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Circular RNA circWHSC1 facilitates colorectal cancer cell proliferation by targeting miR-130a-5p/zeb1 signaling *in vitro* and *in vivo*

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ABSTRACT

Colorectal cancer is a prevalent cancer globally and has become a threaten of human health. Recently, circular RNAs (circRNAs) have been widely studied in the cancer area, and the function of circular RNA circWHSC1 has been identified in several cancers. However, the role of circWHSC1 in colorectal cancer remains elusive. In this study, we were interested in the effects of circWHSC1 on colorectal cancer progression. We found that level of circWHSC1 was elevated in colorectal cancer cells compared with normal colon epithelial cells. FISH assay further confirmed that circWHSC1 was mainly localized in cytoplasm. CircWHSC1 depletion repressed the viability of colorectal cancer cells. The colony formation number and Edu-positive colorectal cancer cells were inhibited by the depletion of circWHSC1, respectively. The knockdown of circWHSC1 promoted the apoptosis of colorectal cancer cells. The tumor growth of colorectal cancer cells in nude mice was attenuated by circWHSC1 silencing. Meanwhile, the invasion and migration ability of colorectal cancer cells was suppressed by circWHSC1 depletion. Mechanically, circWHSC1 targets miR-130a-5p to promote zeb1 expression in colorectal cancer cell. The depletion of circWHSC1 remarkably reduced the cell viability and Edu-positive colorectal cancer cells, and the miR-130a-5p inhibitor or zeb1 overexpression could restore the phenotypes. Furthermore, the tumor growth of colorectal cancer cells in nude mice was attenuated by circWHSC1 knockdown, while miR-130a-5p depletion or zeb1 overexpression reversed the effect in the model. Therefore, we concluded that Circular RNA circWHSC1 facilitated colorectal cancer cell proliferation by targeting miR-130a-5p/zeb1 signaling *in vitro* and *in vivo*.

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers globally and has become a threaten of human health [1,2]. The incidence of CRC increases annually in recent years [3,4]. Noteworthy, the overall survival rate of patients with CRC has increased over the past decades, owing to the efforts on developing diagnostic and therapeutic methods [2]. Nevertheless, patients with CRC at advanced stages are commonly accompanied by metastasis and showed a low five-year survival rate [2,5]. Therefore, exploring the mechanisms underlying the metastasis of CRC is critical for CRC treatment.

In recent years, circular RNAs (circRNAs) have been widely studied in the cancer area [6]. CircRNAs are a member of non-coding

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RNA (ncRNA) family that featured with closed circular structure and higher stability than linear RNAs [7]. Studies have reported the regulatory role of circRNAs in various cellular process during cancer initiation and progression, such as cell proliferation, apoptosis, and metastasis [8,9]. It has been well-recognized that circRNAs act as microRNA (miRNA) sponges, competitively suppressing miRNAs to modulate target mRNA level, which consequently regulate gene expression and function [10]. CircWHSC1 is a circRNA that recently emerged as a regulator that involved in the initiation and progression of cancers. It is recently reported that circWHSC1 promotes the progression of ovarian cancer cells through sponging miR-145 and miR-1182 [11]. CircWHSC1 also promotes the malignancy of hepatocellular carcinoma, breast cancer, and lung cancer and so on [12–16]. Accumulating evidence has identified that circWHSC1 participates in cell proliferation, metastasis, and metabolism via targeting multiple signaling cascades, such as AKT and AMPK/mTOR signaling pathway [13,14,17]. Therefore, it is of great importance to study the effects of circWHSC1 on cancers. However, the role of circWHSC1 during CRC progression and the underlying molecular mechanisms has not been determined yet.

Zeb1 is a pivotal transcription factor in the epithelial-mesenchymal transition (EMT) signaling and has been reported to participate in the migration and invasion of multiple cancer cells, including the CRC cells [18,19]. Meanwhile, miR-130a-5p plays an important role in cancer suppression in a variety of tumors, yet its role in CRC has not been reported [20–22]. In this study, we explored the expression of circWHSC1 in CRC tissues and its function in cancer metastasis. Further study using bioinformatic tool and *in vitro* experiments demonstrated that circWHSC1 sponges miR-130a-5p to regulate zeb1 expression in CRC cells. Our finding may provide novel evidence for therapy of metastatic CRC.

