

RESEARCH ARTICLE

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# Circ\_0003266 sponges miR-503-5p to suppress colorectal cancer progression via regulating PDCD4 expression

Caihong Wen<sup>1\*†</sup>, Xiaoqing Feng<sup>1†</sup>, Honggang Yuan<sup>2</sup>, Yong Gong<sup>3</sup> and Guangsheng Wang<sup>4</sup>

## Abstract

**Background:** Circular RNAs (circRNAs) feature prominently in tumor progression. However, the biological function and molecular mechanism of circ\_0003266 in colorectal cancer (CRC) require further investigation.

**Methods:** Circ\_0003266 expression in 46 pairs CRC tissues / adjacent tissues, and CRC cell lines was detected by quantitative real-time polymerase chain reaction (qRT-PCR); after circ\_0003266 was overexpressed or knocked down in CRC cells, cell proliferation, apoptosis, migration, and invasion were evaluated by the cell counting kit-8 (CCK-8), flow cytometry, and Transwell assays, respectively; the interaction among circ\_0003266, miR-503-5p, and programmed cell death 4 (PDCD4) was confirmed using bioinformatics analysis and dual-luciferase reporter assay; PDCD4 protein expression in CRC cells was quantified using Western blot.

**Results:** Circ\_0003266 was significantly lowly expressed in CRC tissues and cell lines. Circ\_0003266 overexpression markedly repressed CRC cell proliferation, migration, and invasion, and accelerated the cell apoptosis, but its overexpression promoted the malignant phenotypes of CRC cells. PDCD4 was a direct target of miR-503-5p and circ\_0003266 promoted PDCD4 expression by competitively sponging miR-503-5p.

**Conclusion:** Circ\_0003266 suppresses the CRC progression via sponging miR-503-5p and regulating PDCD4 expressions, which suggests that circ\_0003266 may serve as a novel target for the treatment of CRC.

**Keywords:** Colorectal cancer, circ\_0003266, miR-503-5p, PDCD4

## Background

Colorectal cancer (CRC) is a common cancer, and its mortality ranks among cancers worldwide, with nearly 900,000 deaths each year [1]. Although great progress has been made in the diagnosis and treatment in recent years, the therapeutic effect of some patients with CRC is not good due to high frequency of metastasis and recurrence [2]. Besides, the incidence of CRC is increasing among people under 45 years old [3]. The research on

circular RNAs (circRNAs), microRNA (miRNA), and their regulatory mechanism in CRC may provide novel diagnostic biomarkers and therapeutic targets for CRC, which may help improve the prognosis of the patients with CRC [4, 5].

CircRNA is a class of non-coding RNA derived from the reverse splicing of the precursor mRNA, which is with a covalent closed-loop structure formed by splicing the 5' end of one exon with the 3' end of another exon [6]. Many circRNAs are dysregulated in diverse diseases [7, 8]. For example, hsa\_circ\_0013958 expression is up-regulated in lung adenocarcinoma tissues, cells, and plasma of the patients, which is positively correlated with TNM stage and lymphatic

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metastasis; functionally, circ\_0013958 accelerates the proliferation and invasion of lung adenocarcinoma cells and inhibits the apoptosis [9]. Some circRNAs are abnormally expressed in CRC tissues, in which circRNAs with down-regulated expression levels, such as circ\_0008287, circ\_0069865, and circVPS13-1, are tumor suppressors, while circRNAs with up-regulated expression levels, such as circMGAT5, circ\_0000724, and circAATF-1, act as tumor promoters [10]. However, the function of circ\_0003266 in CRC awaits further study.

MicroRNAs (miRNAs), an endogenous RNA with about 20–22 nt, is involved in regulating many physiological and pathological processes. CircRNAs can exert their function via sponging miRNA, competitively combine with the corresponding miRNA through base pairing, and regulate gene expression at the post-transcriptional level [11]. For example, circAPLP2 activates Notch signaling pathway in CRC by targeting miR-101-3p, thus promoting tumor proliferation and metastasis [12]. CircAGFG1 drives the metastasis of CRC by modulating the YY1/CTNBN1 axis via sponging miR-4262 and miR-185-5p [13]. Reportedly, miR-503 promotes the migration and invasion of CRC cells by regulating programmed cell death 4 (PDCD4) [14]. However, whether miR-503/PDCD4 axis is involved in a competitively endogenous RNA (ceRNA) network in CRC is still obscure.

In this work, we used circRNA microarray to identify the abnormal expression of circRNAs in CRC tissues. We demonstrated that, circ\_0003266 expression was significantly down-regulated in CRC. Functionally, circ\_0003266 impeded the proliferation and metastasis of CRC cells and promoted apoptosis by regulating miR-503-5p/PDCD4 pathway.

## Methods

### Tissue samples

The study enrolled 46 CRC patients (22 males and 24 females, aged from 23 to 60 years) recruited between 2018 and 2019 from the Yichang Central People's Hospital. All CRC patients who had undergone surgery without chemotherapy or radiotherapy were diagnosed by pathological examination. The cancerous and paracancerous tissues (more than 2 cm from the edge of the tumor) were collected and immediately stored in liquid nitrogen. Tumor histological grading and staging were performed according to the World Health Organization classification criteria and the Tumor Node Metastasis system. This study was endorsed by the Institutional Ethics Committee of Yichang Central People's Hospital, and written informed consents were obtained from all patients before the research.

### Expression profile analysis of circRNAs

CircRNA expression profile data were downloaded from Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>). Using keywords (“circRNA” and “colorectal cancer”) in the GEO database, we searched the circRNAs microarray related with CRC and found the dataset GSE142837. We used GEO2R online analysis tool to get log fold change and adjusted *P*-value. Excel was used to screen out the circRNAs with *P* < 0.05 and |log<sub>2</sub>fold change (FC)| > 1 in CRC tissues (v.s. non-tumor tissues).

