European Review for Medical and Pharmacological Sciences 2020; 24: 1743-1754

CircDDX17 reduces 5-fluorouracil resistance and hinders tumorigenesis in colorectal cancer by regulating miR-31-5p/KANK1 axis

T.-J. REN, C. LIU, J.-F. HOU, F.-X. SHAN

Department of Oncology, Luoyang Central Hospital Affiliated to Zhengzhou University, Luoyang, Henan, China

Abstract. – OBJECTIVE: Colorectal cancer (CRC) is one of the most common malignancies worldwide. Chemotherapy resistance is a considerable obstacle to CRC treatment. Circular RNAs (circRNAs) are involved in the pathogenesis of many cancers. This study aimed to investigate the role and molecular basis of DEAD-box helicase 17 circRNA (circDDX17) in 5-fluorouracil (5-Fu) sensitivity and CRC progression.

MATERIALS AND METHODS: The levels of circDDX17, microRNA-31-5p (miR-31-5p) and kidney ankyrin repeat-containing protein 1 (KANK1) were detected by quantitative real-time PCR or western blot assay. Cell viability was assessed by Cell Counting Kit-8 (CCK-8) assay. Cell apoptosis rate was monitored by flow cytometry. Cell invasion capacity was evaluated by transwell assay. Western blot assay was conducted to measure the expression of matrix metallopeptidase 9 (MMP9) and E-cadherin. The interaction among circDDX17, miR-31-5p and KANK1 was indicated by bioinformatics analysis and dual-luciferase reporter assay. Xenograft assay was performed to analyze tumor growth and 5-Fu sensitivity *in vivo*.

RESULTS: CircDDX17 and KANK1 were down-regulated, while miR-31-5p was upregulated in CRC tissues and cells. Upregulation of circDDX17 enhanced 5-Fu sensitivity and impeded CRC development. CircDDX17 inhibited 5-Fu resistance and CRC progression *via* sponging miR-31-5p. Besides, KANK1 depletion attenuated the effect of circDDX17 upregulation on chemosensitivity and CRC progression. CircDDX17 regulated KANK1 expression by binding to miR-31-5p. Moreover, circDDX17 overexpression blocked tumor growth and elevated 5-Fu sensitivity *in vivo*.

CONCLUSIONS: Upregulation of circDDX17 strengthened chemosensitivity of CRC to 5-Fu and blocked CRC progression by regulating miR-31-5p/KANK1 axis, which might provide an effective treatment strategy for CRC patients.

Key Words:
Colorectal cancer, CircDDX17, MiR-31-5p, KANK1, 5-fluorouracil.

Introduction

Colorectal cancer (CRC) is listed as the third most frequently diagnosed cancer with a high mortality rate[1]. Colorectal tumors in the early stages can be surgically removed, but the recurrence and metastasis of CRC patients are very high, leading to a poor prognosis of colorectal cancer[2]. Moreover, many CRC patients are not diagnosed until late stage due to limitations in early diagnostic markers and methods[3]. Thus, studying the deep molecular mechanism of CRC progression is significant for improving CRC treatment.

Circular RNAs (circRNAs) are a new class of non-coding RNAs (ncRNAs) without the ability to encode proteins[4]. Unlike linear RNA, circRNAs have a covalent closed-loop structure, so they are more stable[5]. CircRNAs are crucial regulators of various biological functions in tumor progression by functioning as microRNA molecular sponges[6]. Circ_0005576 was upregulated in cervical cancer and exerted oncogenic properties by sponging microRNA-153 and increasing KIF20A expression[7]. CircFBXW7 suppressed the development of triple-negative breast cancer by regulating the microRNA-197-3p/FBXW7-185aa axis[8]. In addition, some circRNAs have been found to participate in the development of CRC[9]. Like, circ_0009361 impeded CRC progression through regulation of cell proliferation and epithelial-mesenchymal transition (EMT)[10]. CircVAPA accelerated CRC development *via* modulating microRNA-101[11]. A recent report[12] revealed that circRNA derived from DEAD-box helicase 17 (circDDX17) was a tumor-suppressing factor in CRC. However, the molecular mechanism of circDDX17 has not been studied.

MicroRNAs (miRNAs) are a type of short ncRNAs composed of 18-22 nucleotides[13]. Mul-

Corresponding Author: Tiejun Ren, MD; e-mail: gfwnhp@163.com

tiple miRNAs may be directly related to the drug resistance of CRC[14]. MiR-874-3p potentiated the chemosensitivity of CRC cells by activating the Hippo signaling pathway[15]. MiR-106a enhanced chemoresistance of CRC cells *via* repressing DUSP2 expression[16]. MiR-223 expression was reduced, and miR-223 knockdown increased the doxorubicin sensitivity through regulation of FBXW7 in CRC[17]. Additionally, miR-31-5p has been reported to be involved in various human cancers, such as oral cancer[18], renal cell carcinoma[19] and hepatocellular carcinoma[20]. However, the relationship between circDDX17 and miR-31-5p has not been investigated.

The kidney ankyrin repeat-containing protein 1 (KANK1) is one of the critical members of the KANK family[21]. In several cancers, the promoter methylation and hybridization deletion of KANK1 lead to insufficient or down-regulated expression[22]. Some researchers have certified that KANK1 has the effect of inhibiting tumor progression [23,24]. Nevertheless, the antitumor effect of KANK1 in CRC remains unclear.

In the present research, we measured the expression of circDDX17, miR-31-5p and KANK1 in CRC tissues and cells. Later, we explored the function and potential mechanism of circDDX17 in CRC cells. These findings contributed to our understanding of CRC resistance and progression.

Materials and Methods

Specimen Collection

Thirty CRC specimens and paired adjacent normal tissues were collected from CRC patients who underwent surgical resection at Luoyang Central Hospital Affiliated to Zhengzhou University. None of them received preoperative radiotherapy or chemotherapy. This study was approved by the Ethics Committee of Luoyang Central Hospital Affiliated to Zhengzhou University. All participants signed written informed consents.

Cell Culture

Human normal colorectal epithelial cell line NCM460 was commercially obtained from BinSuiBio (Shanghai, China). Two CRC cell lines (HCT116 and SW480) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were maintained at 37°C in Roswell Park Memorial Institute 1640 (RPMI-1640; Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco).

Cell Transfection

CircDDX17 overexpression vector (DDX17), the empty overexpression vector (pcDNA), miR-31-5p mimics, the mimics control (miR-NC), small interfering RNA (siRNA) targeting circDDX17 (si-DDX17), siRNA against KANK1 (si-KANK1) and the siRNA control (si-NC) were synthesized from RiboBio (Guangzhou, China). Transfection of plasmids and oligonucleotides into CRC cells was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Quantitative Real-Time PCR (qRT-PCR)

RNA was extracted with TRIzol reagent (Invitrogen), and then the first-stranded cDNA was synthesized by M-MLV RT Kit (AiYou Biosciences, Guangzhou, China) or miScript II RT Kit (Qiagen, Frankfurt, Germany). Quantitative RT-PCR was performed using SYBR Green PCR Master Mix (LMAI Bio, Shanghai, China). The expression of circDDX17 and KANK1 was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH). MiR-31-5p expression was normalized by U6. The primers used included: circDDX17-F: 5'-TGCCAACCACAACATCCTCCA-3', circDDX17-R, 5'-CGCTCCCCAGGATTACCAAAT-3'; miR-31-5p-F: 5'-CGGCGGAGGCAAGATGCTGGCA-3', miR-31-5p-R, 5'-CAACTGGTGTCGTGGAGTCGG-3'; KANK1-F: 5'-GTGCCGAGGAGAACATGAAC-3', KANK1-R, 5'-CTCTAAGCTGTACTTCTAGGCGA-3'; GAPDH-F: 5'-ACAACTTTGGTATCGTGGAAGG-3', GAPDH-R, 5'-GCCATCACGCCACAGTTTC-3'; U6-F: 5'-CTCGCTTCGGCAGCACA-3', U6-R, 5'-ACGCTTCACGAATTTGCGT-3'.

