inhibitors via RT-qPCR. (G) Correlations between MAPK1 and PVT1, and MAPK1 and miR-761. ** $P < 0.01$ vs. control. PVT1, plasmacytoma variant translocation 1; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; WT, wild-type; MUT, mutant.

Overexpression of PVT1 following transfection with the pcDNA3.1 PVT1 was confirmed, as presented in Fig. 4E. Furthermore, the expression of MAPK1 was upregulated by PVT1 overexpression, and was restored to normal levels by miR-761 mimics, suggesting that PVT1 regulated the expression of MAPK1 via miR-761 (Fig. 4F). It was also identified that the expression of MAPK1 was negatively correlated with miR-761 ($r = 0.86$) and positively correlated with PVT1 ($r = 0.72$) (Fig. 4G).

Knockdown of PVT1 suppress the progression of CRC via the miR-761/MAPK1 axis. To further identify whether

PVT1 exerted its role in CRC via the miR-761/MAPK1 axis, additional experiments were performed. Overexpression of MAPK1 following transfection with the pcDNA3.1 MAPK1 was confirmed, as presented in Fig. 5A. First, CCK-8 and EdU assays revealed that cell viability and proliferation were significantly inhibited by knockdown of PVT1, and was partially restored following the overexpression of MAPK1 (Fig. 5B and C). Moreover, flow cytometric analysis results demonstrated that knockdown of PVT1 significantly increased the apoptotic rate of CRC cells, which was partially alleviated by MAPK1 overexpression (Fig. 5D). It was also found that the increase of Bax and cleaved caspase-3

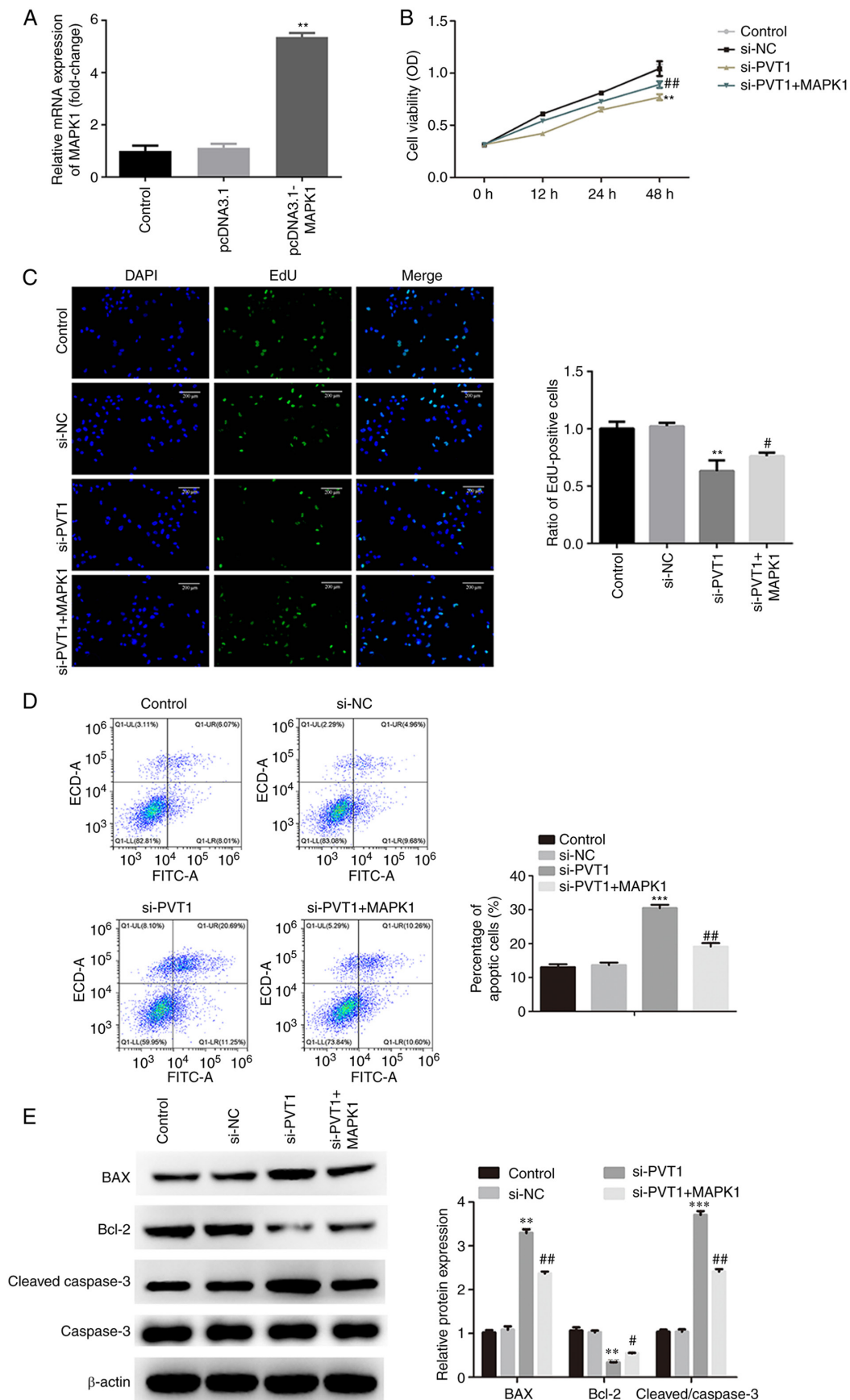


Figure 5. PVT1 regulates the proliferation and apoptosis of CRC cells via the miR-761/MAPK1 axis. (A) mRNA expression levels of MAPK1 following transfection with pcDNA3.1-MAPK1 were determined via reverse transcription-quantitative PCR. MAPK1 overexpression reversed the inhibitory effects of cell viability and proliferation induced by si-PVT1, as determined via (B) Cell Counting Kit-8 and (C) EdU assays (magnification, x200). (D) MAPK1 overexpression alleviated the apoptosis of CRC cells induced by si-PVT1, as measured via flow cytometry. (E) MAPK1 overexpression reversed the effects of si-PVT1 on the expression levels of Bcl-2/BAX and caspase-3, as detected via western blotting. ** $P < 0.01$, *** $P < 0.001$ vs. control; # $P < 0.05$, ## $P < 0.01$ vs. si-PVT1. PVT1, plasmacytoma variant translocation 1; miR, microRNA; si-, small interfering RNA; CRC, colorectal cancer; EdU, 5-Ethynyl-2'-deoxyuridine; NC, negative control.