### Cell culture

Human normal colonic epithelial cells (NCM460) and CRC cell lines (HT29, SW480, HCT-116, Lovo, and DLD-1) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All CRC cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA), 100 U/ml penicillin and 100 µg/ml streptomycin (Sigma, St. Louis, MO, USA) at 37 °C in 5% CO<sub>2</sub>.

### Cell transfection

The overexpression vectors of circ\_0003266 and PDCD4 were constructed using pcDNA3.1 vector. siRNA targeting circ\_0003266 (si-circ\_0003266), miR-503-5p mimic and inhibitor, and their corresponding controls were purchased from GenePharma (Shanghai, China). The negative control (si-NC or miR-control) was adopted as the control vectors. The above mentioned oligonucleotides or plasmids (50 nM) and Lipofectamine™ 2000 reagents (Invitrogen, Carlsbad, CA, USA) were diluted using 100 µL of Opti-MEM medium (Invitrogen, Carlsbad, CA, USA), respectively, and incubated for 2 min at room temperature. Then they were mixed and incubated at room temperature for 20 min. The mixture was then added to a 6-well plate (containing 3 × 10<sup>5</sup> cells/well).

**Table 1** Sequences used for qRT-PCR

Name	Primer sequences
circ_0003266	Forward: 5'-AGTTGACAGCGGTACCATCC-3' Reverse: 5'-TGTAGGTTCCGGAAGTCCTC-3'
miR-503-5p	Forward: 5'-CCTATTTCCCATGATTCCTTCATA-3' Reverse: 5'-GTAATACGGTTATCCACGCG-3'
U6	Forward: 5'-ATTGGAACGATACAGAGAAGATT-3' Reverse: 5'-GGAACGCTTCACGAATTTG-3'
PDCD4	Forward: 5'-ACAGGTGTATGATGTGGAGGA-3' Reverse: 5'-TTCTCAAATGCCCTTTCATCCAA-3'
GAPDH	Forward: 5'-AACGGATTTGGTCGATTGGG-3' Reverse: 5'-CCTGGAAGATGGTGATGG GAT-3'

48 h after the transfection, the transfection efficiency was detected.

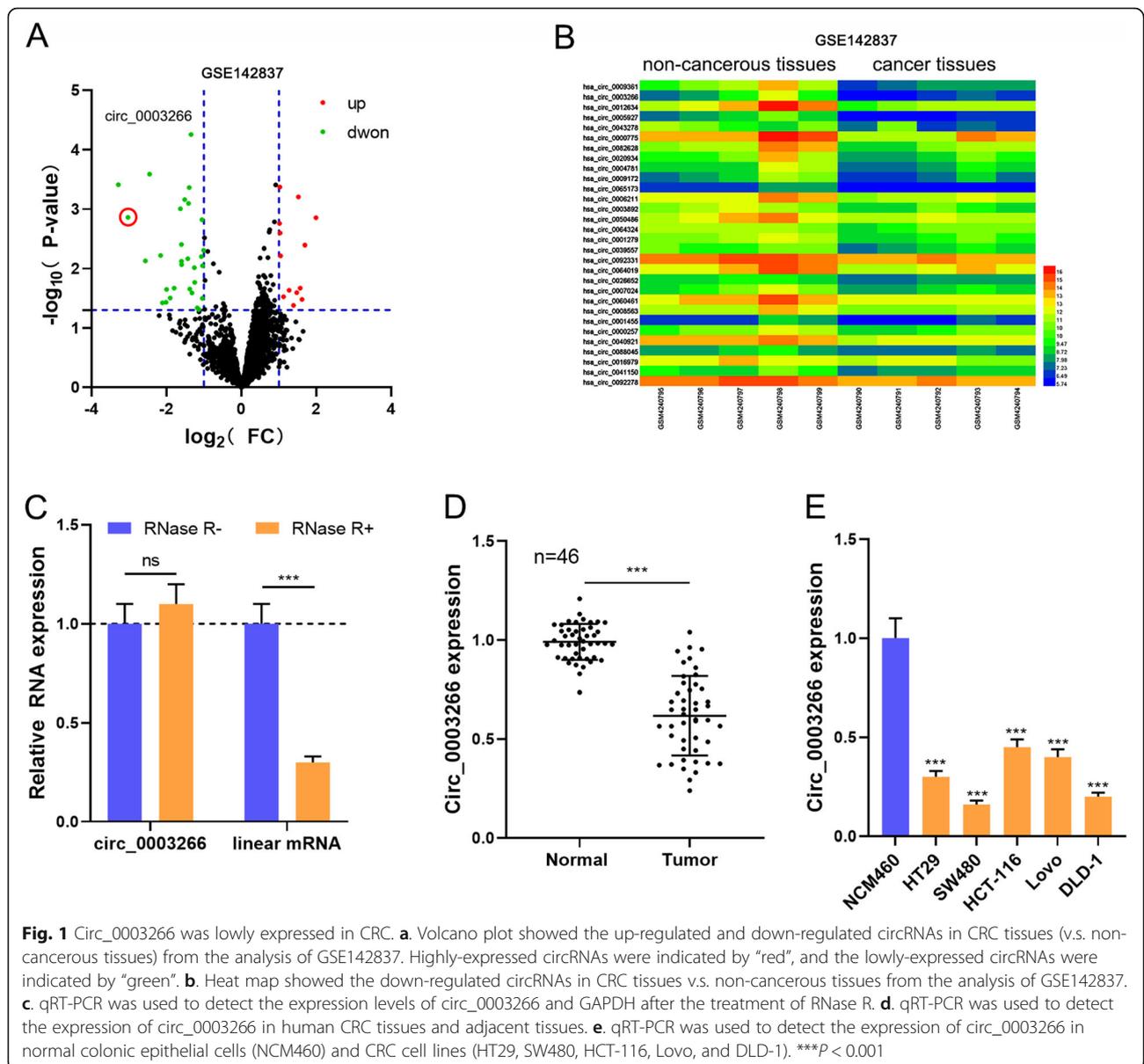
**Quantitative real-time polymerase chain reaction (qRT-PCR)**