Cell Counting Kit-8 (CCK-8) Assay

Cells were plated in 96-well plates and then treated with different doses of 5-fluorouracil (5-Fu) for 48 h. Next, 10 μL of CCK-8 solution (Beyotime, Shanghai, China) was added into each well after stimulation with 5-Fu. After incubation for 2 h, absorbance was examined at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). The half inhibition concentration (IC_{50}) was calculated by the cell viability curve.

Flow Cytometry

Cells were plated in six-well plates and washed twice with cold PBS. Cell apoptosis rate was monitored by AnnexinV-fluorescein isothiocyanate (AnnexinV-FITC)/Propidium Iodide (PI) Apoptosis Detection kit (Invitrogen) at 48 h post-transfection. Next, BD FACSCalibur flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) was used to assess the apoptotic index.

Cell Invasion Assay

HCT116 and SW480 cells were collected and resuspended after transfection. Subsequently, cells were plated into 24-well transwell chambers (Corning, Corning, NY, USA) precoated with Matrigel (BD Biosciences). The lower chamber was added with medium containing 20% FBS. After incubation for 24 h, the invaded cells were fixed using methanol and stained with crystal violet for 30 min. Next, the stained cells in 5 randomly selected fields were photographed and counted under a microscope.

Western Blot Assay

Cells were lysed with RIPA buffer (Solarbio, Beijing, China). After centrifugation and quantification, equal amounts of protein were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, the membranes were probed with primary antibodies against matrix metallopeptidase 9 (MMP9) (ab38898, Abcam, Cambridge, UK), E-cadherin (ab15148, Abcam), KANK1 (ab251677, Abcam) and GAPDH (ab9485, Abcam) at a dilution ratio of 1:1000. Next, the membranes interacted with secondary antibody (ab7090, Abcam). The signal intensity was tested by the enhanced chemiluminescence system (Millipore, Billerica, MA, USA).

Dual-Luciferase Reporter Assay

The sequences of circDDX17 or KANK1 3'UTR containing putative miR-31-5p binding sites were constructed into pmirGLO vectors (Promega, Madison, WI, USA) to form WT-DDX17 or WT-KANK1 reporter. Also, MUT-DDX17 or MUT-KANK1 reporter carrying the mutant miR-31-5p binding sites were generated. Then, the constructed luciferase reporter was cotransfected with miR-31-5p mimics or miR-NC into HCT116 and SW480 cells. After introduction for 48 h, the luciferase intensity was determined using a Dual Luciferase Reporter Assay Kit (Vazyme Biotech, Nanjing, China).

Lentivirus Infection

Lentivirus vectors carrying circDDX17 overexpression (lenti-DDX17) or the negative control (lenti-Control) were constructed by HanBio (Shanghai, China). Then, treated lentivirus and polybrene were cotransfected into SW480 cells using Lipofectamine 2000 (Invitrogen). Subsequently, puromycin was added to obtain stably expressing cells.

Xenograft Tumor Experiment

BALB/c nude mice (5-week-old) were randomly divided into 4 groups (Control, Control+5-Fu, DDX17, DDX17+5-Fu) with 5 mice in each group. SW480 cells harboring DDX17 or Control were subcutaneously injected into the right flank of mice. Seven days after injection, mice in the Control and DDX17 groups were intraperitoneally injected with PBS (500 μL), and mice in the Control+5-Fu and DDX17+5-Fu groups were injected with PBS containing 5-Fu (50 mg/kg) every 4 days. Tumor volume was measured every 4 days. Twenty-seven days later, the mice were anesthetized and sacrificed. Then, the xenografts were removed and weighed. The xenograft experiment was ratified by the Animal Welfare Committee of Luoyang Central Hospital Affiliated to Zhengzhou University.

Statistical Analysis

GraphPad Prism 7 software (GraphPad, San Diego, CA, USA) was used to assess the data. Data were presented as mean ± standard deviation. Student's *t*-test was used to compare the difference between two groups, and one-way analysis of variance followed by Tukey's test was employed to evaluate the differences among multiple groups. The linear relationship was analyzed by Spearman's correlation coefficient. $p<0.05$ indicated that the difference was statistically significant.

Results

CircDDX17 was Down-Regulated, while miR-31-5p was Upregulated in CRC Tissues and Cells

First, we attempted to determine the relationship between circDDX17 and miR-31-5p *in vivo*.