2. Methods

2.1. Extraction and quantification of RNA and genomic DNA

Total RNA was obtained using Trizol reagent (Invitrogen, Waltham, MA). Genomic DNA (gDNA) was obtained using a genomic DNA isolation kit (TIANGEN, China) following the manufacturer's instruction. For RNA quantification, the extracted RNAs were reverse-transcribed to cDNAs using First Strand cDNA Synthesis Kit (Transgen, China). Then qPCR assay was performed using SYBR Master Kit (Takara, Japan) in line with manufacturer's protocol. The RNA levels were quantified using $2^{-\triangle\triangle Ct}$ method, and normalized to GAPDH or U6. PCR products and gDNA were loaded into 1.5% agarose gel electrophoresis with TAE buffer using. The images of DNA bands were captured under a gel image system (Bio-Rad, USA).

CircWHSC1: forward, 5′-GACGCACCGCAGTGTTCT-3′, reverse, 5′-GAGGATTTCTGGTGCCTGCT-3′;
MiR-130-5p: forward, 5′- AACACGCGCTGACTCCTAGT-3′, reverse, 5′- CAGTGCAGGGTCCGAGGT-3′;
zeb1: forward, 5′ to −3′, reverse, 5′ to −3′;
GAPDH: forward, 5′-TGTGGGCATCAATGGATTTGG-3′, reverse, 5′- ACACCATGTATTCCGGGTCAAT-3′;
U6: forward, 5′-AAAGCAAATCATCGGACGACC-3′, reverse, 5′- GTACAACACATTGTTTCCTCGGA-3′.

2.2. RNA stability

The extracted RNA (2 μg) was treated with RNase R (2 U/μg) at 37 °C for 30 min, purified with RNeasy MinElute Cleanup kit (QIAGEN, Germany) and measured by qPCR assay.

2.3. Fluorescence in situ hybridization (FISH) assay

Cy3-labeled circWHSC1, 18a, and U6 probes were synthesized by RiobBio (China). FISH assay was performed using the FISH kit (RiboBio, China) as per manufacturer's instruction. Cells were fixed with 4% formaldehyde for 15 min, washed with PBS, and dehydrated with ethanol. The dried cells were then incubated with 40 nmol/l of the probes in a hybridization buffer at 80 °C for 2 min, followed by standing at 55 °C for 2 h. Nuclei were dyed by DAPI (Thermo, USA). The cell samples were then washed and dehydrated. Images were taken under a confocal microscope (Leica, Germany).

2.4. Cell line and treatment

The CRC cell lines SW480, SW620, HCT-116, HT-29, and DLD-1, and normal colon epithelial cell line FHC-1 were provided by American Type Culture Collection (ATCC; USA). SW480, SW620, and DLD-1 cells were incubated in RPMI-1640 medium (Hyclone, USA), and HCT-116, HT-29, and FHC-1 cells were incubated in MyCoy's 5A medium (Hyclone, USA) that contains 10% FBS (Hyclone, USA). All cells were incubated in a 37 °C incubator with 5% CO_2. Cells were treated with actinomycin D at the indicated times and RNA was extracted for detection of circular RNA and linear RNA levels.

2.5. Cell transfection

The siRNAs that target circWHSC1, and negative controls (NCs), the miR-130a-5p mimics and inhibitors, and zeb1 overexpression vectors were synthesized by RiboBio (China). Cells were seeded into 6-well plate and transfected with indicated oligonucleotides using Lipofectamine 2000 (Invitrogen, USA) in accordance with manufacturer's description.

2.6. Cell viability and proliferation

Cell viability and proliferation were checked by CCK-8 (Beyotime, China) and colony formation experiments. For CCK-8 assay, cells were seeded into 96-well plates and incubated for 24, 48, 72, and 96 h, respectively. Then CCK-8 reagent was added into each well and incubated for another 2 h. The optical density at 450 nm was measured by microplate reader (PerkinElmer, Germany). For colony formation assay, cells were suspended as single cells and seeded into 6-well plates. After incubation for 15 days, colonies were stained with 1% crystal violet (Beyotime, China) for 20 min and captured by camera.

2.7. 5-Ethynyl-2′-deoxyuridine (EdU) assay

Cell proliferation was checked by EdU assay kit (Beyotime, China) in accordance with manufacturer's description. In short, cells were placed in 12-well plates and incubated with EdU (50 μM) at 37 °C for 2 h, fixed in 4% PFA and neutralized with glycine. Nuclei were then dyed with DAPI. Images were taken under confocal microscope (Leica, Germany).