Total RNA was isolated using TRIzol (Vazyme, Nanjing, China). cDNA synthesis was conducted using the TaqMan MicroRNA reverse transcription kit (Applied Biosystems, Foster City, CA) for miR-503-5p and PrimeScript RT Master Mix Kit (Takara Biotechnology Co., Ltd., Dalian, China) were used for preparing the cDNA to detect PDCD4 and circ\_0003266. Then quantitative PCR was performed, and circ\_0003266 and PDCD4 expression levels were determined by SYBR SYBR Premix Ex Taq II (Takara, Dalian, China), and

miR-503-5p expression was quantified by stem-loop primer SYBR Green qRT-PCR (Synbio Tech, Suzhou, China). GAPDH and U6 worked as internal controls for circRNA/mRNA and miRNA, respectively. qRT-PCR was operated on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, UK), and relative expression levels were calculated by  $2^{-\Delta\Delta CT}$  method. Primer sequences are listed in Table 1.

**RNase R resistance analysis of circRNAs**

To confirm the circular property of circ\_0003266, 2  $\mu$ g of total RNAs was treated with or without 3 U/mg RNase R (Epicentre Technologies, Madison, WI, USA) for 30 min at 37 °C in RNase R reaction buffer. Then the



**Fig. 1** Circ\_0003266 was lowly expressed in CRC. **a.** Volcano plot showed the up-regulated and down-regulated circRNAs in CRC tissues (v.s. non-cancerous tissues) from the analysis of GSE142837. Highly-expressed circRNAs were indicated by “red”, and the lowly-expressed circRNAs were indicated by “green”. **b.** Heat map showed the down-regulated circRNAs in CRC tissues v.s. non-cancerous tissues from the analysis of GSE142837. **c.** qRT-PCR was used to detect the expression levels of circ\_0003266 and GAPDH after the treatment of RNase R. **d.** qRT-PCR was used to detect the expression of circ\_0003266 in human CRC tissues and adjacent tissues. **e.** qRT-PCR was used to detect the expression of circ\_0003266 in normal colonic epithelial cells (NCM460) and CRC cell lines (HT29, SW480, HCT-116, Lovo, and DLD-1). \*\*\* $P < 0.001$

expression level of circ\_0003266 was detected by qRT-PCR.

#### Western blotting

Total protein was extracted by RIPA lysis buffer (Beyotime, Shanghai, China). Subsequently, protein concentration was measured using a bicinchoninic assay. Then 30 µg of protein / lane was separated via 10% SDS-PAGE and then transferred onto a PVDF membrane (Millipore, Schwalbach, Germany). After being blocked with TBS containing 5% skimmed milk at room temperature for 1 h, the membrane was incubated with rabbit anti-PDCD4 antibody (1:1000, ab51495, Abcam, Cambridge, UK) and anti-GAPDH antibody (1:2000; ab37168, Abcam, Cambridge, UK), respectively at 4 °C overnight, and then incubated with HRP-conjugated secondary antibodies (1:5000, Beyotime, Shanghai, China) at room temperature for 1 h. GAPDH was used as the internal control. Ultimately, the protein bands were developed by the enhanced chemiluminescence reagent (Beyotime, Shanghai, China).

#### Cell counting kit-8 (CCK-8) assay

Cells were harvested 24 h after the transfection. A total of  $1 \times 10^3$  CRC cells was transferred into each well of the 96-well plates and CCK-8 assay was performed every 24 h. Briefly, 10 µL of CCK-8 solution (Sigma, St. Louis, MO, USA) was added into each well at the corresponding time points, and the cells were cultured for another 1 h. The the viability of the cells (indicated by the value of absorbance) was analyzed at a wavelength of 450 nm, using a microplate reader (Potenov, Beijing, China). 4 d later, the proliferation of the cells in each group was plotted, with the absorbance values as the ordinate, and the time as the abscissa.

#### Transwell assay

Cell migration and invasion assays were performed using Transwell chambers (Corning Corning, NY, USA). In cell migration assay, a total of  $5 \times 10^5$  cells was suspended in serum-free medium and transferred into the upper chamber of each Transwell insert, while the lower chamber was added with 600 µL of complete medium with 20% FBS. After the culture for 24 h, cells in the upper surface of the filter were removed with cotton swabs and cells remaining on the bottom surface of the filter were fixed with methanol at room temperature for 15 min, followed by being staining with 1% crystal violet at room temperature for 30 min. Finally, stained cells were photographed under a light microscope at  $\times 200$  magnification and, and the number of these cells of five randomly selected fields was counted. In cell invasion assay, the filter was pre-coated with diluted Matrigel,

and the other procedures were executed as described above.

#### Flow cytometry

Cell apoptosis was detected by Annexin V-FITC Apoptosis Detection Kit (Sigma, St. Louis, MO, USA). Transfected cells were centrifugated at  $5000 \times g$  for 5 min at room temperature. Cell pellets were rinsed with PBS and re-suspended in the staining buffer. Then the cells were stained with 5 µL of propidium iodide staining solution in the dark for 30 min at 4 °C and subsequently stained with 5 µL of Annexin V-FITC staining solution for 20 min at room temperature. After that, apoptotic cells were analyzed by a flow cytometer (BD Biosciences, San Jose, CA, USA).