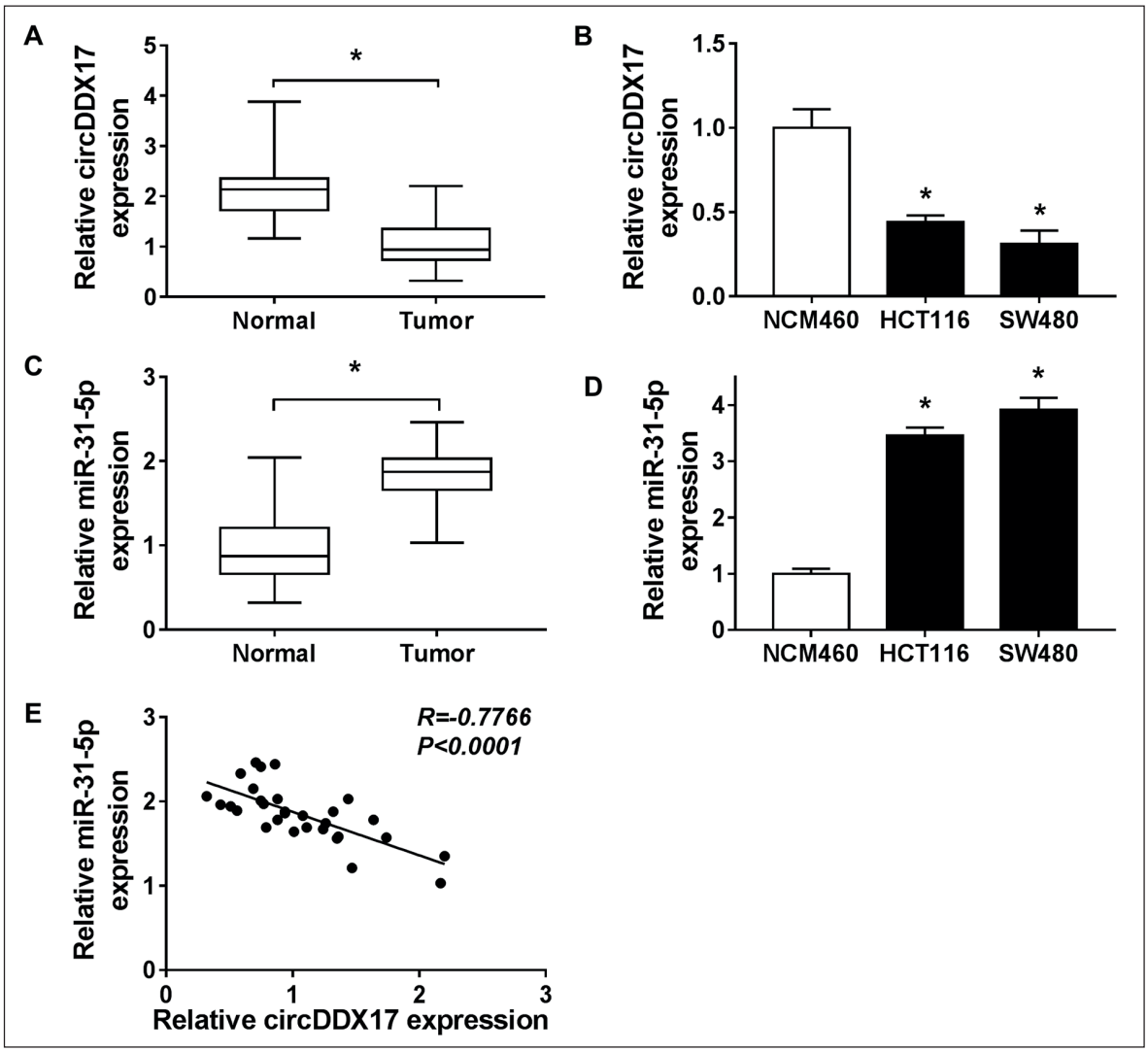

Figure 1. CircDDX17 was down-regulated, while miR-31-5p was upregulated in CRC tissues and cells. **(A** and **C)** The levels of circDDX17 and miR-31-5p were detected in CRC tissues (n=30) and adjacent normal tissues (n=30) using qRT-PCR. **(B** and **D)** The levels of circDDX17 and miR-31-5p were measured in NCM460 cells and CRC cell lines (HCT116 and SW480) by qRT-PCR. **(E)** The correlation between the levels of circDDX17 and miR-31-5p in CRC tissues was analyzed by Spearman's correlation coefficient. *$p < 0.05$.

The results indicated that circDDX17 expression was markedly reduced in CRC tissues compared with adjacent normal tissues (Figure 1A). Consistently, circDDX17 expression was remarkably lower in CRC cell lines (HCT116 and SW480) than that in NCM460 cells (Figure 1B). In addition, miR-31-5p was an oncogene in CRC. As expected, the expression of miR-31-5p was distinctly increased in CRC tissues and cells (Figure1C and 1D). Furthermore, Spearman's correlation analysis revealed a negative correlation between circDDX17 and miR-31-5p expression in CRC tissues (Figure 1E).

CircDDX17 Overexpression Enhanced 5-Fu Sensitivity and Apoptosis Rate and Hindered Invasion and EMT in CRC Cells

To investigate the role of circDDX17 in the chemoresistance and progression of CRC, HCT116 and SW480 cells were introduced with pcDNA or DDX17. First of all, the results exhibited that circDDX17 expression was strikingly increased in CRC cells transfected with DDX17 compared to the untransfected (Control) or pcDNA group (Figure 2A). Next, the effect of DDX17 on 5-Fu sensitivity of HCT116 and SW480 cells was studied by CCK-8 assay. The results indicated that the IC_{50}

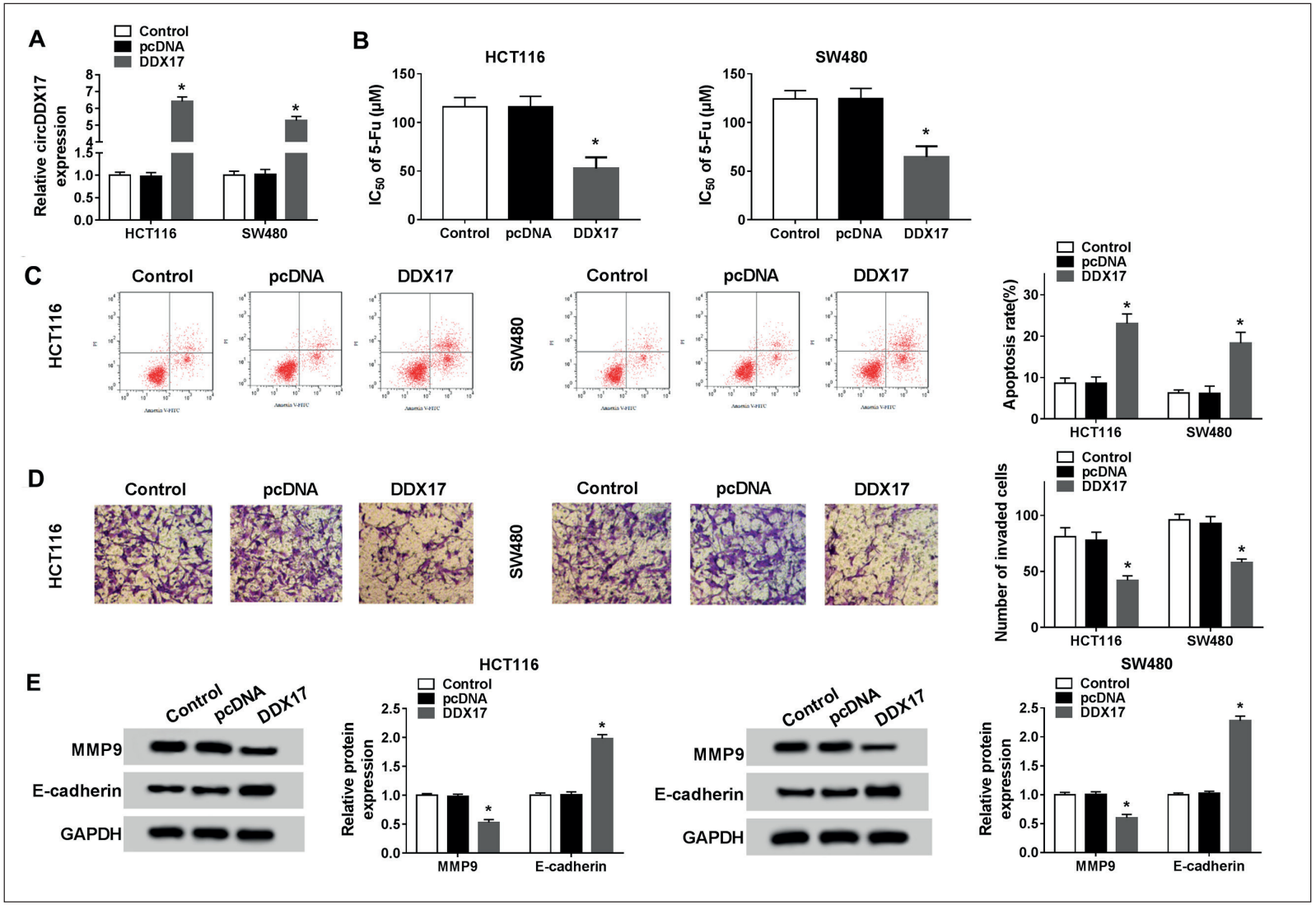

Figure 2. CircDDX17 overexpression enhanced 5-Fu sensitivity and apoptosis rate and hindered invasion and EMT in CRC cells. **(A-E)** HCT116 and SW480 cells were introduced with pcDNA or DDX17. **(A)** The expression of circDDX17 was examined by qRT-PCR. **(B)** IC_{50} of 5-Fu was evaluated by CCK-8 assay after 5-Fu treatment. **(C)** Cell apoptosis rate was monitored by flow cytometry. **(D)** Cell invasion was assessed using transwell assay (100 ×). **(E)** EMT-related markers (MMP9 and E-cadherin) were measured by western blot assay. $*p < 0.05$.

value of 5-Fu in CRC cells introduced with DDX17 was decreased compared to the pcDNA group (Figure 2B). Flow cytometry showed that upregulation of DDX17 triggered a distinct increase of apoptosis rate in HCT116 and SW480 cells (Figure 2C). Transwell assay suggested that cell invasion capacity was clearly decreased through overexpression of DDX17 (Figure 2D). Moreover, transfection with DDX17 resulted in an apparent reduction in MMP9 expression and a dramatical increase in E-cadherin expression (Figure 2E). These data evidenced that upregulation of DDX17 increased 5-Fu sensitivity and facilitated apoptosis and suppressed invasion and EMT in CRC cells.