2.8. Cell apoptosis

Cell apoptosis was measure by Annexin V/PI Apoptosis Detection Kit (Beyotime, China). Cells were digested and suspended in binding buffer, then stained with Annexin V (5 μl) and PI (5 μl) for 30 min. After staining, samples were immediately analyzed with flow cytometry.

2.9. Cell cycle

The cell cycle of cancer cells was measured by PI staining (Beyotime, China) and examined by flow cytometry following manufacturer's introduction. In short, cells were digested, washed with PBS, and fixed in 70% ethanol at 4 °C for 48 h. After that, the cells were suspended in staining solution that composed of 50 μg/ml of PI, 1% of Triton-X100, and 100 μg/ml of DNase-free RNase and incubated for 30 min. Then the samples were analyzed by flow cytometry.

2.10. Cell migration and invasion

Cell invasion and migration were measured by Transwell (Corning, USA) coated with or without Matrigel (Corning). In brief, cells were suspended in FBS-free DMEM placed into the upper chamber, whereas the lower chamber was added with complete culture medium. After incubation for 24 h, the upper chamber was washed with PBS and stained with crystal violet. Images were taken under microscope.

For wound healing assay, cells were seeded into 6-well plates to form a monolayer. Wounds were created using sterile 200 μl pipettes and photographed at indicated time points.

2.11. Western blotting

Tumor tissues and cells were lysed using RIPA buffer (Thermo, USA) that contains protease inhibitors (Sigma, USA). After quantification with BCA kit (Beyotime, China), a total of 50 μg proteins were separated with SDS-PAGE and shifted to PVDF membranes. After blocking with 5% nonfat milk, protein bands were probed with primary antibodies against Vimentin (ab8978, Abcam, USA), E-Cadherin (ab231303, Abcam, USA), zeb1 (ab203829, Abcam, USA), N-Cadherin (ab76011, Abcam, USA), Nanog (ab109250, Abcam, USA), Snail (ab216347, Abcam, USA), Bcl-2 (ab182858, Abcam, USA), Bax (ab32503, Abcam, USA), cleaved caspase-3 (ab32042, Abcam, USA) and β-actin (ab8226, Abcam, USA), respectively, at 4 °C overnight. Next day, bands were visualized by ECL solution (Millipore, USA) after incubation with secondary antibodies. All antibodies were brought from Proteintech (China).

2.12. Luciferase reporter gene assay

The potential interaction between miR-130a-5p with zeb1 3′UTR and circWHSC1 were predicted by ENCORI website. Wild type or mutate sequences of zeb1 3′UTR and circWHSC1 were cloned into pmirGLO plasmids. Cells were co-transfected with constructed vectors and miR-130a-5p for 24 h. Cell lysates were then analyzed by a Dual-Luciferase reporter assay system (Promega, USA).

2.13. RNA pulldown assay

Biotin-labeled miR-130-5p with wild type (WT) or mutated (MUT) sequences were synthesized by RiboBio (Guangzhou, USA). RNA pulldown experiment was conducted using the biotin-labeled miRNA. In brief, cells were transfected with the biotin-labeled miRNA for 24 h. Cells were then lysed and sonicated into small fragments, which were then incubated with C-1 magnetic beads (Invitrogen, CA, USA), washed, eluted, and level of circWHSC1 was measured by qRT-PCR.

2.14. Xenograft mouse model

Male SCID/Nude mice aged 6-week-old were brought from Vital River Laboratory (China). To establish the xenograft model, tumor cells (10^6 in 50 μl PBS) that transfected with indicated oligonucleotides were inoculated into the right fat pads of mice. The length and width of tumors were measured, and tumor size was calculated: 0.5 × length × width^2. For treatment, siRNAs or overexpression vectors (20 nmol/20 g body weight) were dissolved in 20 μl PBS and were injected around tumor site every 3 days for 30 days after tumor inoculation. Mice were then sacrificed, and tumor tissues were collected, weighed, and stored for histological analysis. All animal experiments in this work were authorized by the Animal Ethical Committee of Tianjin Union Medical Center. Confirmation that informed consent was obtained. Confirmation that the study complies with all regulations.