#### Dual-luciferase reporter assay

The wild type (WT) fragments of circ\_0003266 / PDCD4 3'-untranslated region (UTR) containing the predicted binding sites of miR-503-5p, and mutant (MUT) circ\_0003266/PDCD4 3'UTR sequences were provided by GenePharma Co., Ltd. (Shanghai, China). The fragments were cloned into the pGL3 Dual-Luciferase miRNA Target Expression vector (Promega, Madison, WI, USA), according to the manufacturer's protocol. The miR-503-5p mimic or negative control mimic was co-transfected into HEK-293 T cells with the wild-type or mutant reporter vectors. After 48 h, the relative activity of luciferase was determined using the Dual-Luciferase Reporter Assay kit

**Table 2** The relationship between the expression of circ\_0003266 and clinical features of CRC

Expression of circ_0003266				
Pathological factors	Low(23)	High(23)	chi-square value	P value
Sex			0.3485	0.555
Male	10	12		
Female	13	11		
Age			0.0897	0.765
< 60	14	13		
≥ 60	9	10		
Lymph node metastasis			0.8070	0.369
Absent	15	12		
Present	8	11		
Grade			5.5758	0.018*
Low	7	15		
High	16	8		
T stage			4.2933	0.038*
T1-T2	9	16		
T3-T4	14	7		

(Promega, Madison, WI, USA) in line with the manufacturer's instructions.

**Statistical analysis**

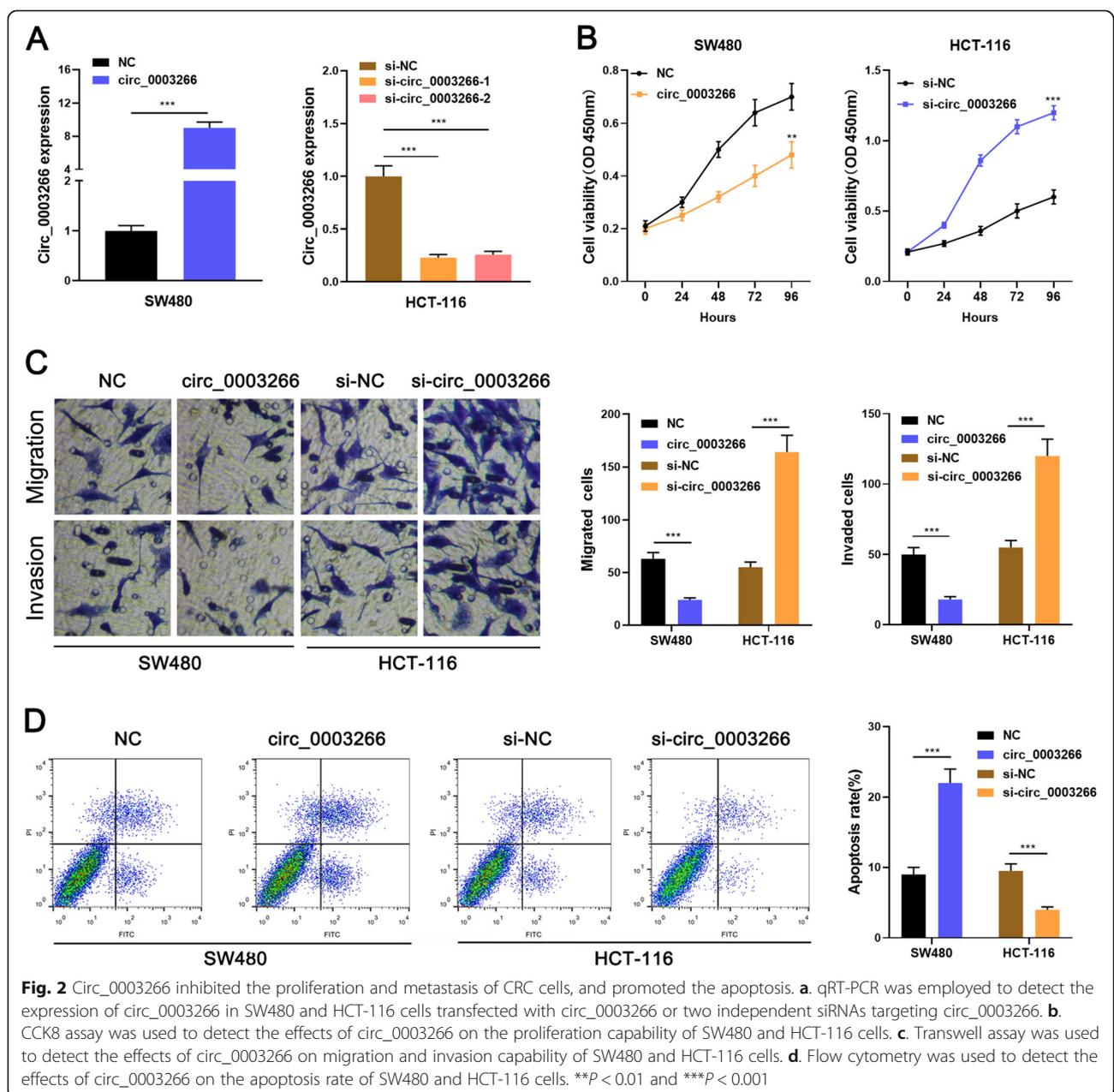
Graphs were generated by GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA, USA), and statistical analysis was performed with SPSS 22.0 (IBM, Chicago, IL, USA). Student's *t*-test or one-way analysis of variance was adopted for making the comparisons. Pearson correlation analysis was conducted to analyze the correlations between the two indicators. Chi-square test was

used to analyze the association between circ\_0003266 expression and the clinical characteristics of the patients.  $P < 0.05$  was considered statistically significant.