CircDDX17 Regulated Chemosensitivity and Tumor Progression by Sponging miR-31-5p

StarBase v2.0 predicted that circDDX17 and miR-31-5p had targeted binding sites (Figure 3A). To further verify the interaction between circDDX17 and miR-31-5p, dual-luciferase reporter assay was performed, and the results showed that miR-31-5p mimics conspicuously reduced the luciferase activity of WT-DDX17 reporter, but did not affect the luciferase activity of MUT-DDX17 reporter in HCT116 and SW480 cells (Figure 3B). Additionally, qRT-PCR analysis unveiled that knockdown of DDX17 notably increased miR-31-

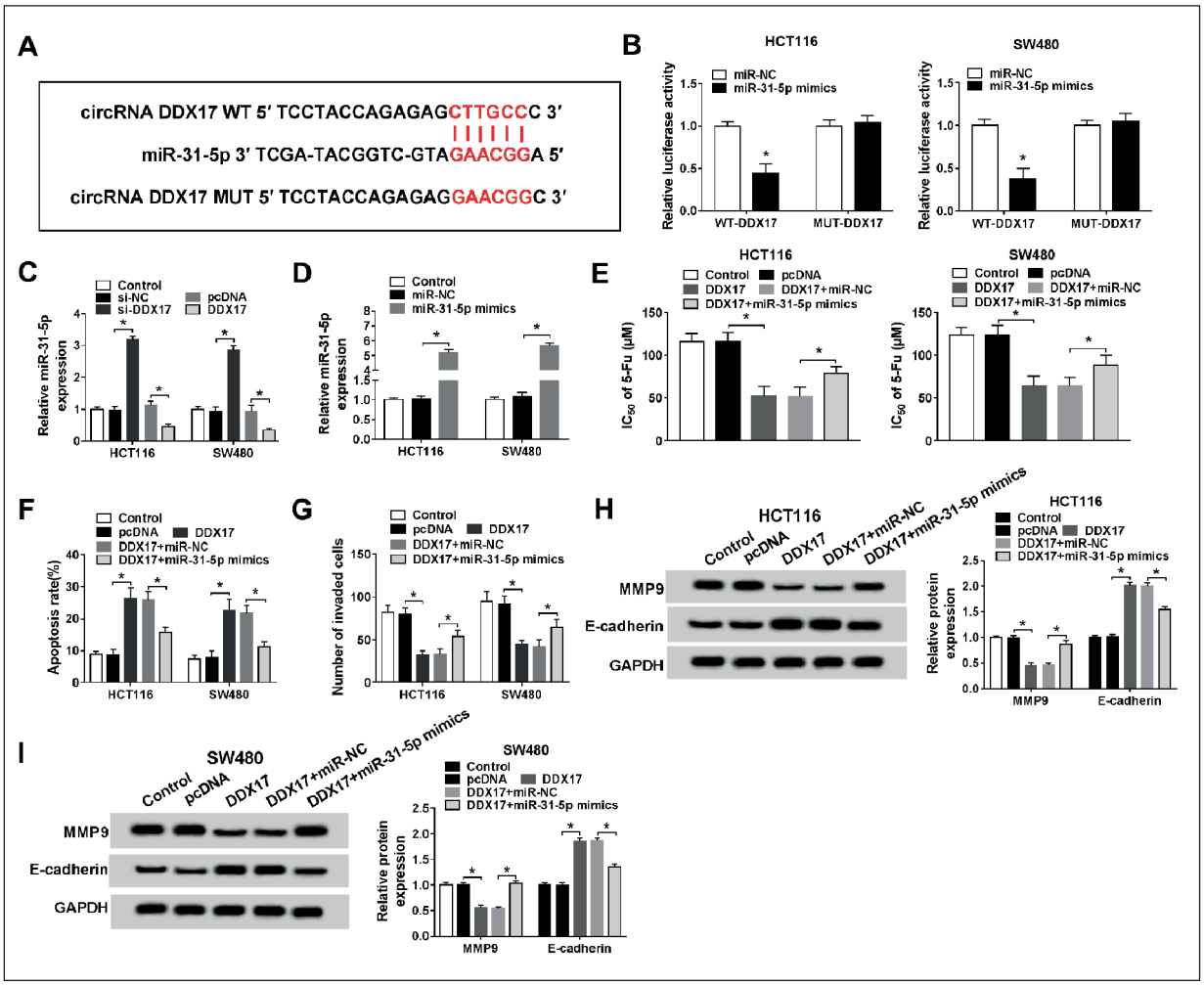

Figure 3. CircDDX17 regulated chemosensitivity and tumor progression by sponging miR-31-5p. **(A)** The putative binding sites of circDDX17 and miR-31-5p were shown. **(B)** Dual-luciferase reporter assay was performed to validate the interaction between circDDX17 and miR-31-5p. **(C)** The level of miR-31-5p was measured in HCT116 and SW480 cells introduced with si-NC, si-DDX17, pcDNA or DDX17. **(D)** The expression of miR-31-5p was detected in CRC cells after transfection with miR-NC or miR-31-5p mimics. **(E-I)** HCT116 and SW480 cells were introduced with pcDNA, DDX17, DDX17+miR-NC or DDX17+miR-31-5p mimics. **(E)** CCK-8 assay was utilized to calculate the IC_{50} of 5-Fu. **(F** and **G)** Flow cytometry and transwell assay were used to detect cell apoptosis rate and cell invaded numbers. **(H** and **I)** Western blot assay was used to measure the expression of EMT-related factors. *$p < 0.05$.

5p expression, while overexpression of DDX17 remarkably reduced miR-31-5p expression (Figure 3C). The expression of miR-31-5p was overtly higher in CRC cells introduced with miR-31-5p mimics than that in the miR-NC group, indicating a productive transfection efficiency (Figure 3D). Moreover, rescue experiments elucidated that miR-31-5p overexpression recovered the effect of DDX17 upregulation on pro-chemosensitivity (Figure 3E), pro-apoptosis (Figure 3F), anti-invasion (Figure 3G) and anti-EMT (Figure 3H and 3I). These data concluded that DDX17 modulated 5-Fu resistance and tumor progression by sponging miR-31-5p in CRC cells.

KANK1 was a Target of miR-31-5p

To further explore the molecular mechanism of circDDX17 in CRC, TargetScan online database was used to testify the existence of complementary sequences of miR-31-5p and KANK1 3'UTR (Figure 4A). Furtherly, dual-luciferase reporter assay validated that the luciferase activity of WT-KANK1 reporter was predominantly suppressed in HCT116 and SW480 cells transfected with miR-31-5p mimics, but luciferase activity was not affected when the binding sites were mutated (Figure 4B). Moreover, the mRNA and protein levels of KANK1 were markedly reduced in CRC tissues relative to adjacent normal tissues (Figure 4C). The correlation between miR-31-5p and KANK1 expression was negative in CRC tissues (Figure 4D). Compared with NCM460 cells, the mRNA and protein levels of KANK1 were overtly decreased in HCT116 and SW480 cells (Figure 4E). In a word, these data manifested that KANK1 was a target of miR-31-5p in CRC cells.