2.15. Immunohistochemical (IHC) analysis

Tumor tissues were fixed with 4% PFA and made into 5-μm paraffin-embedded slices. Tumor samples were heated at 98 °C for antigen retrieval, pretreated with a 3% H_2O_2, penetrated with 1% Triton X-100 in PBS, and blocked with goat serum. The tissues were then incubated with anti-Ki67 antibody (ab16667, Abcam, USA) for 2 h at room temperature, followed by incubation with biotin-labeled secondary antibody and reaction with 3,3-diaminobenzidine (DAB; Beyotime, China).

2.16. Statistical analysis

Data analyses in this work were performed by SPSS 22.0 software and Graphpad Prism 7.0 software. Comparison between two or more groups were analyzed by student's t-test or one-way ANOVA. Correlation between miR-130a-5p with zeb1 and circWHSC1 was measured by Pearson correlation analysis. P values < 0.05 were regarded as statistically significant.

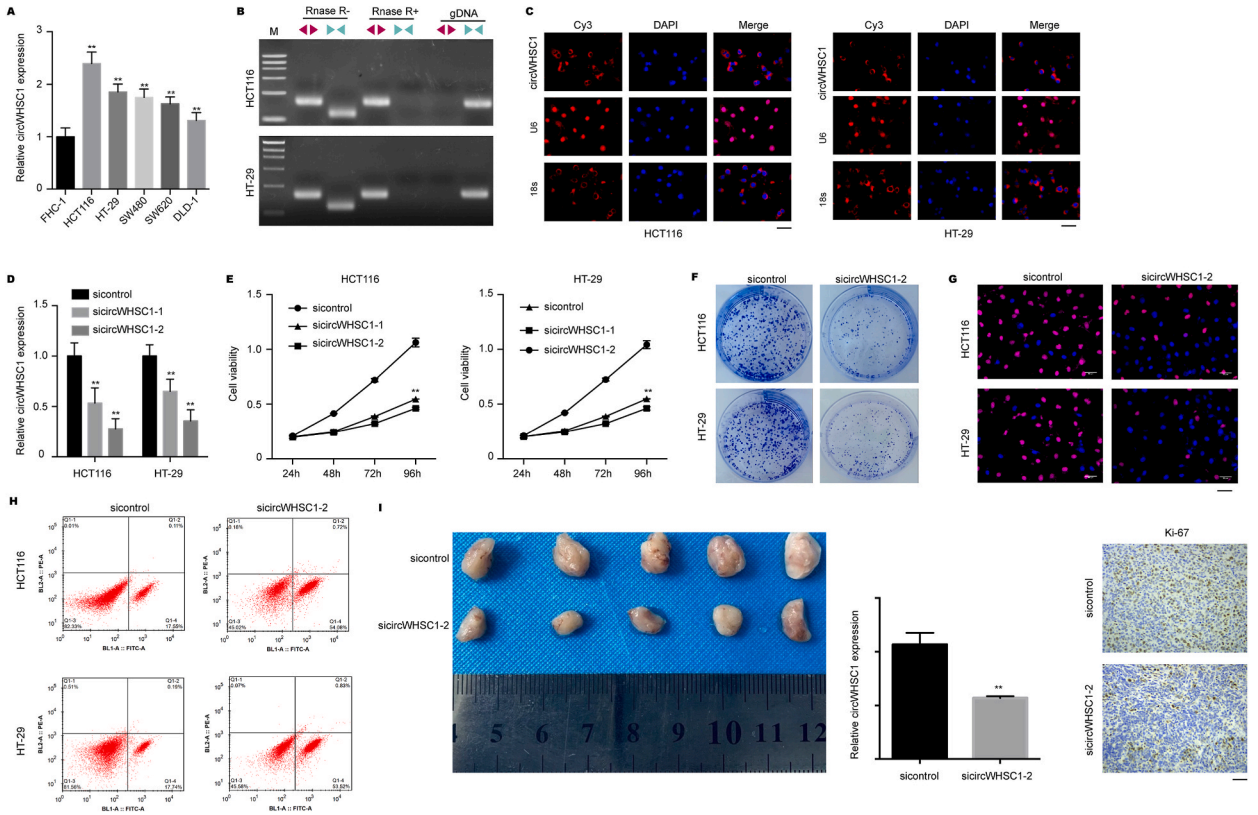

Fig. 1. circWHSC1 facilitates cell growth of colorectal cancer *in vitro* and *in vivo*. (A) The expression of circWHSC1 was determined by qPCR in the indicated cells. (B) circular and linear forms of WHSC1 expression was analyzed by agarose gel electrophoresis assays in HCT116 and HT-29 cells in the presence or absence of Rnase R treatment. (C) The cellular localization of circWHSC1 was measured by FISH analysis, in which 18s and U6 were used as control. (D–H) The HCT116 and HT-29 cells were treated with circWHSC1 siRNAs. (D) The expression of circWHSC1 was validated by qPCR in the cells. (E) The cell viability was detected by CCK-8 assay. (F and G) The cell proliferation was examined by colony formation and Edu assay, respectively. (H) The cell apoptosis was tested by flow Cytometry analysis. (I) The tumor growth of HCT116 treated with circWHSC1 siRNA was analyzed in the nude mice (n = 5). The tumor image was shown. The expression of circWHSC1 was measured by qPCR. The levels of Ki-67 were confirmed by IHC staining. $^{**}P < 0.01$. Scale bar, 50 μm.

3. Results