**Results**

**Circ\_0003266 is lowly expressed in CRC**

The expression profile of circRNAs in 5 pairs of CRC tissue and normal tissue samples were analyzed by circRNA microarray (GSE142837). Out of 3292 circRNAs, 43 circRNAs ( $|\log_2FC| > 1, P < 0.05$ ) were screened out, among which the expression levels of 13 circRNAs were up-regulated and 30



**Fig. 2** Circ\_0003266 inhibited the proliferation and metastasis of CRC cells, and promoted the apoptosis. **a.** qRT-PCR was employed to detect the expression of circ\_0003266 in SW480 and HCT-116 cells transfected with circ\_0003266 or two independent siRNAs targeting circ\_0003266. **b.** CCK8 assay was used to detect the effects of circ\_0003266 on the proliferation capability of SW480 and HCT-116 cells. **c.** Transwell assay was used to detect the effects of circ\_0003266 on migration and invasion capability of SW480 and HCT-116 cells. **d.** Flow cytometry was used to detect the effects of circ\_0003266 on the apoptosis rate of SW480 and HCT-116 cells. \*\*\* $P < 0.01$  and \*\*\*\* $P < 0.001$

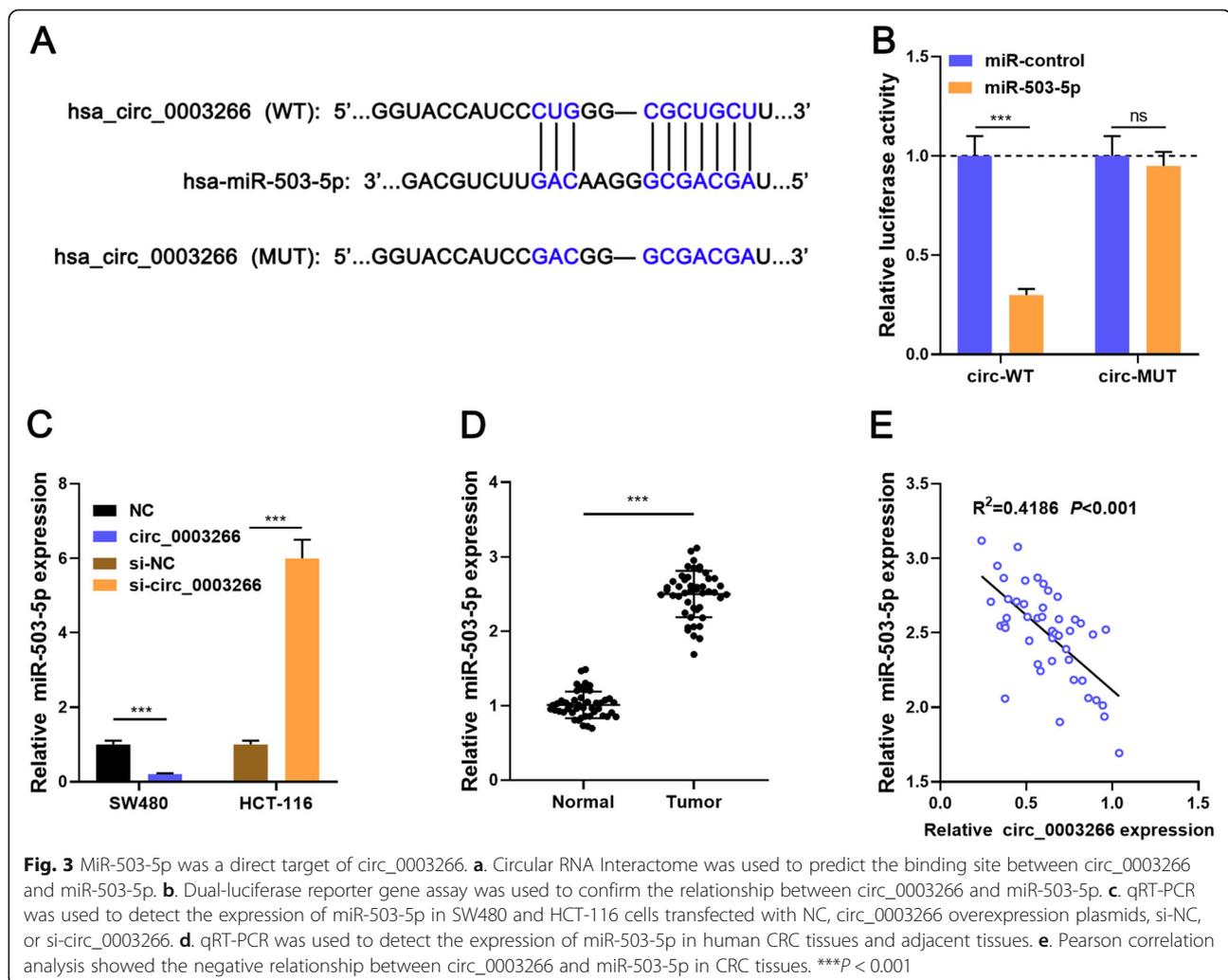
were down-regulated (Fig. 1a-b). In this study, circ\_0003266, whose expression was significantly down-regulated, was investigated. CircRNAs have no free ends, showing a longer half-life and resistance to RNase R, compared with linear RNA. To verify the circular structure of circ\_0003266, RNase R resistance assay was performed, and it was found that only linear mRNA (GAPDH) expression level was decreased after RNA R treatment, implying that circ\_0003266 has a loop structure (Fig. 1c). Next, we found that circ\_0003266 expression in CRC tissues was markedly lower than that in adjacent tissues by qRT-PCR (Fig. 1d). Besides, circ\_0003266 expression in CRC cell line was significantly down-regulated compared with that in NCM460 cells (Fig. 1e). Furthermore, we analyzed the relationship between the expression of circ\_0003266 and the clinicopathological features of CRC patients. The results illustrated that circ\_0003266 expression level was negatively correlated with tumor grade and stage of CRC patients (Table 2). These dates suggested that the abnormal down-regulation of circ\_0003266 expression might affect CRC progression.