KANK1 Depletion Reversed the Effect of circDDX17 Overexpression on 5-Fu Resistance and Tumor Progression

Firstly, we analyzed the knockdown efficiency of KANK1, and the results revealed that KANK1 knockdown drastically reduced the mRNA and protein expression of KANK1 (Figure 5A and 5B). To elucidate whether circDDX17 affected chemosensitivity and tumor development in CRC by regulating KANK1, HCT116 and SW480 cells were introduced with pcDNA, DDX17, DDX17+si-NC or DDX17+si-KANK1. CCK-8 assay exhibited that the introduction of DDX17 led to a marked decrease in IC_{50} of 5-Fu, while this effect was counteracted by inhibiting KANK1 (Figure 5C). Flow cytometry showed that upregulation of DDX17 remarkably expedited cell apoptosis, whereas cotransfection of DDX17 and si-KANK1 abrogated the effect (Figure 5D). Transwell analysis discovered that cotransfection of DDX17 and si-KANK1 restored the decrease in the number of invaded cells induced by DDX17 overexpression (Figure 5E). In addition, DDX17 upregulation resulted in a noticeable decrease in MMP9 expression and an apparent increase in E-cadherin expression, while the levels were recuperated after transfection with si-KANK1 (Figure 5F). These data evidenced that circDDX17 inhibited 5-Fu resistance and tumor progression by modulating KANK1 in CRC cells.

CircDDX17 Upregulated KANK1 Expression by Sponging miR-31-5p

To investigate the interaction among circDDX17, miR-31-5p and KANK1, the expression of KANK1 was detected in HCT116 and SW480 cells introduced with miR-NC, miR-31-5p mimics, miR-31-5p mimics+pcDNA or miR-31-5p mimics+DDX17. Western blot analysis disclosed that miR-31-5p upregulation reduced KANK1 expression, while introduction of DDX17 reversed the effect on KANK1 expression caused by miR-31-5p mimics (Figure 6A and 6B). These data confirmed that circDDX17 acted as a molecular sponge of miR-31-5p to increase KANK1 expression in CRC cells.

CircDDX17 Overexpression Blocked Tumor Growth and Increased 5-Fu Sensitivity in Vivo

To evaluate the function of circDDX17 in tumorigenesis and 5-Fu sensitivity *in vivo*, the mice xenograft model was constructed. The results suggested that 5-Fu injection or DDX17 overexpression hindered tumor growth, and upregulation of DDX17 enhanced 5-Fu-induced antitumor function *in vivo* (Figure 7A and 7B). In addition, we found that circDDX17 expression was increased in tumor tissues infected with SW480 cells containing DDX17. These data concluded that upregulation of DDX17 inhibited tumor growth and induced 5-Fu sensitivity *in vivo*.

Discussion

Colorectal cancer ranks third in all cancer cases, accounting for about 9.2% in 2018[25]. Chemotherapy resistance has become one of the leading causes of poor prognosis in CRC patients[26]. Flu-

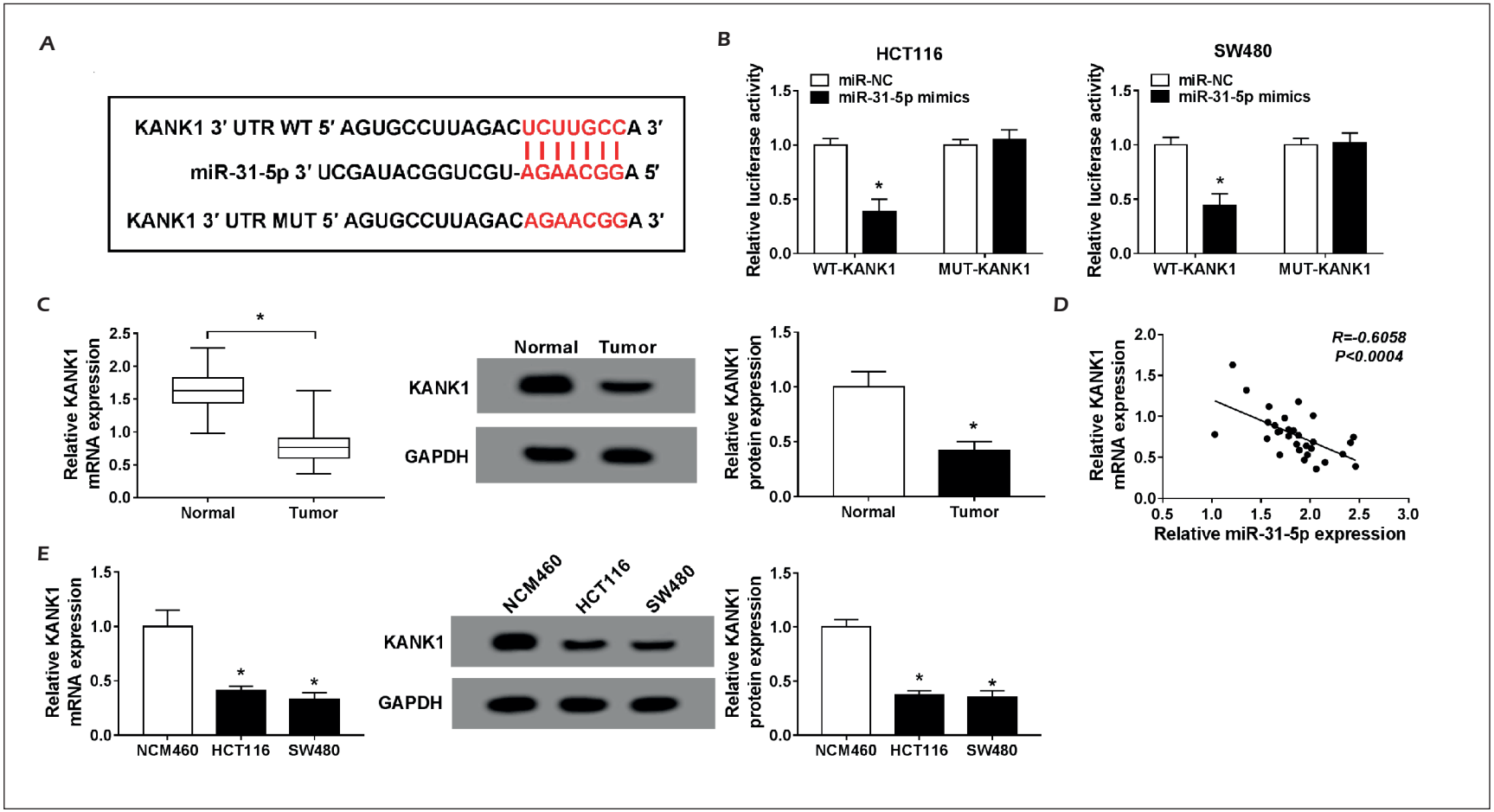

Figure 4. KANK1 was a target of miR-31-5p. **(A)** The predicted binding sites of miR-31-5p and KANK1 3'UTR were exhibited. **(B)** The luciferase activity was detected using dual-luciferase reporter assay in CRC cells cotransfected with WT-KANK1 or MUT-KANK1 and miR-31-5p mimics or miR-NC. **(C)** The expression of KANK1 was measured in CRC tissues and adjacent normal tissues using qRT-PCR and Western blot. **(D)** Spearman's correlation coefficient was conducted to analyze the correlation between miR-31-5p and KANK1 in CRC tissues. **(E)** The mRNA and protein levels of KANK1 were examined in NCM460 cells and CRC cell lines. *$p < 0.05$.