expression levels and the decrease of Bcl-2 expression induced by si-PVT1 were partially alleviated after transfection with pcDNA3.1-MAPK1 (Fig. 5E).

Discussion

Accumulating evidence has revealed the presence of dysregulated lncRNAs in CRC, including SLC25A25 antisense RNA 1, AFAP1 antisense RNA 1, colorectal neoplasia differentially expressed and growth arrest specific 5 (19-22). The aberrant expression of lncRNAs participates in the tumorigenesis and progression of CRC by regulating autophagy, proliferation, migration, invasion, metastasis and other biological processes (9-12,35). Therefore, investigating the possible roles of lncRNAs in CRC is crucial to identify a therapeutic strategy for CRC. Previous studies have reported that abnormally expressed PVT1 contributes to the initiation and development of CRC, and predicts poor prognosis (15). For example, Wu *et al* (36) found that PVT1 promotes CRC tumorigenesis via a miR-16-5p/VEGFA/VEGFR1/AKT axis. In addition, knockdown of PVT1 could suppress CRC progression by regulating the miR-106b-5p/four-jointed box kinase 1 axis (37). In the present study, PVT1 was upregulated in CRC tissues and cells. Furthermore, PVT1 knockdown suppressed the progression of CRC. These results suggested that PVT1 may be an oncogene in CRC, which was consistent with a study by Ping *et al* (15). However, the underlying mechanisms via which PVT1 modulates the tumorigenic processes in CRC have not been fully elucidated.

lncRNAs function as ceRNAs by sponging miRNAs to participate in carcinogenesis, including in CRC (1-4,38). PVT1, located in a cancer-associated region-8q24, acts as an oncogene by promoting the proliferation and invasion of cervical cancer via binding to miR-140-5p, and knockdown of PVT1 combined with the overexpression of miR-214 inhibits hepatocarcinogenesis (39,40). A previous study also revealed that overexpression of PVT1 induced the proliferation of CRC by regulating miR-216a-5p (41). Moreover, bioinformatics analyses have identified that PVT1 binds to various miRNAs, including miR-186-5p, miR-16-5p and miR-761. Therefore, PVT1 may regulate the initiation and development of CRC by binding to other miRNAs.

Abnormally expressed miRNAs act as oncogenes or antitumor genes in cancer and regulate cell proliferation, differentiation, apoptosis and the epithelial-mesenchymal phenotype (42). miR-761 is downregulated in CRC, while its overexpression suppresses the progression of CRC and enhances chemosensitivity (43). These results suggested that miR-761 may function as an antitumor gene in CRC (44). In the present study, the expression of miR-761 was negatively correlated with PVT1. Moreover, the overexpression of miR-761 facilitated the effects of PVT1 knockdown on the progression of CRC. The regulatory role of si-PVT1 in regulating Bcl-2/BAX and caspase-3 was promoted by the overexpression of miR-761. These results indicated that PVT1 regulated the progression of CRC via miR-761. Therefore, the PVT1/miR-761 axis may be a promising biomarker for CRC. However, the underlying mechanisms remain unknown.

The MAPK signaling cascade is prominently conserved in evolution (45). MAPK1, located in the cytoplasm, is often

activated or upregulated in tumors (43,44). Accumulating evidence has verified that the upregulation of MAPK1 contributes to tumorigenesis (46-48). In CRC, the activation of MAPK1 induces the proliferation and inhibits the apoptosis of CRC cells (49). The present study identified a novel upstream mechanism of MAPK1. PVT1 functioned as a ceRNA to regulate MAPK1 by sponging miR-761. MAPK1 was predicted and shown to be a target of miR-761. Furthermore, the expression of MAPK1 was negatively correlated with miR-761, while it was positively correlated with PVT1. Overexpression of MAPK1 has been demonstrated to alleviate the effects of PVT1 knockdown on the proliferation and apoptosis of CRC cells, as well as the regulatory role of apoptosis-related genes, such as Bcl-2/BAX and caspase-3 (45,46). Thus, the PVT1/miR-761/MAPK1 axis may serve a crucial role in the progression of CRC.

The main limitation of the present study was the small sample size of the patients and lack of an animal model, which could make the results more convincing. Therefore, an increased number of patients and animal model will be included in a future study.

In conclusion, the present study demonstrated that PVT1 was upregulated in CRC tissues. Knockdown of PVT1 inhibited the proliferation and triggered the apoptosis of CRC cells by functioning as a ceRNA towards miR-761 and subsequently downregulating MAPK1 expression. The present findings may provide a promising diagnostic biomarker and therapeutic strategy for CRC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YW designed the experiments. YL and WD were the major contributors in writing the manuscript and performed the experiments. YL and WD confirm the authenticity of all the raw data. ZZ and JG performed the experiments and analyzed data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The Second Affiliated Hospital of Soochow University (Suzhou, China; approval no. JS sz-358). All patients provided informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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