

Inhibition of CDK1 Reverses the Resistance of 5-Fu in Colorectal Cancer

This article was published in the following Dove Press journal:
Cancer Management and Research

Yiping Zhu¹
Kai Li²
Jieling Zhang²
Lu Wang¹
Lili Sheng¹
Liang Yan²

¹Department of Oncology, The First Affiliated Hospital of Wannan Medical College, Wuhu, Anhui, People's Republic of China; ²Provincial Key Laboratory of Biological Macro-molecules Research, Wannan Medical College, Wuhu, Anhui, People's Republic of China

Introduction: Although the survival rate of colorectal cancer (CRC) patients can be improved by surgery, radiotherapy, and chemotherapy, the resistance to 5-fluorouracil (5-Fu) affects the effect of chemotherapy and the prognosis of patients. An increasing number of studies showed that 5-Fu resistance was the main reason for the failure of colorectal cancer treatment. The poor prognosis of colorectal cancer greatly harms people's health. This study aimed to clarify the correlation between cyclin-dependent kinase 1 (CDK1) and 5-Fu-induced tumor resistance.

Materials and Methods: Cell proliferation and invasion experiments showed that down-regulation of CDK1 inhibited fluorouracil-resistant CRC cell proliferation. The expression level of CDK1 was detected in 5-Fu-resistant CRC cells in vitro. Tumor growth was inhibited by down-regulation of CDK1 in tumor xenograft mouse models.

Results: We found that CDK1 was highly expressed in tumor tissues, especially in fluorouracil-resistant tissues. We also confirmed that the differential expression of 5-Fu in tumor tissues was related to tumor site, lymph node metastasis and stage. CDK1 promoted migration, invasion and inhibited apoptosis in 5-Fu-resistant CRC cells. Down-regulation of CDK1 inhibited fluorouracil-resistant CRC cell proliferation and tumorigenesis in vivo.

Conclusion: High expression of CDK1 may lead to poor clinical prognosis, and inhibition of CDK1 enhances 5-Fu sensitivity in CRC. Our research suggested that CDK1 may be used to predict 5-Fu efficacy and as a therapeutic target for CRC.

Keywords: CDK1, colorectal cancer, 5-Fu, drug resistance

Background

Approximately 1,800,000 patients were diagnosed with colorectal cancer (CRC) in 2018, and some patients died of this disease.¹ Although diagnostic methods and treatment have been greatly improved,²⁻⁴ for nearly 60% of colorectal cancer patients with distant metastases, the survival rate is very low.⁵ Clinical stages closely result in the outcomes of CRC patients. The 5-year survival rate for stage I patients exceeds 90%, while is less than 10% for stage IV patients.⁶ Surgery, radiotherapy, and chemotherapy are widely used in CRC. Radiotherapy is recommended as adjuvant/neoadjuvant treatment for the majority of rectal cancer patients,⁷ and radiotherapy combined with chemotherapy significantly reduces the risk of recurrence and metastasis.⁸ Adjuvant chemotherapy contained 5-fluorouracil (5-Fu) is currently recommended for patients with stage III CRC after surgery.⁹⁻¹¹ Moreover, a combination of 5-Fu, irinotecan/oxaliplatin, and monoclonal antibodies significantly improved overall response rate (ORR), and prolonged survival time of advanced CRC patients.¹²⁻¹⁴

Correspondence: Lili Sheng; Liang Yan
Email 13605535185@163.com;
yane2002@126.com

5-Fu is a common thymidylate synthase inhibitor, converting into 5-fluorouracil deoxynucleotide (5F-dUMP), inhibiting deoxythymidylate synthetase, preventing deoxy methylation of uridine (dUMP) into deoxythymidylate (dTTP), which affects DNA and RNA synthesis, and exerting an anti-tumor effect.¹⁵ However, nearly 50% of colorectal cancer patients showed no response to 5-Fu, and more and more studies reported that fluorouracil resistance led to failure of CRC treatment.^{16–18} But the mechanism is not clear. Therefore, biomarkers predicting the efficacy of fluorouracil are urgently needed in CRC treatment.

CDK1 (cyclin-dependent kinase 1) is a serine/threonine-like protein kinase. Activation of CDK1 can phosphorylate target proteins and produce corresponding physiological effects, such as regulating cell cycle and promoting cell proliferation.¹⁹ Multiple studies have reported that CDK1 is overexpressed in several malignancies.^{20,21} In CRC patients, the high nuclear/cytoplasmic ratio of CDK1 often predicted a poor prognosis.²² Moreover, CDK1 has been found to be closely associated with chemotherapy resistance in numerous tumors. Li et al²³ found that silencing *UBE2C* in cisplatin-resistant ovarian cancer cells reversed its resistance to cisplatin via down-regulating the expression of CDK1. Shang et al²⁴ also reported that lncRNA OR3A4 promoted drug resistance to cisplatin by upregulating CDK1 in non-small cell lung cancer (NSCLC). In CRC, *BRAF* mutation is a poor prognostic factor and often results in poor survival outcomes. A combination of CDK1 inhibitor and MEK/ERK inhibitor promoted apoptosis in *BRAF* mutant CRC cells, which suggested that CDK1 could mediate apoptosis resistance in colorectal cancer.²⁵ Zhang²⁶ showed that Wnt/ β -catenin activation promoted the resistance of colorectal cancer to fluorouracil and oxaliplatin. However, the mechanism by which CDK1 promotes resistance remains to be elucidated. Our previous research revealed that there was a ceRNA regulatory relationship between LINC00365 and CDK1. CDK1 expression was positively correlated with Wnt/ β -catenin activation.^{27,28} Therefore, we surmised that CDK1 might be associated with 5-Fu resistance in CRC.

Our study demonstrated that overexpression of CDK1 resulted in a poor prognosis in CRC patients. Besides, CDK1 up-regulation was also observed in fluorouracil-resistant CRC tissues. Then, we explored that silence of CDK1 in fluorouracil-resistant cells inhibited the proliferation, migration, and invasion, and

reversed fluorouracil-resistance. CDK1 might be considered as a predictor for fluorouracil efficacy, and a therapeutic target for CRC.

Materials and Methods

Samples of CRC Patients

From April 2012 to September 2014, we collected tumor and paracancerous tissues of 80 CRC patients who were admitted to the First Affiliated Hospital of Wannan Medical College. All these 80 cases underwent surgery. Meanwhile, we also collected tumor tissues from 76 metastatic CRC (mCRC) patients. None of the samples were exposed to radiotherapy or chemotherapy. All the fresh tissues were stored in the -80°C refrigerator until RNA extraction. All specimens were confirmed by two independent pathologists. Tumors were staged according to the seventh edition of cancer staging criteria of the American Joint Committee on Cancer (AJCC). Clinicopathological characteristics of 80 patients undergoing surgery were collected, and the correlation between CDK1 and clinical case characteristics was analyzed. All 76 mCRC patients received first-line chemotherapy containing 5-Fu. Tumor response to chemotherapy was confirmed by CT every two cycles and evaluated according to RECIST. Among the 76 patients, 43 cases achieved partial response (PR) and complete response (CR), 33 cases achieved stable disease (SD) and progression disease (PD). This study complied with the Helsinki Declaration. We obtained permission from Yijishan Hospital Ethics Committee (approval number 2018–34) and got informed consent from all patients.