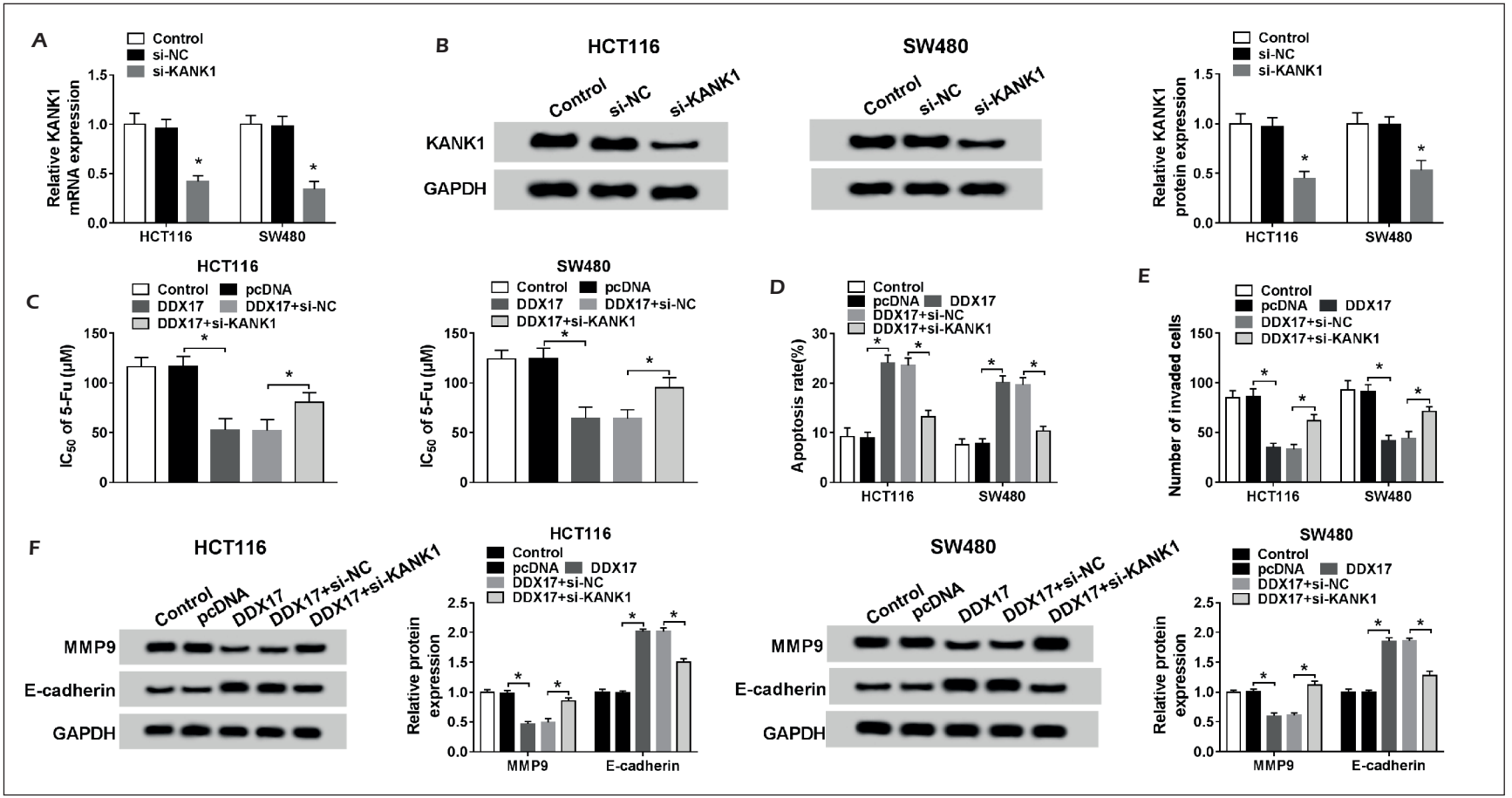

Figure 5. KANK1 depletion reversed the effect of circDDX17 overexpression on 5-Fu resistance and tumor progression. **(A** and **B)** The expression of KANK1 was detected in Control, si-NC or si-KANK1 group. **(C-F)** HCT116 and SW480 cells were introduced with pcDNA, DDX17, DDX17+si-NC or DDX17+si-KANK1. **(C)** The IC_{50} value was calculated using CCK-8 assay. **(D)** Cell apoptosis rate was detected by flow cytometry. **(E)** The number of invaded cells was evaluated by transwell assay. **(F)** EMT-related proteins were measured by Western blot analysis. *$p < 0.05$.

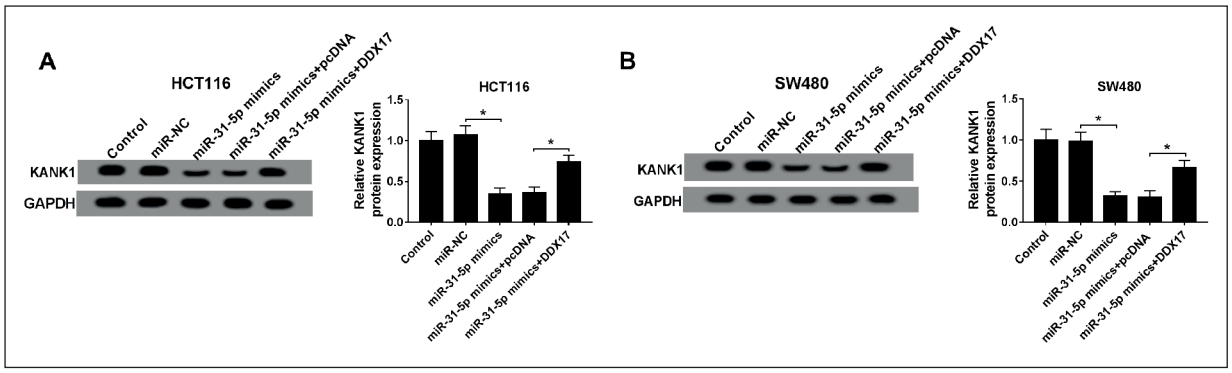

Figure 6. CircDDX17 upregulated KANK1 expression by sponging miR-31-5p. **(A** and **B)** The protein expression of KANK1 was detected in HCT116 and SW480 cells introduced with miR-NC, miR-31-5p mimics, miR-31-5p mimics+pcDNA or miR-31-5p mimics+DDX17. *$p < 0.05$.

orouracil is the first choice of chemotherapy for advanced CRC[27]. However, fluorouracil treatment has no significant effect on most patients with advanced CRC because of acquired or inherent resistance[28]. Therefore, seeking more effective CRC intervention strategies is essential.