3.1. Knockdown of circWHSC1 represses cell growth of colorectal cancer in vitro and in vivo

In order to assess the potential correlation of circWHSC1 with colorectal cancer, the expression of circWHSC1 was detected in normal colon epithelial FHC-1 cell line and colorectal cancer cell lines, including SW480, SW620, HCT-116, HT-29, and DLD-1. We observed that the expression of circWHSC1 was upregulated in colorectal cancer cells compared with normal colon epithelial cells, in which HCT-116 and HT-29 cells presented higher levels of circWHSC1 and were used in the subsequent analysis (Fig. 1A). To validate the characteristic of circWHSC1, the HCT-116 and HT-29 cells were treated with RNase R and we found that circWHSC1, but not linear WHSC1, was still detectable under the treatment of RNase R in HCT-116 and HT-29 cells (Fig. 1B). FISH assay further confirmed that circWHSC1 was mainly localized in cytoplasm of HCT-116 and HT-29 cells (Fig. 1C).

Then, we further analyzed the effect of circWHSC1 on colorectal cancer cell growth *in vitro* and *in vivo*. To this end, the depletion of circWHSC1 by siRNAs was validated in HCT-116 and HT-29 cells (Fig. 1D), and the transfection of circWHSC1 siRNAs significantly repressed the viability of HCT-116 and HT-29 cells (Fig. 1E). Similarly, the colony formation numbers and Edu-positive HCT-116 and HT-29 cells were inhibited by the depletion of circWHSC1, respectively (Fig. 1F and G). Consistently, the knockdown of circWHSC1 promoted the apoptosis of HCT-116 and HT-29 cells (Fig. 1H). Importantly, the tumor growth of HCT-116 cells in nude mice was attenuated by circWHSC1 silencing, in which the levels of circWHSC1 and Ki-67 were reduced in the model (Fig. 1I).

3.2. Depletion of circWHSC1 suppresses migration and invasion of colorectal cancer cells

Cell migration and invasion play a crucial role in colorectal cancer development, we then evaluated the function of circWHSC1 in the modulation of migration and invasion ability of colorectal cancer cells. Remarkably, the depletion of circWHSC1 suppressed the