Cell Culture and Establishment of Drug-Resistant Cell Lines

Human CRC cells (SW620, SW480, HCT116, HT-29) and human colorectal mucosal epithelial cells-NCM460 were purchased from Shanghai Cell Research Institute. We obtained HT-29/5-Fu and SW480/5-Fu by gradually increasing 5-Fu concentration to treat HT-29 and SW480 cells in 6 months. Cells were cultured at 37°C with 5% CO_2 .

qRT-PCR

Total RNA was extracted from the tissues with Trizol (Invitrogen). RNA was reverse-transcribed to cDNA with Reverse Transcription Kit (Ribo, China). qRT-PCR was performed on the ABI7500 PCR operating system. Sequences of primers are listed as follows: GAPDH

Forward: GAACGGGAAGCTCACTGG, Reverse: GCC TGCTTACCACCTTCT; CDK1 Forward: GGTTCTA GTACTGCAATTCG, Reverse: TTTGCCAGAAATTCG TTTGG. Relative CDK1 expression was normalized using equation $2^{-\Delta\Delta CT}$. GAPDH served as a reference.

Transfections

Small interfering RNA targeting *CDK1* and its negative control were synthesized by Ribo, and its sequences are listed below: siRNA1: GGAAGTTCGTCATCCAAAT, siRNA2: GTACTGCAATTCGGGAAAT, siRNA3: GGTT ATATCTCATCTTTGA. Lipo3000 was used for cell transfection according to the protocol. Cells transfected with siRNA were collected for further experiments. Then, the knockdown efficiency of CDK1 was detected by RT-PCR.

Establishment of Stable Transfected Cell Lines

siRNA3 targeting *CDK1* was selected for further study. Based on its sequence, GenePharma (Shanghai, China) synthesized the short hairpin RNA (shRNA) and packaged it into a recombinant lentivirus. Simultaneously, a negative control was synthesized with an empty vector. Infected with shRNA for 48 hours, cells were treated with puromycin (Genomeditech, China) at different concentrations (SW480: 1.0 $\mu\text{g/mL}$; HT-29: 0.6 $\mu\text{g/mL}$). Puromycin concentrations were stably maintained for 6 weeks to establish stable transfected cell lines.

Methyl Thiazolyl Tetrazolium (MTT) Assay

Cells were cultured in 96-well plate, 180 μL of culture medium was added into each well. Thereafter, 20 μL of MTT solution was added into each well at 24, 48, 72, and 96 h. After incubation for 4 h, we added 150 μL of dimethyl sulfoxide into each well. Ten minutes later, the samples were detected at 490nm wavelength.

Flow Cytometry

In 6-well plates, floating and adherent cells were harvested and centrifuged. On the ice, the cells were stained with Annexin V/propidium iodide (LiankeBio, China). Next, cell apoptosis detection was performed on flow cytometry (BD, USA).

Colony Formation Assay

In 12-well plates, 500 cells were cultured each well, and the medium was changed every 5 days until visible colonies formed. Then, colonies were fixed and stained with giemsa. The images were captured with a fluorescence microscope and analyzed with Image J software.

Wound Healing Assay

We scraped cells with a 20 μL pipette tip when they were confluent. Floating cells were washed out. Then, cells were incubated with serum-free medium. Images were captured at 0, 24, and 48 h with a fluorescence microscope. The pictures were processed with Image J software to analyze the ability of cell migration.

Transwell Assay

Matrigel (Matrigel: RPMI-1640, 1:9) was used to treat the transwell chamber. Then, cells were resuspended to a suspension with a density of 105/mL, and 100 μL suspension was added into the upper chamber. On the next day, cells which passed through the matrix gel were fixed and stained. Images were captured with a fluorescence microscope.

Western Blotting

Radioimmunoprecipitation assay buffer (Beyotime, China) was used to extract protein; 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used for protein separation. Subsequently, we transferred proteins to polyvinylidene fluoride (PVDF) membrane. After blocking with BSA, the PVDF membrane was incubated with a specific antibody. The Enhanced Chemiluminescence System (Invitrogen, CA) was used to detect protein bands with specific antibodies. Anti-CDK1 (1:1000, Cat. ab18, Abcam, UK) and anti- β -actin (1:1000, Cat. ab8226, Abcam, UK) were primary antibodies. β -actin was considered as an endogenous control. Image J software was used to analyze the quantity of protein.

Tumor Xenograft Mouse Models

Experimental Animal Center of Nanjing Medical University provided the 4–5-week-old BALB/c nude mice. The animal experiments were approved by the Yijishan Hospital Ethics Committee and followed the guidelines published by the Yijishan Hospital Ethics Committee. Each nude mouse was inoculated subcutaneously with 2×10^6 cells. From day 8 to day 14, the mice

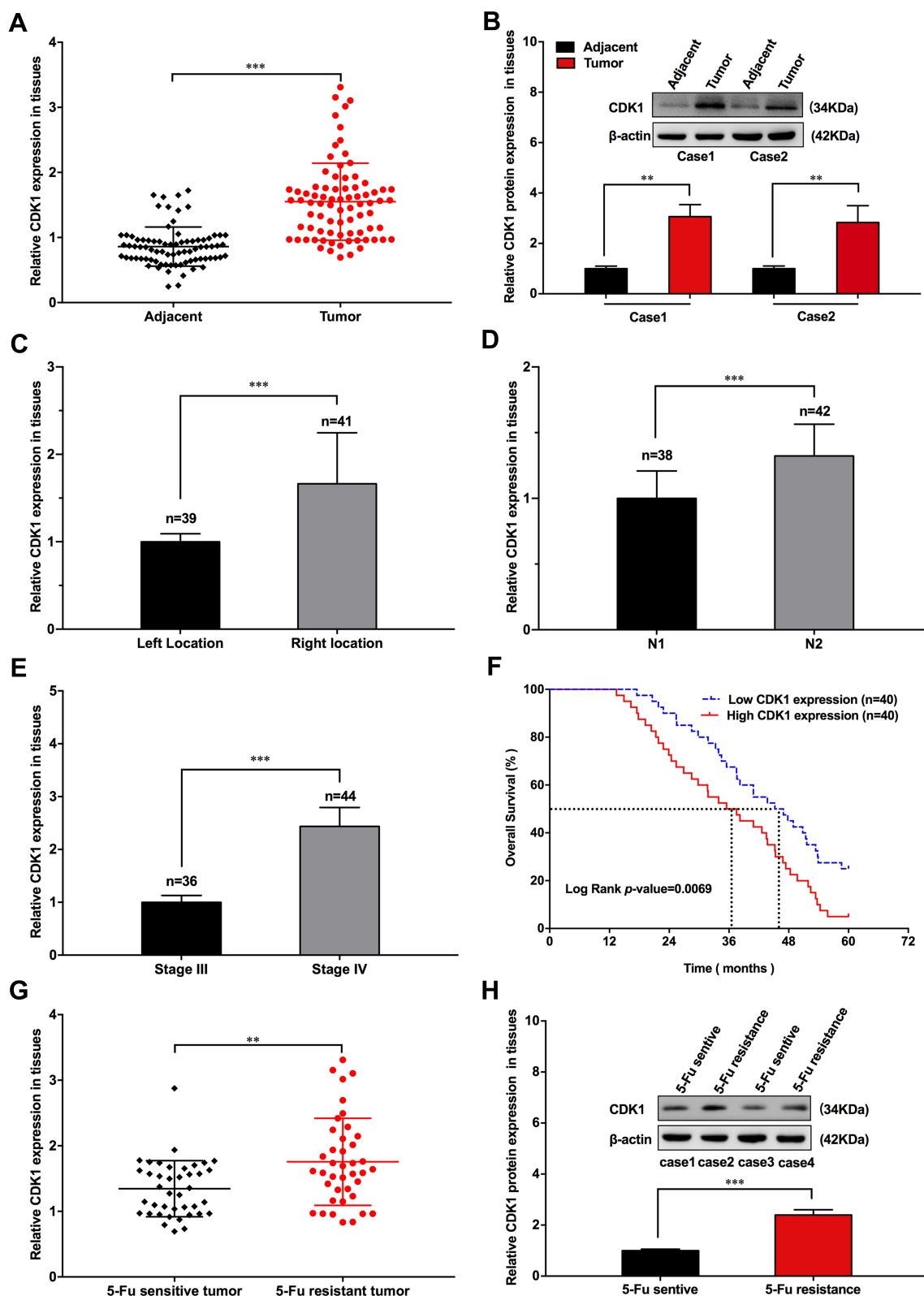


Figure 1 CDK1 overexpression in CRC tissue resulting in poor outcome.