### Circ\_0003266 suppresses the malignant phenotypes of CRC cells

Circ\_0003266 overexpression plasmid was constructed and transfected into SW480 cells, and si-circ\_0003266 was transfected into HCT-116 cells. The transfection efficacy was confirmed by qRT-PCR (Fig. 2a). To further clarify the function of circ\_0003266 in CRC, CCK-8, flow cytometry, and Transwell assays were performed, and the results displayed that circ\_0003266 overexpression significantly decreased the growth, migration, and invasion of SW480 cells, and expedited the apoptosis, but circ\_0003266 silencing functioned oppositely on HCT-116 cells (Fig. 2 b-d). These results showed that circ\_0003266 was a tumor suppressor in CRC.

### Circ\_0003266 negatively regulates miR-503-5p expression

We analyzed the candidate miRNAs with complementary sequences to circ\_0003266 using the online CircInteractome database, and found that miR-503-5p was one of the



potential targets (Fig. 3a). Dual-luciferase reporter gene assay uncovered that miR-503-5p could decrease the luciferase activity of circ\_0003266-WT reporter but had no significant effect on circ\_0003266-MUT reporter (Fig. 3b). Besides, qRT-PCR results showed that circ\_0003266 overexpression resulted in a significant decrease in miR-503-5p expression in CRC cells, while the depletion of circ\_0003266 worked oppositely (Fig. 3c). Besides, miR-503-5p expression in CRC tissues was higher than that in adjacent tissues and negatively correlated with circ\_0003266 expression (Fig. 3 d-e). These evidence indicated that miR-503-5p was the target of circ\_0003266.

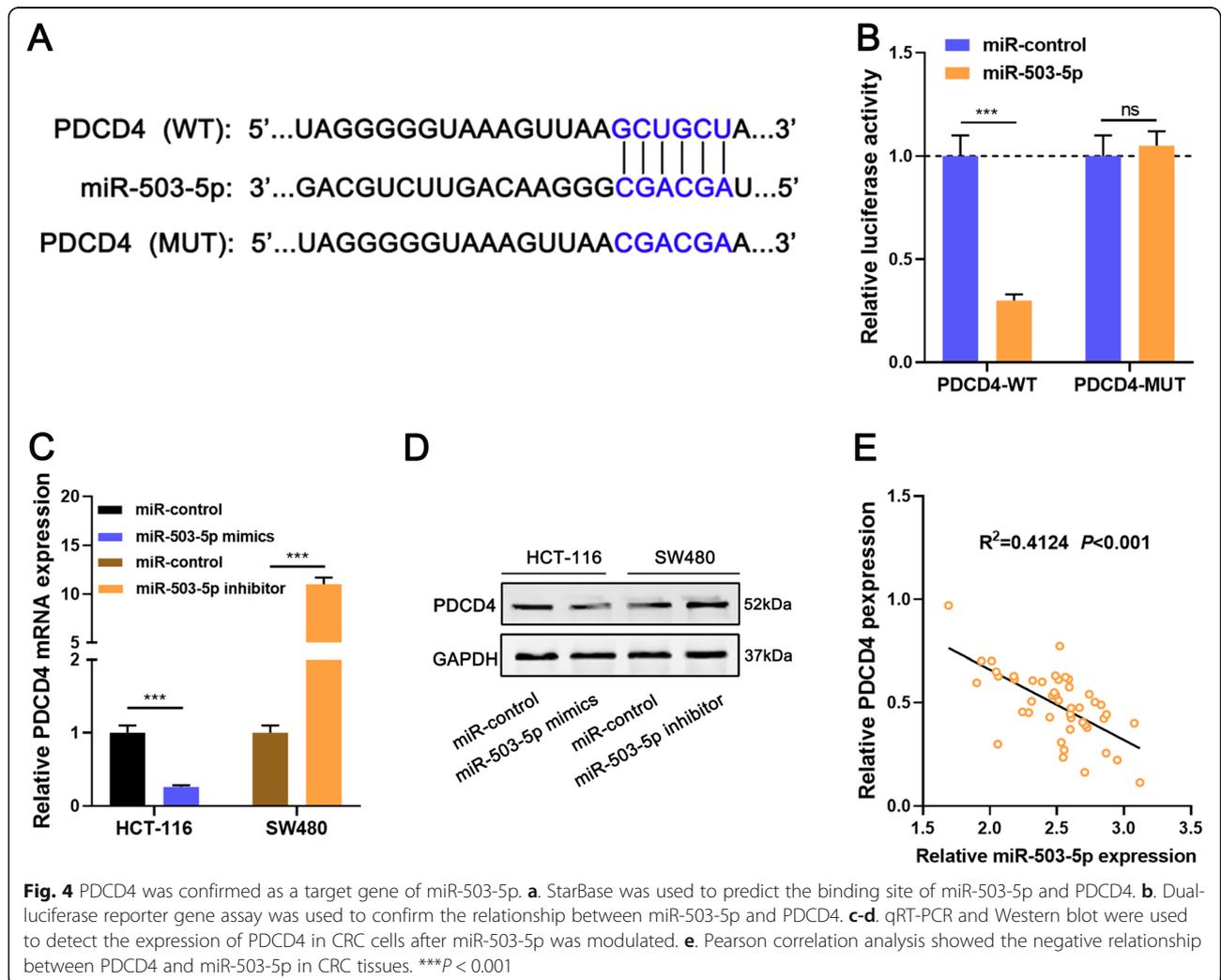
**MiR-503-5p negatively regulates PDCD4 expression**

Through analyzing StarBase database, TargetScan database, and miRDB database, we found that there was a binding site in PDCD4 3'UTR to miR-503-5p (Fig. 4a),

which is consistent with the previous report [14]. Dual-luciferase reporter gene assay indicated that miR-503-5p overexpression markedly repressed the luciferase activity of PDCD4-WT reporter but did not exert an impact on that of PDCD4-MUT reporter (Fig. 4b). MiR-503-5p mimics significantly inhibited PDCD4 mRNA and protein expression levels while miR-503-5p inhibitors worked oppositely (Fig. 4c-d). Meanwhile, there was a negative correlation between the expression levels of miR-503-5p and PDCD4 in CRC tissues (Fig. 4e).