EMT induces tumor metastasis characterized by the deletion of epithelial-associated protein (E-cadherin) and the increase of mesenchymal-associated protein[29]. Studies have shown that some circRNAs can regulate EMT progression[30]. For instance, circRNA_0023642 acted as a metastasis activator *via* activating the EMT signaling pathway[31]. Moreover, increasing evidence has corroborated that dysregulated circRNAs are vital regulators of oncogenesis and progression[32] and provide many potential biomarkers for CRC diagnosis, prognosis and treatment. Zhu et al[33] revealed that circ-BANP was prominently elevated in CRC tissues and circ-BANP depletion curbed cell proliferation in CRC. Zhang et al[34] presented that circ_0007534 knockdown impeded proliferation and accelerated apoptosis in CRC cells. In addition, circDDX17 was down-regulated, and circDDX17 silencing facilitated tumor progression in CRC[35]. We observed in the present study that circDDX17 expression was overtly decreased in CRC tissues and cells. Upregulation of circDDX17 enhanced 5-Fu sensitivity and induced apoptosis and impeded invasion and EMT in CRC cells.

Moreover, circRNAs could regulate tumor progression by acting as miRNA sponges or competing endogenous RNAs (ceRNAs)[36,37]. Is the case of circ-ZNF609 that contributed to tumor progression by functioning as a ceRNA of miR-150-5p in nasopharyngeal carcinoma[38]. In the current

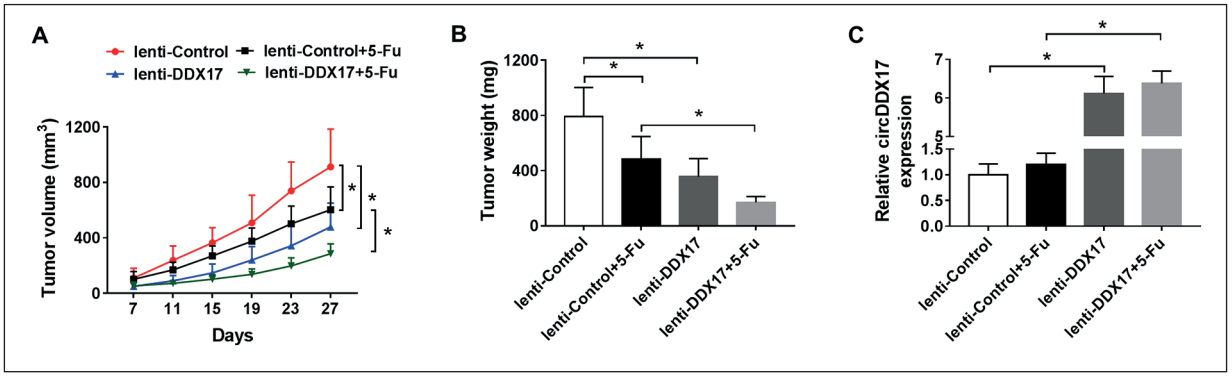

Figure 7. CircDDX17 overexpression blocked tumor growth and increased 5-Fu sensitivity *in vivo***.** SW480 cells transfected with lenti-DDX17 or lenti-Control were subcutaneously inoculated into the mice. **(A)** Tumor volume was measured every four days after inoculation. **(B)** Tumor weight was examined after mice were sacrificed. **(C)** Expression of circDDX17 in xenograft tumors was determined by qRT-PCR. *$p < 0.05$.

study, we validated that miR-31-5p was a target of circDDX17. Meanwhile, a large number of studies have certified that miR-31 was a tumor regulator in many types of malignant tumors, including CRC[39]. Peng et al[40] unveiled that miR-31-5p directly targeted NUMB to expedite CRC growth and development. Furthermore, Nakagawa et al[41] discovered that 5-Fu exposure induced miR-31 to reinforce drug resistance of CRC cells. LncRNA ENST00000547547 increased 5-Fu sensitivity in CRC by sponging microRNA-31[42]. We demonstrated that miR-31-5p was strikingly upregulated in CRC tissues and cells. Rescue experiments validated that DDX17 attenuated 5-Fu resistance and impaired tumor progression by sponging miR-31-5p in CRC cells.

KANK1 was a tumor-inhibiting gene in a variety of cancers. In lung cancer and gastric cancer, KANK1 overexpression curbed tumor invasion and metastasis[23,24]. In malignant peripheral nerve sheath tumors, KANK1 hindered tumor growth by accelerating CXXC5-mediated apoptosis[43]. Guo et al[44] indicated that overexpression of KANK1 facilitated apoptosis by mediating mitochondrial pathway in brain glioma. In the present study, we found that KANK1 expression was observably reduced in CRC tissues and cells. Notably, this study disclosed that miR-31-5p targeted KANK1 in CRC cells. CircDDX17 reduced chemoresistance of CRC cells by regulating KANK1 expression. In addition, upregulation of DDX17 reversed the decrease in KANK1 protein level induced by miR-31-5p overexpression, suggesting that DDX17 might act as a ceRNA of miR-31-5p to regulate KANK1 expression in CRC cells.

Conclusions

The above findings indicated that upregulation of circDDX17 potentiated 5-Fu sensitivity and inhibited tumorigenesis in CRC cells by down-regulating miR-31-5p and elevating KANK1 expression. Besides, DDX17 overexpression impeded tumor growth and enhanced 5-Fu sensitivity *in vivo*. These data contributed to our understanding of CRC and might provide a promising target for CRC treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Schreuders EH, Ruco A, Rabeneck L, Schoen RE, Sung JJ, Young GP, Kuipers EJ. Colorectal cancer screening: a global overview of existing programmes. Gut 2015; 64: 1637-1649.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.
3) Miller KD, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL, Siegel RL. Cancer treatment and survivorship statistics, 2019. CA Cancer J Clin 2019; 69: 363-385.
4) Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. Nat Rev Genet 2019; 20: 675-691.
5) Wu J, Qi X, Liu L, Hu X, Liu J, Yang J, Yang J, Lu L, Zhang Z, Ma S, Li H, Yun X, Sun T, Wang Y, Wang Z, Liu Z, Zhao W. Emerging epigenetic regulation of circular RNAs in human cancer. Mol Ther Nucleic Acids 2019; 16: 589-596.
6) Zhu LP, He YJ, Hou JC, Chen X, Zhou SY, Yang SJ, Li J, Zhang HD, Hu JH, Zhong SL, Zhao JH, Tang JH. The role of circRNAs in cancers. Biosci Rep 2017; 37: pii: BSR20170750.
7) Ma H, Tian T, Liu X, Xia M, Chen C, Mai L, Xie S, Yu L. Upregulated circ_0005576 facilitates cervical cancer progression via the miR-153/KIF20A axis. Biomed Pharmacother 2019; 118: 109311.
8) Ye F, Gao G, Zou Y, Zheng S, Zhang L, Ou X, Xie X, Tang H. CircFBXW7 inhibits malignant progression by sponging miR-197-3p and encoding a 185-aa protein in triple-negative breast cancer. Mol Ther Nucleic Acids 2019; 18: 88-98.
9) Wang P, He X. Current research on circular RNAs associated with colorectal cancer. Scand J Gastroenterol 2017; 52: 1203-1210.
10) Geng Y, Zheng X, Hu W, Wang Q, Xu Y, He W, Wu C, Zhu D, Wu C, Jiang J. Hsa_circ_0009361 acts as the sponge of miR-582 to suppress colorectal cancer progression by regulating APC2 expression. Clin Sci (Lond) 2019; 133: 1197-1213.
11) Li XN, Wang ZJ, Ye CX, Zhao BC, Huang XX, Yang L. Circular RNA circVAPA is up-regulated and exerts oncogenic properties by sponging miR-101 in colorectal cancer. Biomed Pharmacother 2019; 112: 108611.
12) Hao S, Cong L, Qu R, Liu R, Zhang G, Li Y. Emerging roles of circular RNAs in colorectal cancer. Onco Targets Ther 2019; 12: 4765-4777.
13) Gulyaeva LF, Kushlinskiy NE. Regulatory mechanisms of microRNA expression. J Transl Med 2016; 14: 143.
14) Yu X, Li Z, Yu J, Chan MT, Wu WK. MicroRNAs predict and modulate responses to chemotherapy in colorectal cancer. Cell Prolif 2015; 48: 503-510.
15) Que K, Tong Y, Que G, Li L, Lin H, Huang S, Wang R, Tang L. Downregulation of miR-874-3p promotes chemotherapeutic resistance in colorectal cancer via inactivation of the Hippo signaling pathway. Oncol Rep 2017; 38: 3376-3386.