Notes: (A) The relative expression of CDK1 mRNA in 80 paired CRC tissues and adjacent noncancerous tissues. (B) Relative expression of CDK1 protein in 80 paired CRC tissues and adjacent noncancerous tissues was measured by Western blot. (C) The relative CDK1 expression in CRC patients with different locations. (D) The relative CDK1 expression in CRC patients with different lymph node metastasis (N1: metastasis in 1 to 3 regional lymph nodes; N2: metastasis in 4 or more regional lymph nodes). (E) The relative CDK1 expression in CRC patients with different stages. (F) The correlation of high- or low-CDK1 expression with the overall survival in patients with CRC. (G) The relative expression of CDK1 mRNA in fluorouracil-sensitive tumor and fluorouracil-resistant tumor. (H) Relative expression of CDK1 protein in fluorouracil-sensitive tumor and fluorouracil-resistant tumor was measured by Western blot. Quantity of protein was analyzed by Image J software. ** $P < 0.01$, *** $P < 0.001$.

were injected intraperitoneally with 50 mg/kg of 5-Fu every other day. We measured tumor size every three days, sacrificed mice, and stripped tumors on the 15th day. The CDK1 expression level was examined with WB and RT-PCR. Immunohistochemical assay was used to detect the expression of Ki-67.

Immunohistochemistry

The transplanted tumors were fixed, embedded, and cut into sections at 4 μ m thickness. The samples were baked for 30 minutes, and dewaxed to remove endogenous catalase. Sections and anti-Ki-67 (1:1000, Cat.ab92742, Abcam, UK) were incubated together at 4°C overnight. The second day, sections and secondary HRP-conjugated antibody were co-incubated for 30 minutes. Two independent pathologists analyzed the data.

TUNE Assay

Transplanted tumor tissue sections were dewaxed, and treated successively with ethanol and proteinase K. After fixing and blocking, sections were incubated in a 50 μ L reaction mixture of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). We captured images with a fluorescence microscope.

Statistical Analysis

SPSS 17.0 and GraphPad Prism 7.0 were used to analyze all data. Student's *t*-test was used to determine the difference between the two groups. Survival curves were analyzed using Log-rank test. We used univariate and multivariate analyses to analyze the prognostic factor. $P < 0.05$ was considered significantly different. All experiments were repeated in triplicate.

Results

CDK1 Overexpression in CRC Tissue Resulted in Poor Outcome

We detected CDK1 in tumor tissues and paired paracancerous tissues. Higher CDK1 expression was observed in tumor tissues than their paired paracancerous tissues (Figure 1A and B). Correlations between the CDK1 and the clinicopathological characteristics are shown in Table 1. Tumor location, numbers of lymph node metastasis and tumor stage led to different expressions of CDK1 (Figure 1C–E). High-CDK1-expression CRC patients had a poor clinical outcome (Figure 1F). Univariate and multivariate analysis revealed that tumor location, number of lymph

node metastases, tumor stage, and CDK1 expression were poor prognosis independent predictors (Table 2). We also found that CDK1 expression in 33 fluorouracil-resistant tissues was significantly increased compared with 43 fluorouracil-sensitive tissue (Figure 1G and H).

CDK1 Up-Regulation in Fluorouracil-Resistant CRC Cell

CDK1 was up-regulated in CRC cells, and significant differences were observed in HT-29 and SW480 (Figure 2A). Therefore, we selected these two cell lines for further research. We observed that the 50% inhibitory concentration of HT-29 was $44.60 \pm 1.65 \mu\text{M}$, while in HT-29/5-Fu, it was $12.30 \pm 0.48 \mu\text{M}$, which was significantly different (Figure 2B and C). A similar phenomenon was also observed in SW480 and

Table 1 Clinicopathologic Features of 80 Patients with Colorectal Cancer

Clinicopathological Features	Low CDK1 Expression	High CDK1 Expression	P value
	n=40	n=40	
Age (year)			
≥60	26	23	0.491
<60	14	17	
Gender			
Male	19	22	0.502
Female	21	18	
Location			
Left	25	14	0.014
Right	15	26	
Pathology type			
Infiltrating type	24	27	0.485
Ulcerative type	16	13	
Differentiation			
Poorly differentiated	17	21	0.371
Well-moderately differentiated	23	19	
Depth of tumor invasion			
T2	12	7	0.421
T3	13	15	
T4	15	18	
Lymph node metastasis			
N1	13	25	0.007
N2	27	15	
TNM stage			
III stage	23	13	0.025
IV stage	17	27	

Table 2 Cox's Proportional Hazard Model Analysis of Prognostic in Patients with CRC

Variables	Univariate Analysis		P	Multivariate Analysis		P
	HR	95% CI		HR	95% CI	
Age(years) ≥60/<60	0.860	0.563–1.231	0.329			
Gender Male/Female	1.183	0.618–2.265	0.610			
Location Left/Right	0.497	0.321–0.752	0.028	0.469	0.309–0.692	0.024
Pathology type Infiltrating/Ulcerative	0.952	0.637–1.754	0.574			
Differentiation Poorly/Well–moderately	1.147	0.763–1.624	0.463			
Depth of tumor invasion T2–3/T4	0.712	0.576–0.943	0.047	0.863	0.633–1.057	0.079
Lymph mode metastasis N1/N2	0.695	0.504–0.983	0.035	0.764	0.568–0.946	0.044
TNM stage III stage/IV stage	0.574	0.368–0.889	0.037	0.565	0.341–0.863	0.019
CDK1 expression High expression/Low expression	1.737	1.326–2.867	0.018	1.697	1.265–2.675	0.021

Abbreviations: HR, hazard ratio; CI, confidence interval.

SW480/5-Fu cells ($27.97 \pm 1.05 \mu\text{M}$ VS $5.60 \pm 0.23 \mu\text{M}$) (Figure 2D and E). As shown in Figure 2F, we also found that CDK1 expression significantly increased in fluorouracil-resistant cells. These studies suggested that CDK1 might participate in the drug resistance and serve as a predictor for chemotherapy response in CRC.

Down-Regulation of CDK1 Inhibited Fluorouracil-Resistant CRC Cells Proliferation

Three siRNAs for silencing CDK1 were transfected into HT-29 and SW480 cells. All siRNAs significantly reduced CDK1 expression compared with negative control, and the silencing ability of siRNA3 was highest (Figure 3A–D). CDK1 protein significantly increased in drug-resistant cells. When transfected with siRNA3, CDK1 expression was significantly attenuated in drug-resistant cells, with no difference compared to that in HT-29 and SW480 (Figure 3E and F). Subsequently, we explored the effect of CDK1 on IC₅₀ values in drug-resistant cells, and found that with CDK1 knockdown, the IC₅₀ was significantly reduced ($44.60 \pm 1.65 \mu\text{M}$ vs $19.33 \pm 0.69 \mu\text{M}$; $27.97 \pm 1.05 \mu\text{M}$ vs

$8.57 \pm 0.28 \mu\text{M}$). This suggested that inhibition of CDK1 expression reversed 5-Fu resistance in CRC (Figure 4A and B). MTT assay revealed that the proliferation ability of drug-resistant cells was significantly enhanced. However, with the silence of CDK1, the proliferation ability was significantly weakened, and no significant difference was observed between fluorouracil-resistant cells and fluorouracil-sensitive cells (Figure 4C and D). Exposed with 5-Fu (IC₅₀), the colony-forming ability of drug-resistant cells was significantly increased compared with fluorouracil-sensitive cells. While CDK1 was knocked down, the colony-forming ability was significantly suppressed in drug-resistant HT-29 and SW480 (Figure 4E and F). These results indicated that CDK1 promoted the proliferation in CRC and contributed to 5-Fu-resistance.