**Circ\_0003266 restrains CRC progression via modulating miR-503-5p/PDCD4 axis**

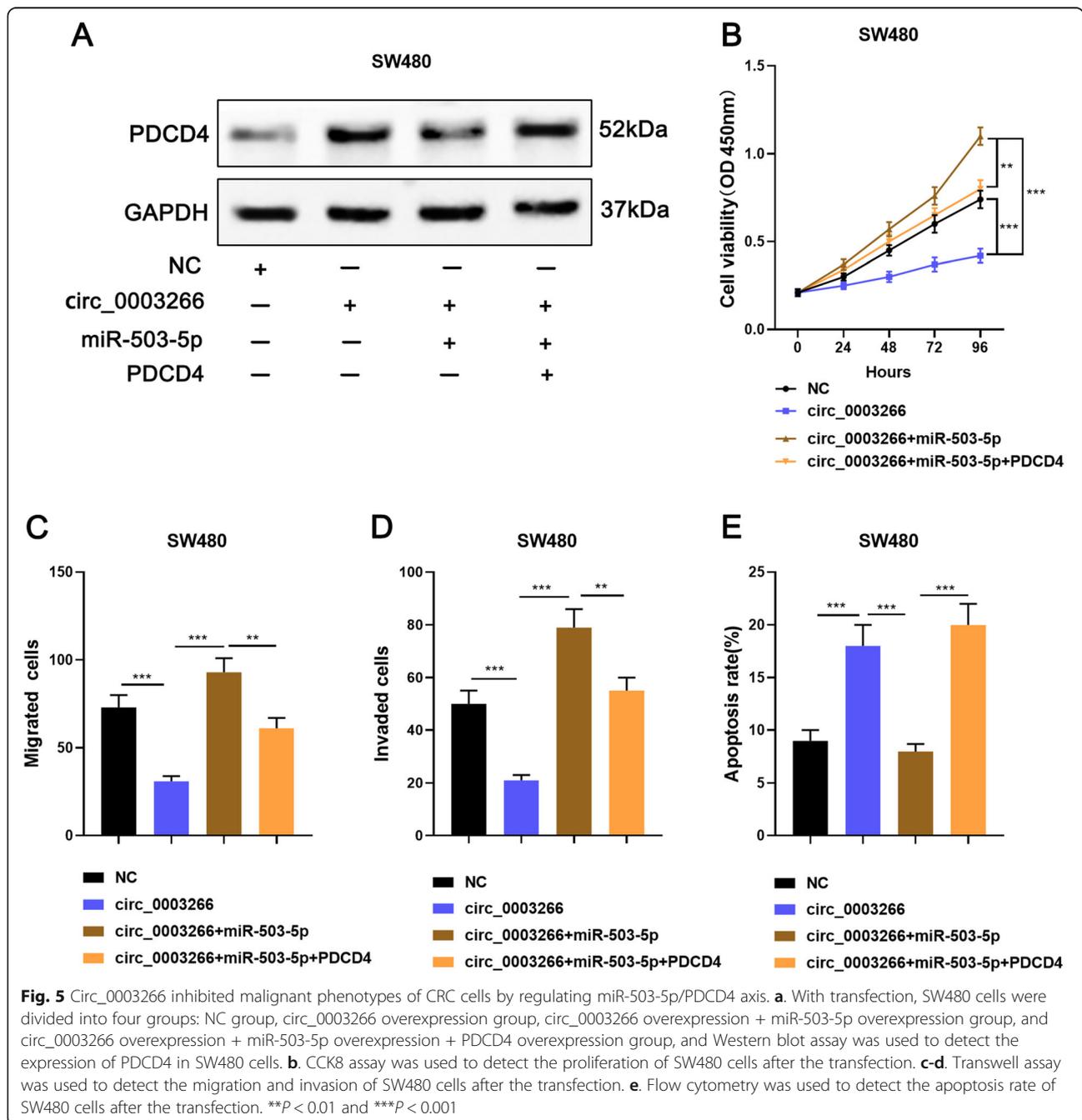
To pinpoint whether circ\_0003266 exerted its functions via modulating miR-503-5p/PDCD4 pathway, we performed rescue assays. The SW480 cells were divided into four groups: NC group, circ\_0003266



**Fig. 4** PDCD4 was confirmed as a target gene of miR-503-5p. **a**. StarBase was used to predict the binding site of miR-503-5p and PDCD4. **b**. Dual-luciferase reporter gene assay was used to confirm the relationship between miR-503-5p and PDCD4. **c-d**. qRT-PCR and Western blot were used to detect the expression of PDCD4 in CRC cells after miR-503-5p was modulated. **e**. Pearson correlation analysis showed the negative relationship between PDCD4 and miR-503-5p in CRC tissues. \*\*\* $P < 0.001$

overexpression group, circ\_0003266 overexpression + miR-503-5p overexpression group, and circ\_0003266 overexpression + miR-503-5p overexpression + PDCD4 overexpression group. Western blot assay showed that, circ\_0003266 significantly promoted the expression of PDCD4 in SW480 cells, and the co-transfection of miR-503-5p reversed this effects, and the transfection of PDCD4 overexpression plasmids counteracted the effects of miR-503-5p (Fig. 5a).

Furthermore, functional assays showed that, circ\_0003266 overexpression inhibited the proliferation, migration, and invasion, and promoted the apoptosis of SW480 cells; however, miR-503-5p mimics totally reversed these effects (Fig. 5b-e); additionally, PDCD4 overexpression reversed the effects induced by miR-503-5p overexpression (Fig. 5b-e). These findings demonstrated that circ\_0003266 suppressed CRC via modulating miR-503-5p/PDCD4 pathway.