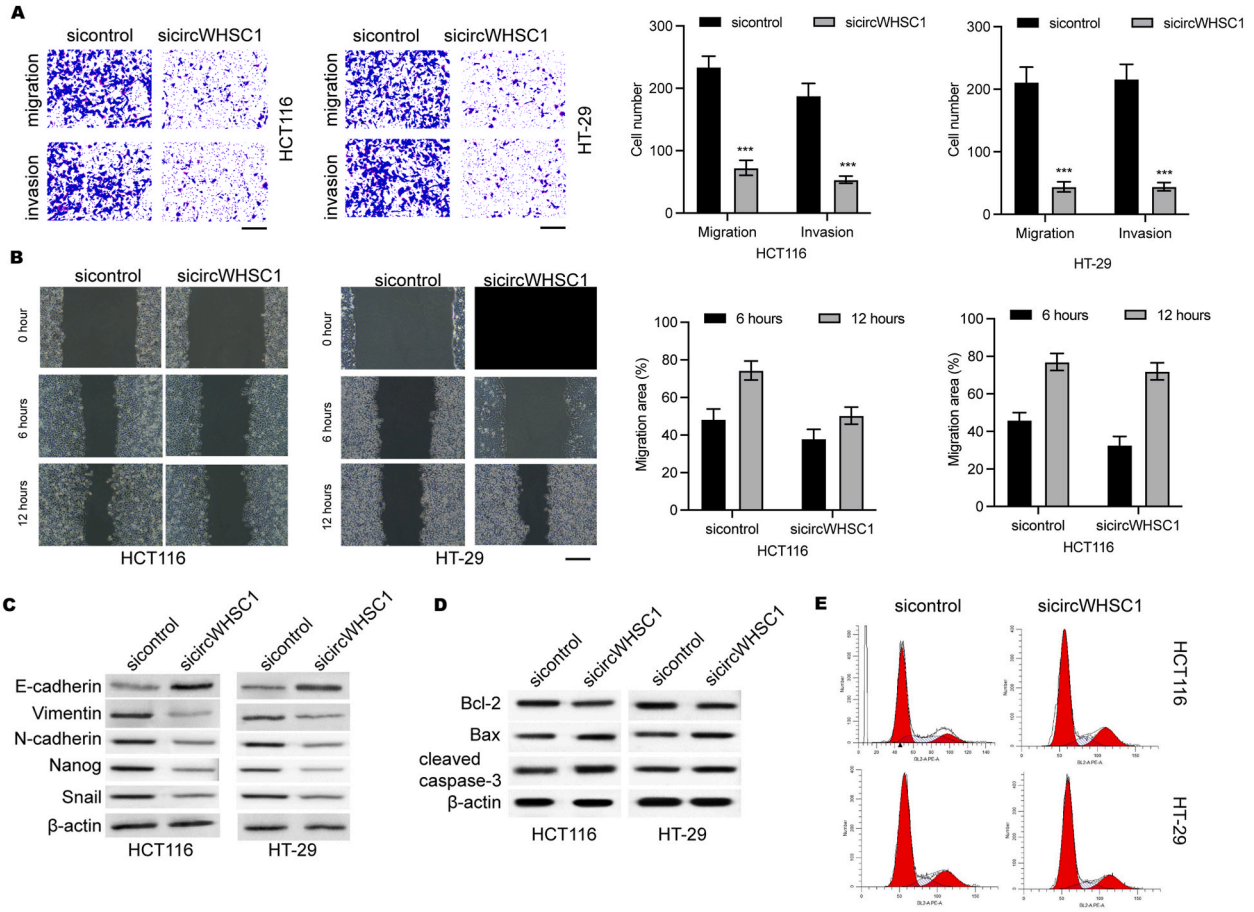

Fig. 2. circWHSC1 enhances migration and invasion of colorectal cancer cells. (A–C) The HCT116 and HT-29 cells were treated with circWHSC1 siRNAs. (A) The cell migration and invasion were detected by transwell. (B) The migration was measured by wound healing assay. (C) The expression of E-cadherin, Vimentin, N-cadherin, Nanog, and Snail was analyzed by Western blot. (D) The expression of Bcl-2, Bax, and cleaved caspase-3 was analyzed by Western blot. (E) Cell cycle was measured by flow cytometry. $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bar, 50 μm.