CDK1 Promoted Migration, Invasion and Inhibited Apoptosis in 5-Fu-Resistant CRC Cells

In HT-29/5-Fu and SW480/5-Fu cells, the migration ability was significantly enhanced compared with HT-29 and SW480, and CDK1 knockdown significantly inhibited the

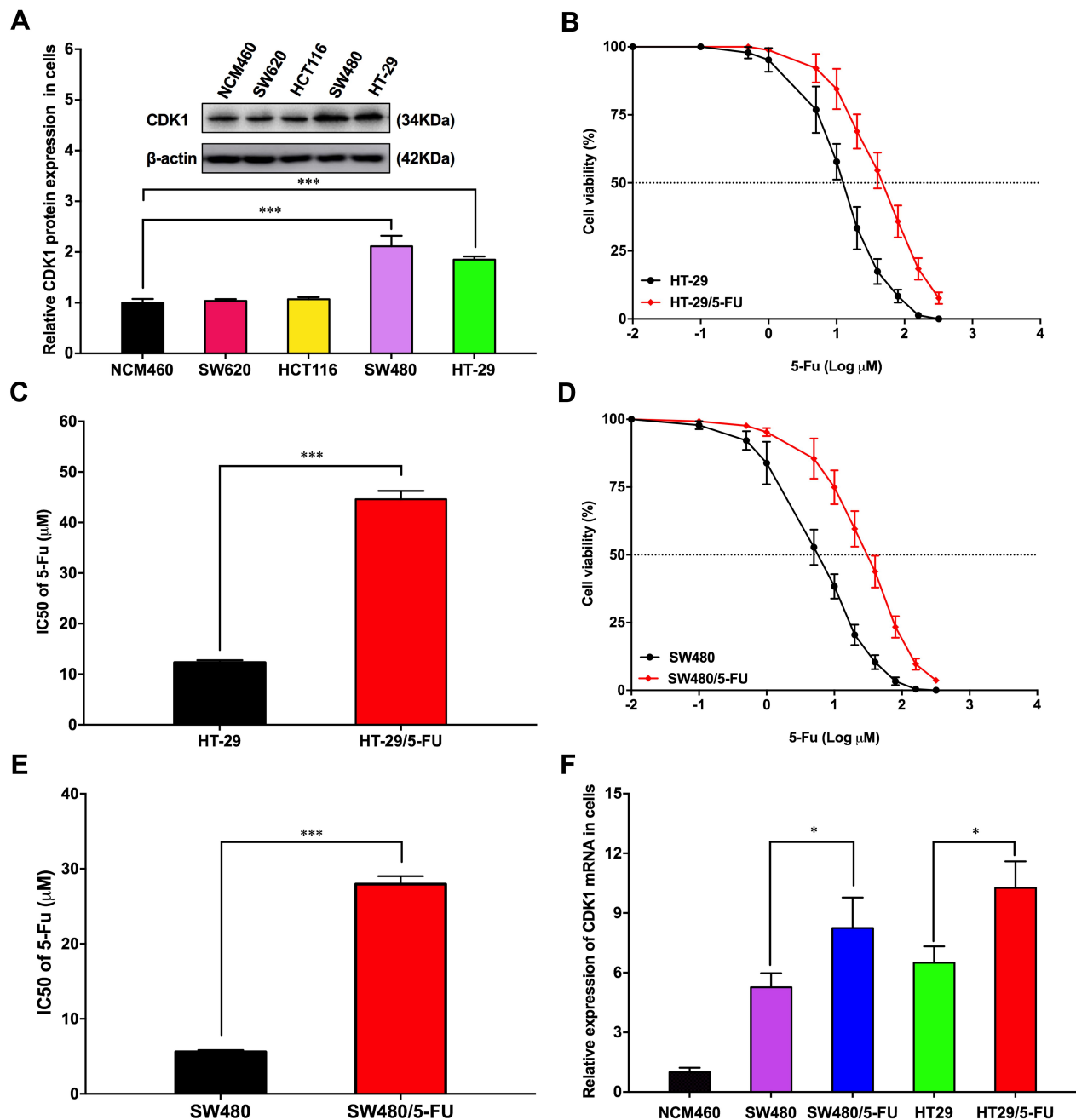


Figure 2 CDK1 up-regulation in fluorouracil-resistant CRC cell.

Notes: (A) Relative expression of CDK1 protein in colorectal mucosal epithelial cells (NCM460) and four CRC cell lines (SW620, SW480, HT-29, HCT116) was detected by Western blot. (B and C) IC₅₀ of HT-29 and HT-29/5-Fu was detected by MTT. (D and E) IC₅₀ of SW480 and SW480/5-Fu was detected by MTT. (F) The relative expression of CDK1 mRNA in fluorouracil-sensitive cells and fluorouracil-resistant cells. Quantity of protein was analyzed by Image J software. * $P < 0.05$, *** $P < 0.001$.

migration ability (Figure 5A and B). Exposed with 5-Fu (IC₅₀), less invasive cells were observed in fluorouracil-sensitive groups compared with fluorouracil-resistant groups. With the silence of CDK1, the invasive ability of fluorouracil-resistant cells was significantly reduced compared with control groups (Figure 5C and D). Flow cytometry showed

that treated with 5-Fu (IC₅₀), the apoptosis rate of HT-29 was $49.42 \pm 2.62\%$ and SW480 was $49.80 \pm 2.14\%$. While in fluorouracil-resistant groups, the apoptosis rate decreased significantly, which was $25.92 \pm 1.66\%$ (HT-29/5-Fu) and $29.82 \pm 1.60\%$ (SW480/5-Fu). We silenced CDK1 in fluorouracil-resistant cells and observed apoptosis rate increased