## Discussion

CircRNAs are discovered in RNA viruses as early as the in the 1970s, and in recent year, multiple circRNAs are identified in the transcriptome of human cells [15]. Reportedly, circRNAs are more stable and abundant than linear RNA, and circRNAs are mainly located in the cytoplasm and they have miRNA response elements; what's more, circRNAs have other biological functions such as working as the scaffold in the assembly of protein complexes, regulating alternative splicing, modulating RNA-protein interactions, and so on [15–18]. CircRNAs are implicated in regulating the pathogenesis of human diseases including diabetes, nervous system diseases, cardiovascular diseases, and cancers, etc. [19]. For example, *CircHipp3* expression is observably raised in CRC tissues and cell lines, and functionally, *CircHipp3* knock-down can markedly impede the growth, migration, and invasion of CRC cells [20]. *Circ-ITGA7* inhibits CRC cell proliferation via adsorbing miR-3187-3p and increasing *ASXL1* expressions [21]. Here, we found that *circ\_0003266* expression was significantly down-regulated in CRC. Additionally, *circ\_0003266* restrained the proliferation and metastatic potential of CRC cells, and expedited the apoptosis. Our results suggested that it could probably be a biomarker and therapy target for CRC.

MiRNAs regulate mRNA expression by inhibiting translation or promoting degradation, and they are important regulators in cancer biology [22]. For example, the decreased expression of miR-4319, as reported, is related to the poor prognosis of CRC patients, and miR-4319 significantly inhibits the proliferation of CRC cells and changes cell cycle distribution by targeting *ABTB1* [23]. Reportedly, circRNAs act as miRNAs sponges to regulate tumor progression. For example, *circ\_0136666* accelerates the multiplication and invasion of CRC cells via miR-136/*SH2B1* axis [24]. In this work, we identified miR-503-5p as the target miRNA of *circ\_0003266* by bioinformatics analysis and dual-luciferase reporter gene assay. MiR-503-5p is abnormally expressed in various cancers including hepatocellular carcinoma, ovarian cancer, cervical cancer, and oral squamous cell carcinoma [25–28]. Besides, miR-503-5p expression in CRC is significantly increased, which expedites the migration and invasion of CRC cells [14]. In this work, we observed that miR-503-5p expression was elevated in CRC tissues, and miR-503-5p promoted proliferation and metastasis of CRC cells, and inhibited the apoptosis, which is consistent with findings of the previous research [14]. Moreover, miR-503-5p could counteract the inhibitory effects of *circ\_0003266* on CRC procession. These findings suggested that *circ\_0003266* contributed to the dysregulation of miR-503-5p in CRC, and its function was dependent on miR-503-5p.

*PDCD4* is a tumor suppressor, and its expression is frequently down-regulated in various types of cancers [29]. *PDCD4* protein is composed of 469 amino acid residues, and *PDCD4* binds to eIF4A and restrains its helicase activity [30–32]. *PDCD4* expression is abnormally down-regulated in CRC, and *PDCD4* represses the translation of *Sin1* translation via interacting the eIF4A, and inhibits CRC progression [30]. *PDCD4* also directly combines with mRNA of *c-Myb*, *Bcl-xL*, and *XIAP* to suppress their translation, thereby inhibiting cell proliferation and promoting apoptosis [32]. Previous studies report that *PDCD4* inhibits the progression of several cancer cells, including hepatocellular carcinoma, breast cancer, and melanoma [33–35]. Reportedly, miR-503-5p can target *PDCD4* [14]. In this study, we further explored the impact of *circ\_0003266* on *PDCD4*, the results of which demonstrated that *circ\_0003266* could positively regulate *PDCD4* via adsorbing miR-503-5p.

There are some limitations of the present work. Firstly, our findings are only based on in vitro experiments, and in vivo assays can further confirm the role of *circ\_0003266* in CRC progression in the future. Secondly, the relationship between *circ\_0003266* and the prognosis of the CRC patients is still obscure, and survival analysis of more patients with follow-up information should be performed in the future to evaluate the prognostic value of *circ\_0003266*. Lastly, *CircInteractome* database also predicts other miRNAs, which can probably be regulated by *circ\_0003266*, and whether *circ\_0003266* could regulate CRC progression via modulating these miRNAs should be explored.

## Conclusion

*Circ\_0003266* is lowly expressed in CRC tissues and cells. Mechanistically, *circ\_0003266* inhibits CRC progression via modulating *PDCD4* expressions by acting as ceRNA of miR-503-5p. The findings of the present study highlight the potential role of *circ\_0003266* as a tumor suppressor in CRC, which provides a novel therapeutic target for CRC treatment.

## Abbreviations

CircRNAs: Circular RNAs; CRC: Colorectal cancer; qRT-PCR: Quantitative real-time polymerase chain reaction; *PDCD4*: Programmed cell death 4; CCK-8: Cell counting kit-8; ceRNA: Competitive endogenous RNA; miRNA: MicroRNA; FBS: Fetal bovine serum; siRNAs: Small interference RNAs; PVDF: Polyvinylidene fluoride; PBS: Phosphate buffered saline

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12885-021-07997-0>.

**Additional file 1.**

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**Authors' contributions**

Conceived and designed the experiments: CHW and XQF; Performed the experiments: CHW and XQF; Statistical analysis: HGY, YG, and GSW; Wrote the paper: CHW and XQF. All authors read and approved the final manuscript.

**Authors' information**

Not Applicable.

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**Availability of data and materials**

The data used to support the findings of this study are available from the corresponding author upon request.

**Declarations****Ethics approval and consent to participate**

This study was approved by the Institutional Ethics Committee of Yichang Central People's Hospital, and written informed consents were obtained from all patients before the research.

**Consent for publication**

Not Applicable.

**Competing interests**

The authors declare that they have no competing interests.

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