16) Qin Y, Chen X, Liu Z, Tian X, Huo Z. MiR-106a reduces 5-Fluorouracil (5-FU) sensitivity of colorectal cancer by targeting dual-specificity phosphatases 2 (DUSP2). Med Sci Monit 2018; 24: 4944-4951.

17) Ding J, Zhao Z, Song J, Luo B, Huang L. MiR-223 promotes the doxorubicin resistance of colorectal cancer cells via regulating epithelial-mesenchymal transition by targeting FBXW7. Acta Biochim Biophys Sin (Shanghai) 2018; 50: 597-604.

18) Lu Z, He Q, Liang J, Li W, Su Q, Chen Z, Wan Q, Zhou X, Cao L, Sun J, Wu Y, Liu L, Wu X, Hou J, Lian K, Wang A. MiR-31-5p is a potential circulating biomarker and therapeutic target for oral cancer. Mol Ther Nucleic Acids 2019; 16: 471-480.

19) Li Y, Quan J, Chen F, Pan X, Zhuang C, Xiong T, Zhuang C, Li J, Huang X, Ye J, Zhang F, Zhang Z, Gui Y. MiR-31-5p acts as a tumor suppressor in renal cell carcinoma by targeting cyclin-dependent kinase 1 (CDK1). Biomed Pharmacother 2019; 111: 517-526.

20) Que KT, Zhou Y, You Y, Zhang Z, Zhao XP, Gong JP, Liu ZJ. MicroRNA-31-5p regulates chemosensitivity by preventing the nuclear location of PARP1 in hepatocellular carcinoma. J Exp Clin Cancer Res 2018; 37: 268.

21) Kakinuma N, Zhu Y, Wang Y, Roy BC, Kiyama R. Kank proteins: structure, functions and diseases. Cell Mol Life Sci 2009; 66: 2651-2659.

22) Fan H, Tian H, Cheng X, Chen Y, Liang S, Zhang Z, Liao Y, Xu P. Aberrant Kank1 expression regulates YAP to promote apoptosis and inhibit proliferation in OSCC. J Cell Physiol 2019 Jul 23. doi: 10.1002/jcp.29102. [Epub ahead of print]

23) Gu Y, Zhang M. Upregulation of the Kank1 gene inhibits human lung cancer progression in vitro and in vivo. Oncol Rep 2018; 40: 1243-1250.

24) Chen T, Wang K, Tong X. In vivo and in vitro inhibition of human gastric cancer progress by upregulating Kank1 gene. Oncol Rep 2017; 38: 1663-1669.

25) 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.

26) Van der Jeught K, Xu HC, Li YJ, Lu XB, Ji G. Drug resistance and new therapies in colorectal cancer. World J Gastroenterol 2018; 24: 3834-3848.

27) van Laar JA, Rustum YM, Ackland SP, van Groeningen CJ, Peters GJ. Comparison of 5-fluoro-2'-deoxyuridine with 5-fluorouracil and their role in the treatment of colorectal cancer. Eur J Cancer 1998; 34: 296-306.

28) Allen WL, Johnston PG. Role of genomic markers in colorectal cancer treatment. J Clin Oncol 2005; 23: 4545-4552.

29) Mittal V. Epithelial mesenchymal transition in tumor metastasis. Annu Rev Pathol 2018; 13: 395-412.

30) Shang BQ, Li ML, Quan HY, Hou PF, Li ZW, Chu SF, Zheng JN, Bai J. Functional roles of circular RNAs during epithelial-to-mesenchymal transition. Mol Cancer 2019; 18: 138.

31) Zhou LH, Yang YC, Zhang RY, Wang P, Pang MH, Liang LQ. CircRNA_0023642 promotes migration and invasion of gastric cancer cells by regulating EMT. Eur Rev Med Pharmacol Sci 2018; 22: 2297-2303.

32) Ng WL, Mohd Mohidin TB, Shukla K. Functional role of circular RNAs in cancer development and progression. RNA Biol 2018; 15: 995-1005.

33) Zhu M, Xu Y, Chen Y, Yan F. Circular BANP, an upregulated circular RNA that modulates cell proliferation in colorectal cancer. Biomed Pharmacother 2017; 88: 138-144.

34) Zhang R, Xu J, Zhao J, Wang X. Silencing of hsa_circ_0007534 suppresses proliferation and induces apoptosis in colorectal cancer cells. Eur Rev Med Pharmacol Sci 2018; 22: 118-126.

35) Li XN, Wang ZJ, Ye CX, Zhao BC, Li ZL, Yang Y. RNA sequencing reveals the expression profiles of circRNA and indicates that circDDX17 acts as a tumor suppressor in colorectal cancer. J Exp Clin Cancer Res 2018; 37: 325.

36) Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, Wu M. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer 2017; 16: 94.

37) Cui X, Wang J, Guo Z, Li M, Li M, Liu S, Liu H, Li W, Yin X, Tao J, Xu W. Emerging function and potential diagnostic value of circular RNAs in cancer. Mol Cancer 2018; 17: 123.

38) Zhu L, Liu Y, Yang Y, Mao XM, Yin ZD. CircRNA ZNF609 promotes growth and metastasis of nasopharyngeal carcinoma by competing with microRNA-150-5p. Eur Rev Med Pharmacol Sci 2019; 23: 2817-2826.

39) Yu T, Ma P, Wu D, Shu Y, Gao W. Functions and mechanisms of microRNA-31 in human cancers. Biomed Pharmacother 2018; 108: 1162-1169.

40) Peng H, Wang L, Su Q, Yi K, Du J, Wang Z. MiR-31-5p promotes the cell growth, migration and invasion of colorectal cancer cells by targeting NUMB. Biomed Pharmacother 2019; 109: 208-216.

41) Nakagawa Y, Kuranaga Y, Tahara T, Yamashita H, Shibata T, Nagasaka M, Funasaka K, Ohmiya N, Akao Y. Induced miR-31 by 5-fluorouracil exposure contributes to the resistance in colorectal tumors. Cancer Sci 2019; 110: 2540-2548.

42) Li J, Li X, Cen C, Ai X, Lin C, Hu G. The long non-coding RNA ENST00000547547 reduces 5-fluorouracil resistance of colorectal cancer cells via competitive binding to microRNA-31. Oncol Rep 2018; 39: 217-226.

43) Cui Z, Shen Y, Chen KH, Mittal SK, Yang JY, Zhang G. KANK1 inhibits cell growth by inducing apoptosis through regulating CXXC5 in human malignant peripheral nerve sheath tumors. Sci Rep 2017; 7: 40325.

44) Guo X, Fan W, Bian X, Ma D. Upregulation of the Kank1 gene-induced brain glioma apoptosis and blockade of the cell cycle in G0/G1 phase. Int J Oncol 2014; 44: 797-804.