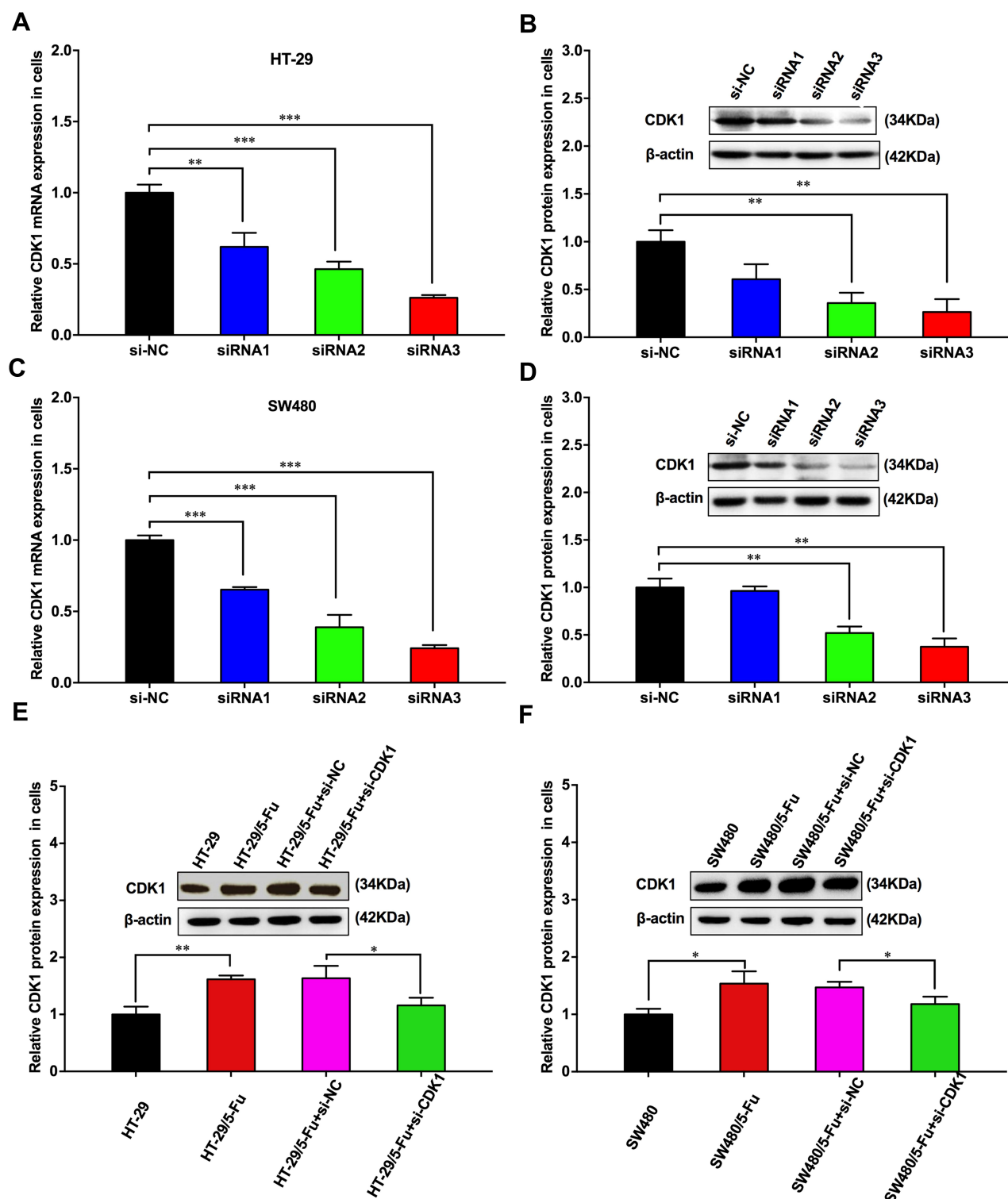


Figure 3 CDK1 was knocked down by using siRNA.

Notes: (A and C) In HT-29 and Sw480 cell lines, the relative expression of CDK1 mRNA was examined by qRT-PCR in the situations of si-NC, siRNA1, siRNA2, and siRNA3. (B and D) In HT-29 and Sw480 cell lines, the relative expression of CDK1 protein was examined by WB in the situations of si-NC, siRNA1, siRNA2, and siRNA3. (E and F) CDK1 expression in fluorouracil-resistant cells significantly attenuated by using siRNA3. Quantity of protein was analyzed by Image J software. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

significantly ($46.41 \pm 3.02\%$ vs $30.1 \pm 1.43\%$, $P < 0.01$; $47.39 \pm 1.12\%$ vs $27.49 \pm 2.52\%$, $P < 0.01$). The siRNA-CDK1 group and fluorouracil-sensitive cells had similar apoptosis

rates, with no statistical difference (Figure 5E and F). Silencing of CDK1 promoted apoptosis and reversed the resistance of CRC to 5-Fu.

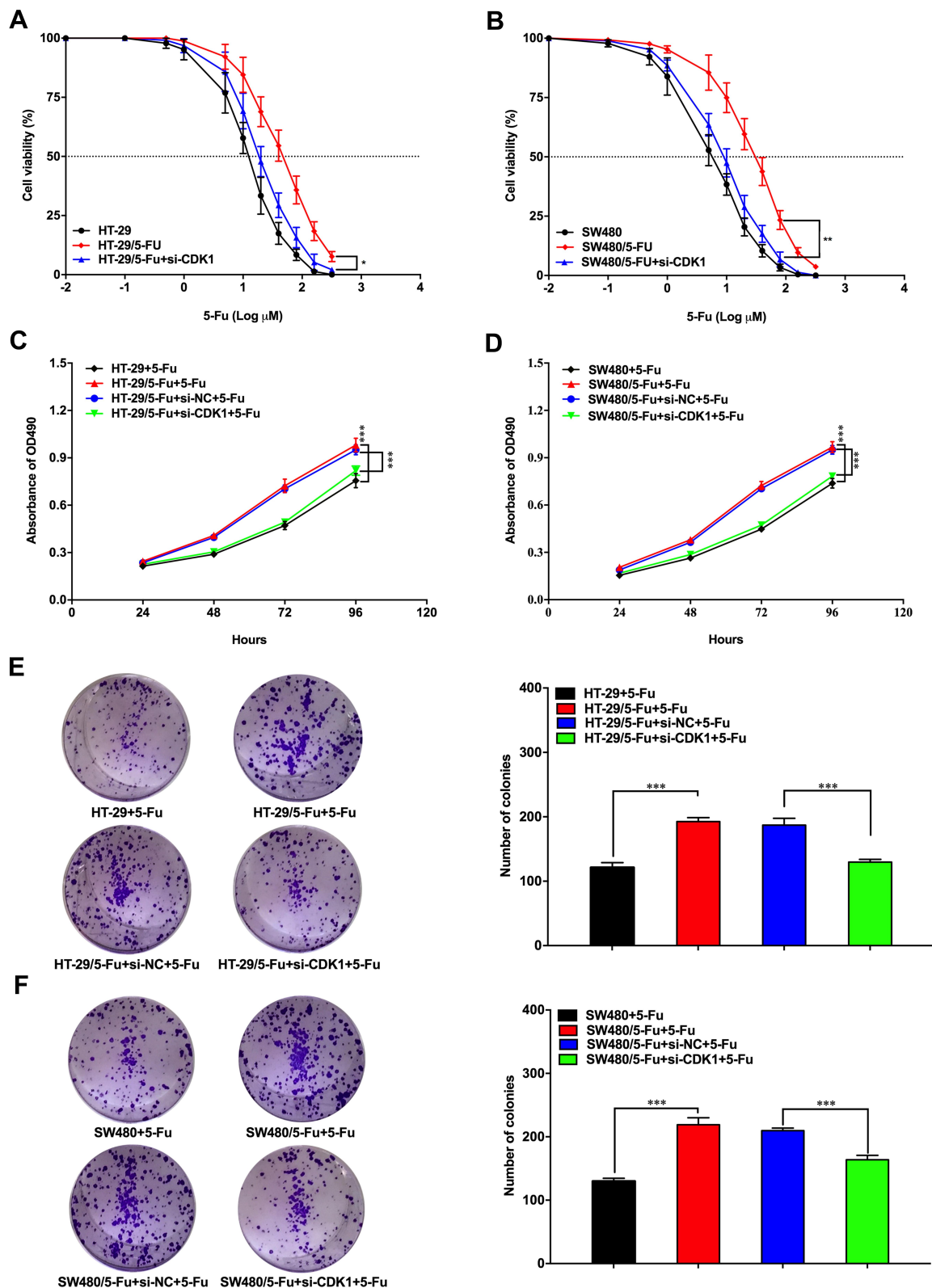


Figure 4 Down-regulation of CDK1 inhibited proliferation of fluorouracil-resistant CRC cells.

Notes: (A and B) Inhibition of CDK1 reversed the 5-Fu-resistance in HT-29/5-Fu and SW480/5-Fu. (C and D) Cell viability was significantly enhanced in HT-29/5-Fu and SW480/5-Fu than that in HT-29 and SW480. Knockdown of CDK1 weakened the proliferation ability. (E and F) Colony-forming ability was significantly increased in HT-29/5-Fu and SW480/5-Fu compared with HT-29 and SW480. Inhibition of CDK1 suppressed the colony-forming ability of drug-resistant HT-29 and SW480. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

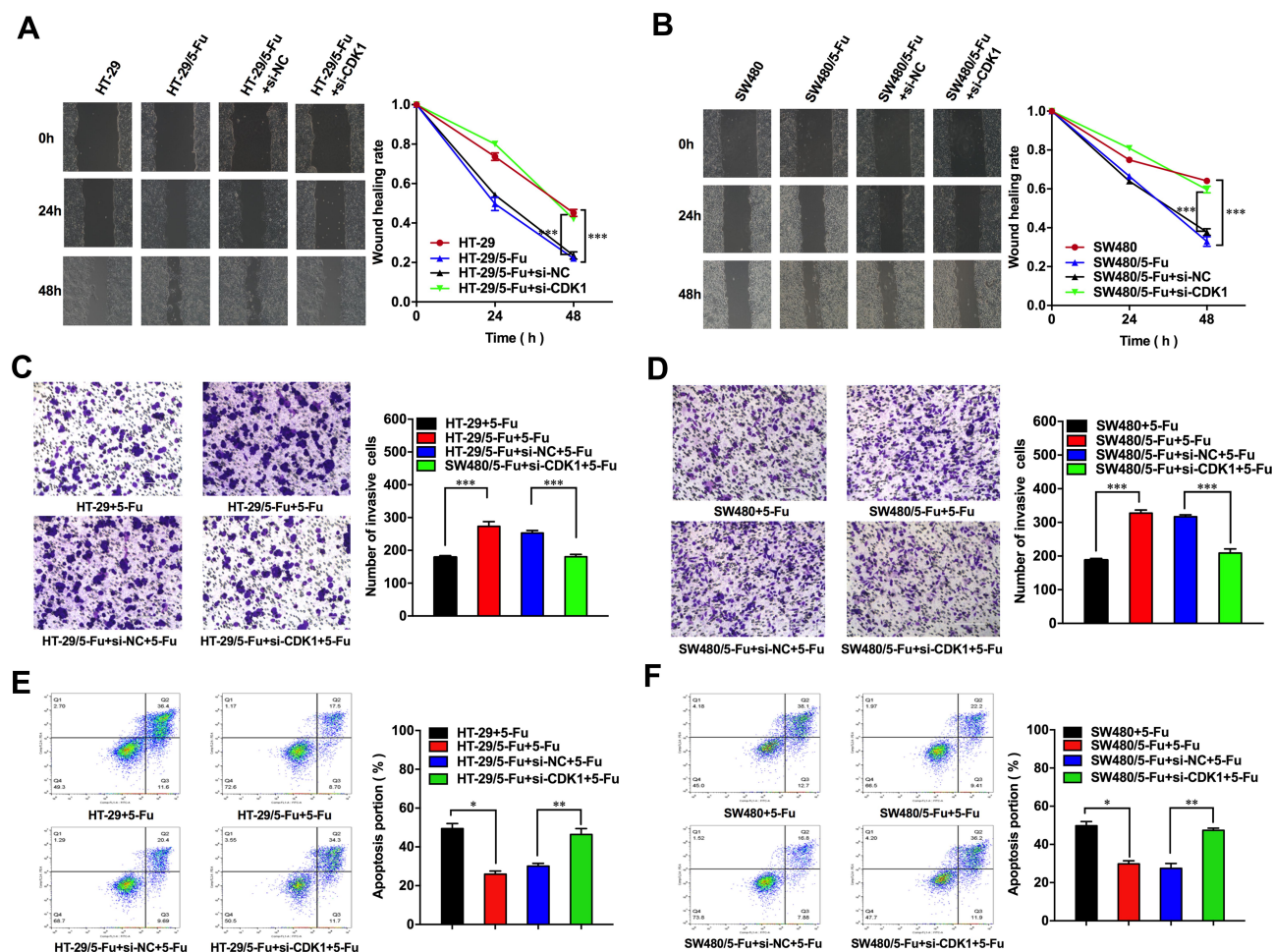


Figure 5 Down-regulation of CDK1 inhibited migration, invasion and promoted apoptosis in fluorouracil-resistant CRC cells.

Notes: (A and B) Wound healing assays were performed to evaluate cellular migration. CDK1 knockdown significantly inhibited the migration ability. (C and D) Transwell assays were performed to evaluate cellular invasion. Silencing of CDK1 attenuated the invasive ability of HT-29/5-Fu and SW480/5-Fu. (E and F) Apoptosis was determined by flow cytometry assays in CRC cells treated with 5-Fu. Inhibition of CDK1 promoted cell apoptosis in CRC cell lines. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

CDK1 Down-Regulation Inhibited CRC Tumorigenesis in vivo

Infected with sh-CDK1 or sh-NC, HT-29/5-Fu cells were subcutaneously inoculated into the armpit of BALB/c mice. From the eighth day, 50 mg/kg of 5-Fu was intraperitoneally injected into each nude mouse every two days. Simultaneously, we recorded tumor size every three days. On the 15th day, mice were sacrificed and the transplanted tumor tissue was stripped (Figure 6A). In the sh-CDK1 group, the growth rate of subcutaneous transplanted tumors was significantly lower, and CDK1 expression was significantly reduced (Figure 6B–D). More TUNEL-positive cells and lower-intensity Ki-67 staining were also observed in the sh-CDK1 group (Figure 6E and F). These results demonstrated that the down-regulation of CDK1 inhibited tumor growth in vivo.

Discussion

CDK1 was abnormally expressed in several tumors.²⁹ Phosphorylated CDK1 was highly expressed and related to poor outcome in cholangiocarcinoma patients with immunohistochemical analysis. Roscovitine, a CDK inhibitor, could significantly decrease the expression of p-CDK1. With p-CDK1 down-regulation, the ability of proliferation and invasion was significantly inhibited, and the cell cycle was stagnated.³⁰ CDK1 phosphorylated TFPC2L1 at Thr177, thereby affecting the cell cycle progression, pluripotency, and differentiation in embryonic stem cells. Activation of the CDK1-TFPC2L1 pathway stimulated cell proliferation, self-renewal and invasion in human aggressive bladder cancer.³¹ Deng³² reported that the up-regulation of CDK1 inhibited the G2/M phase block and promoted cervical cancer resistance to

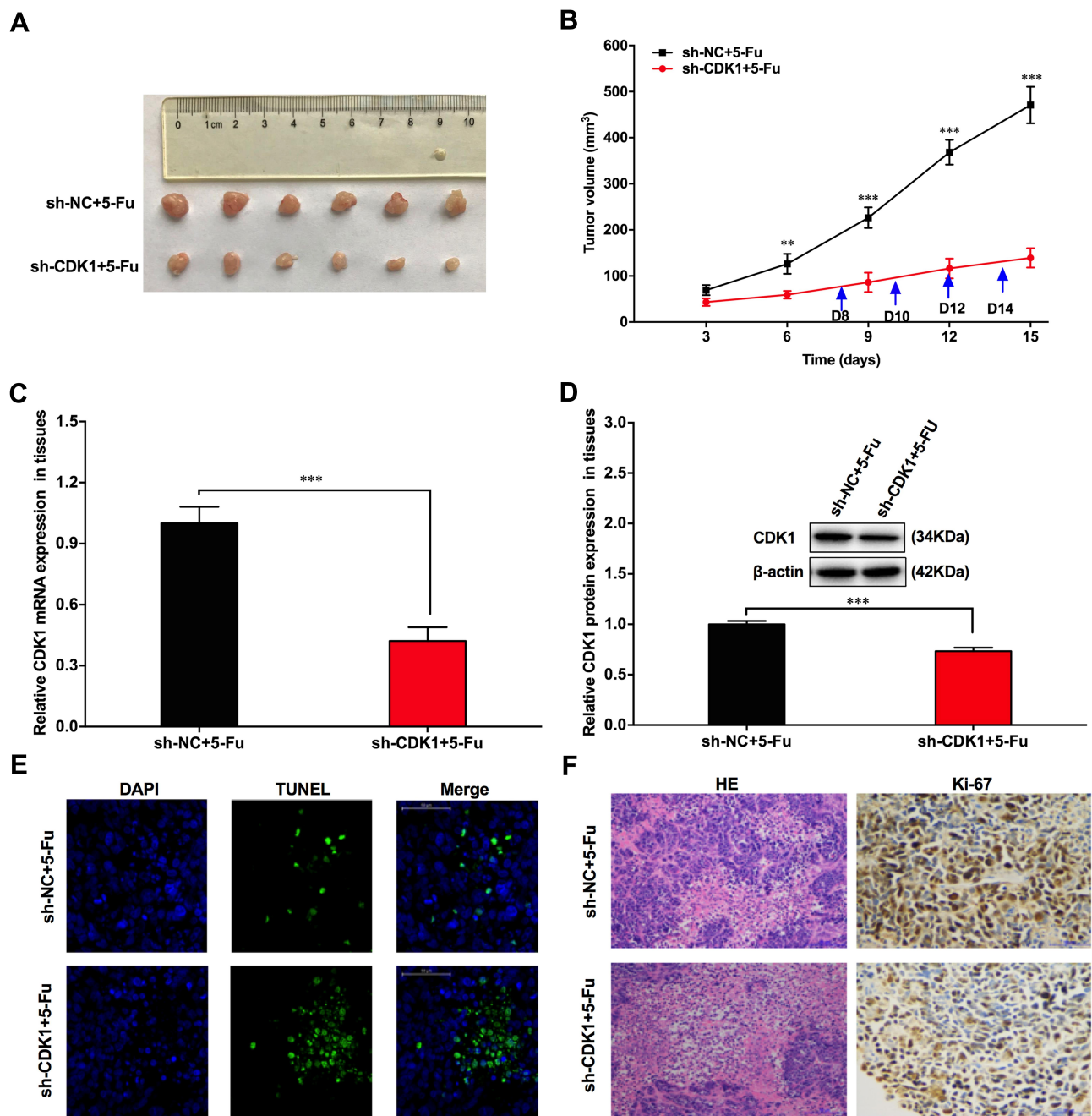


Figure 6 CDK1 down-regulation inhibited CRC tumorigenesis in vivo.

Notes: (A) Representative images of tumors from implanted mice. (B) Growth curves of tumors. (C) Relative expression of CDK1 mRNA in xenograft tumor tissues was detected by qRT-PCR. (D) Relative expression of CDK1 protein in xenograft tumor tissues was detected by WB. (E) Apoptotic cells were determined by TUNEL. (F) The expression of Ki-67 in tissues was visualized by immunohistochemistry (IHC) staining. ** $P < 0.01$, *** $P < 0.001$.

radiotherapy. CDK1 was also found to be stably activated by CDK1-KCTD12 interaction in BRAF-mutated colon cancer models resistant to Vemurafenib. When the CDK1-KCTD12 interaction was disrupted, the activation of CDK1 was inhibited, which reversed the resistance of Vemurafenib in CRC.³³

CDK1, a cell cycle checkpoint regulator, facilitated cell viability via mediating anti-apoptotic factors survivin^{34,35}

and Mcl-1.³⁶ Shapiro et al have proposed that CDK1 inhibitors might serve as the potential therapeutic target for tumors.³⁷ In KRAS-mutated colorectal and pancreatic cancer cells, CDK1 inhibitors had been shown to reduce the proportion of S-phase cells in KRAS-mutant cells and regulate Rb (the main control point of G1/S). CDK1 could be a synthetic lethal therapeutic target for KRAS mutations.³⁸ Another study showed that a combination of

CDK1 inhibitors and sorafenib could overcome the resistance of sorafenib in hepatocellular carcinoma by down-regulating CDK1 and inactivating downstream PDK1 and β -catenin.³⁹ Hansel reported that Cdc2/Cdk1 was differentially up-regulated in adenocarcinoma compared with normal esophageal mucosal epithelium. Treated with CDC2/CDK1 inhibitor, the proliferation of esophageal adenocarcinoma cells showed a dose-dependent reduction.⁴⁰

In the present study, we observed higher expression of CDK1 in tumor tissues, especially in fluorouracil-resistant tissues and cells. In fluorouracil-resistant cells, the ability of proliferation, migration, and invasion was significantly enhanced, and the apoptosis significantly reduced. We also demonstrated that inhibition of CDK1 expression could suppress CRC progression in vivo and in vitro. CDK1 was closely related to the clinicopathological features. We revealed that the silencing of CDK1 reversed the resistance of 5-Fu in CRC. This indicated that CDK1 might be a predictor of 5-Fu resistance and targeting CDK1 might be a novel strategy in CRC. However, our study did not detect the CDK1 downstream key protein level, when the CDK1 was knocked down, the mechanisms of CDK1 leading to drug resistance should be further explored.

Funding

Funding of “Peak” Training Program for Scientific Research of Yijishan Hospital, Wannan Medical College (GF2019G11) supported this study.

Disclosure

The authors declare no competing interests.

References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424. doi:10.3322/caac.21492
- Amd W, Eth F, Church TR, et al. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American cancer society. *CA Cancer J Clin*. 2018;68:250–281.
- Corley DA, Jensen CD, Quinn VP, et al. Association between time to colonoscopy after a positive fecal test result and risk of colorectal cancer and cancer stage at diagnosis. *JAMA*. 2017;317:1631–1641. doi:10.1001/jama.2017.3634
- Li L, Ma BB. Colorectal cancer in Chinese patients: current and emerging treatment options. *Onco Targets Ther*. 2014;7:1817–1828. doi:10.2147/OTT.S48409
- Schmidt-Kittler O, Ragg T, Daskalakis A, et al. From latent disseminated cells to overt metastasis: genetic analysis of systemic breast cancer progression. *Proc Natl Acad Sci U S A*. 2003;100:7737–7742. doi:10.1073/pnas.1331931100
- Dienstmann R, Salazar R, Tabernero J. Personalizing colon cancer adjuvant therapy: selecting optimal treatments for individual patients. *J Clin Oncol*. 2015;33:1787–1796.
- Saltz LB, Minsky B. Adjuvant therapy of cancers of the colon and rectum. *Surg Clin North Am*. 2002;82:1035–1058. doi:10.1016/S0039-6109(02)00041-5
- Robinson E, Bartal A, Cohen Y, Haim N, Mohilever J, Mekori T. Combined adjuvant therapy of radically operated colorectal cancer patients. *Cancer Chemother Pharmacol*. 1982;8:35–40. doi:10.1007/BF00292869
- Kerr DJ, Gray R, McConkey C, Barnwell J. Adjuvant chemotherapy with 5-fluorouracil, L-folinic acid and levamisole for patients with colorectal cancer: non-randomised comparison of weekly versus four-weekly schedules—less pain, same gain. QUASAR colorectal cancer study group. *Ann Oncol*. 2000;11:947–955. doi:10.1023/A:1008303229469
- Shigeta K, Ishii Y, Hasegawa H, Okabayashi K, Kitagawa Y. Evaluation of 5-fluorouracil metabolic enzymes as predictors of response to adjuvant chemotherapy outcomes in patients with stage II/III colorectal cancer: a decision-curve analysis. *World J Surg*. 2014;38:3248–3256. doi:10.1007/s00268-014-2738-1
- Tajima Y, Shimada Y, Kameyama H, et al. Association between poorly differentiated clusters and efficacy of 5-fluorouracil-based adjuvant chemotherapy in stage III colorectal cancer. *Jpn J Clin Oncol*. 2017;47:313–320. doi:10.1093/jjco/hyw209
- Innocenti F, Ou FS, Qu X, et al. Mutational analysis of patients with colorectal cancer in CALGB/SWOG 80405 identifies new roles of microsatellite instability and tumor mutational burden for patient outcome. *J Clin Oncol*. 2019;37:1217–1227. doi:10.1200/JCO.18.01798
- Modest DP, Martens UM, Riera-Knorrenschild J, et al. FOLFOXIRI plus panitumumab as first-line treatment of RAS wild-type metastatic colorectal cancer: the randomized, open-label, phase II VOLFI Study (AIO KRK0109). *J Clin Oncol*. 2019;37:3401–3411.
- Oki E, Kato T, Bando H, et al. A multicenter clinical phase ii study of FOLFOXIRI plus bevacizumab as first-line therapy in patients with metastatic colorectal cancer: QUATTRO study. *Clin Colorectal Cancer*. 2018;17:147–155. doi:10.1016/j.clcc.2018.01.011
- Joag MG, Sise A, Murillo JC, et al. Topical 5-fluorouracil 1% as primary treatment for ocular surface squamous neoplasia. *Ophthalmology*. 2016;123:1442–1448. doi:10.1016/j.ophtha.2016.02.034
- Bian Z, Jin L, Zhang J, et al. LncRNA-UCA1 enhances cell proliferation and 5-fluorouracil resistance in colorectal cancer by inhibiting miR-204-5p. *Sci Rep*. 2016;6:23892. doi:10.1038/srep23892
- Wang M, Hu H, Wang Y, et al. Long non-coding RNA TUG1 mediates 5-fluorouracil resistance by acting as a ceRNA of miR-197-3p in colorectal cancer. *J Cancer*. 2019;10:4603–4613. doi:10.7150/jca.32065
- Zhang LH, Li LH, Zhang PF, Cai YF, Hua D. LINC00957 acted as prognostic marker was associated with fluorouracil resistance in human colorectal cancer. *Front Oncol*. 2019;9:776. doi:10.3389/fonc.2019.00776
- Huang WW, Ko SW, Tsai HY, et al. Cantharidin induces G2/M phase arrest and apoptosis in human colorectal cancer colo 205 cells through inhibition of CDK1 activity and caspase-dependent signaling pathways. *Int J Oncol*. 2011;38:1067–1073. doi:10.3892/ijo.2011.922
- Xi Q, Huang M, Wang Y, et al. The expression of CDK1 is associated with proliferation and can be a prognostic factor in epithelial ovarian cancer. *Tumour Biol*. 2015;36:4939–4948. doi:10.1007/s13277-015-3141-8
- Zheng HP, Huang ZG, He RQ, et al. Integrated assessment of CDK1 upregulation in thyroid cancer. *Am J Transl Res*. 2019;11:7233–7254.
- Sung WW, Lin YM, Wu PR, et al. High nuclear/cytoplasmic ratio of Cdk1 expression predicts poor prognosis in colorectal cancer patients. *BMC Cancer*. 2014;14:951. doi:10.1186/1471-2407-14-951

23. Li J, Zhi X, Shen X, et al. Depletion of UBE2C reduces ovarian cancer malignancy and reverses cisplatin resistance via downregulating CDK1. *Biochem Biophys Res Commun.* **2020**;523:434–440.
24. Shang J, Xu YD, Zhang YY, Li M. Long noncoding RNA OR3A4 promotes cisplatin resistance of non-small cell lung cancer by upregulating CDK1. *Eur Rev Med Pharmacol Sci.* **2019**;23:4220–4225. doi:10.26355/eurrev_201905_17926
25. Zhang P, Kawakami H, Liu W, et al. Targeting CDK1 and MEK/ERK overcomes apoptotic resistance in BRAF-mutant human colorectal cancer. *Mol Cancer Res.* **2018**;16:378–389. doi:10.1158/1541-7786.MCR-17-0404
26. Zhang K, Li M, Huang H, et al. Dishevelled1-3 contribute to multi-drug resistance in colorectal cancer via activating Wnt/ β -catenin signaling. *Oncotarget.* **2017**;8:115803–115816. doi:10.18632/oncotarget.23253
27. Zhu Y, Bian Y, Zhang Q, et al. Construction and analysis of dysregulated lncRNA-associated ceRNA network in colorectal cancer. *J Cell Biochem.* **2019**;120(6):9250–9263. doi:10.1002/jcb.28201
28. Zhu Y, Bian Y, Zhang Q, et al. LINC00365 promotes colorectal cancer cell progression through the Wnt/ β -catenin signaling pathway. *J Cell Biochem.* **2020**;121:1260–1272. doi:10.1002/jcb.29359
29. Piao J, Zhu L, Sun J, et al. High expression of CDK1 and BUB1 predicts poor prognosis of pancreatic ductal adenocarcinoma. *Gene.* **2019**;701:15–22. doi:10.1016/j.gene.2019.02.081
30. Yamamura M, Sato Y, Takahashi K, Sasaki M, Harada K. The cyclin-dependent kinase pathway involving CDK1 is a potential therapeutic target for cholangiocarcinoma. *Oncol Rep.* **2020**;43:306–317. doi:10.3892/or.2019.7405
31. Heo J, Noh BJ, Lee S, et al. Phosphorylation of TFCEP2L1 by CDK1 is required for stem cell pluripotency and bladder carcinogenesis. *EMBO Mol Med.* **2020**;12:e10880. doi:10.15252/emmm.201910880
32. Deng YR, Chen XJ, Chen W, et al. Sp1 contributes to radioresistance of cervical cancer through targeting G2/M cell cycle checkpoint CDK1. *Cancer Manag Res.* **2019**;11:5835–5844. doi:10.2147/CMAR.S200907
33. Yang J, Xu WW, Hong P, et al. Adefovir dipivoxil sensitizes colon cancer cells to vemurafenib by disrupting the KCTD12-CDK1 interaction. *Cancer Lett.* **2019**;451:79–91. doi:10.1016/j.canlet.2019.02.050
34. O'Connor DS, Grossman D, Plescia J, et al. Regulation of apoptosis at cell division by p34cdc2 phosphorylation of survivin. *Proc Natl Acad Sci U S A.* **2000**;97:13103–13107. doi:10.1073/pnas.240390697
35. O'Connor DS, Wall NR, Porter AC, Altieri DC. A p34(cdc2) survival checkpoint in cancer. *Cancer Cell.* **2002**;2:43–54. doi:10.1016/S1535-6108(02)00084-3
36. Rossi AG, Sawatzky DA, Walker A, et al. Cyclin-dependent kinase inhibitors enhance the resolution of inflammation by promoting inflammatory cell apoptosis. *Nat Med.* **2006**;12:1056–1064. doi:10.1038/nm1468
37. Shapiro GL. Cyclin-dependent kinase pathways as targets for cancer treatment. *J Clin Oncol.* **2006**;24:1770–1783. doi:10.1200/JCO.2005.03.7689
38. Costa-Cabral S, Brough R, Konde A, et al. CDK1 is a synthetic lethal target for KRAS mutant tumours. *PLoS One.* **2016**;11:e0149099. doi:10.1371/journal.pone.0149099
39. Wu CX, Wang XQ, Chok SH, et al. Blocking CDK1/PDK1/ β -Catenin signaling by CDK1 inhibitor RO3306 increased the efficacy of sorafenib treatment by targeting cancer stem cells in a preclinical model of hepatocellular carcinoma. *Theranostics.* **2018**;8:3737–3750. doi:10.7150/thno.25487
40. Hansel DE, Dhara S, Huang RC, et al. CDC2/CDK1 expression in esophageal adenocarcinoma and precursor lesions serves as a diagnostic and cancer progression marker and potential novel drug target. *Am J Surg Pathol.* **2005**;29:390–399. doi:10.1097/00000478-200503000-00014

Cancer Management and Research

Dovepress

Publish your work in this journal

Cancer Management and Research is an international, peer-reviewed open access journal focusing on cancer research and the optimal use of preventative and integrated treatment interventions to achieve improved outcomes, enhanced survival and quality of life for the cancer patient.

The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/cancer-management-and-research-journal>