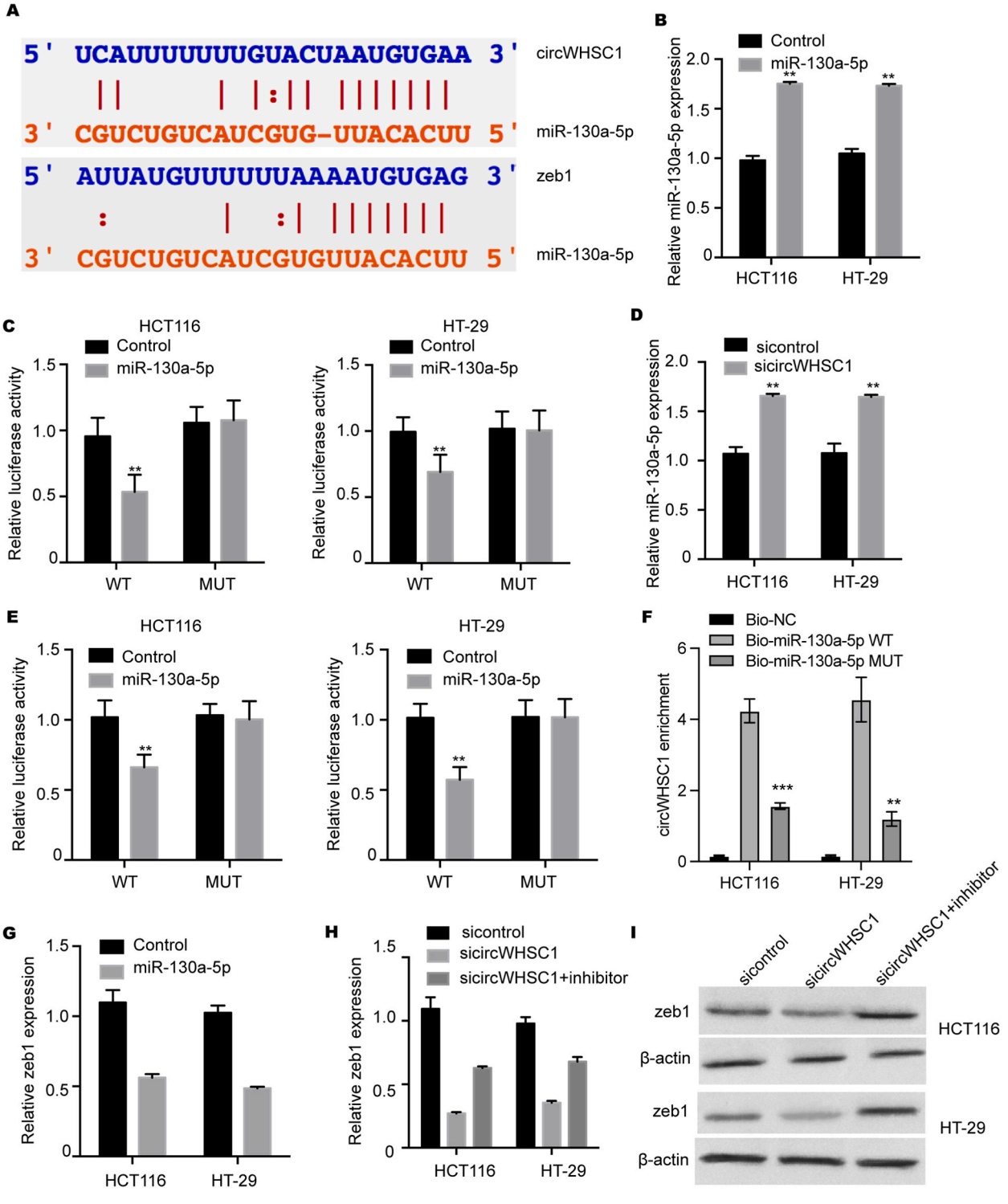

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Fig. 3. circWHSC1 targets miR-130a-5p to promote zeb1 expression in colorectal cancer cells. (A) The binding sites of miR-130a-5p within circWHSC1 and zeb1 3′UTR were predicted. (B and C, E and F) The HCT116 and HT-29 cells were treated with miR-130a-5p mimic. (B) The expression of miR-130a-5p was checked by qPCR. (C) The luciferase activity of wild type circWHSC1 (WT) and circWHSC1 mutant (MUT) was analyzed by dual-luciferase reporter assay. (D) The expression of miR-130a-5p was detected by qPCR in HCT116 and HT-29 cells transfected with circWHSC1 siRNA. (E) The luciferase activity of wild type zeb1 (WT) and zeb1 mutant (MUT) was analyzed by dual-luciferase reporter assay. (F) RNA pulldown assay was conducted to measure interaction between circWHSC1 with miR-130-5p. (G) The expression of zeb1 was analyzed by qPCR. (H and I) The expression of zeb1 was detected by qPCR and Western blot in HCT116 and HT-29 cells transfected with circWHSC1 siRNA and miR-130a-5p inhibitor. $^{**}P < 0.01$.

migration and invasion of HCT-116 and HT-29 cells (Fig. 2A). Consistently, the wound healing ability of HCT-116 and HT-29 cells was attenuated by the silencing of circWHSC1 (Fig. 2B). Meanwhile, the knockdown of circWHSC1 increased E-cadherin expression and decreased expression of Vimentin, N-cadherin, Nanog, and Snail in HCT-116 and HT-29 cells (Fig. 2C). Besides, knockdown of circWHSC1 also induced apoptosis of HCT-116 and HT-29 cells, manifested as downregulated expression of Bcl-2 and upregulated expression of Bax and cleaved caspase-3 (Fig. 2D). The results from cell cycle test demonstrated that knockdown of circWHSC1 caused cell cycle arrest at G0/G1 phase of HCT-116 and HT-29 cells (Fig. 2E).

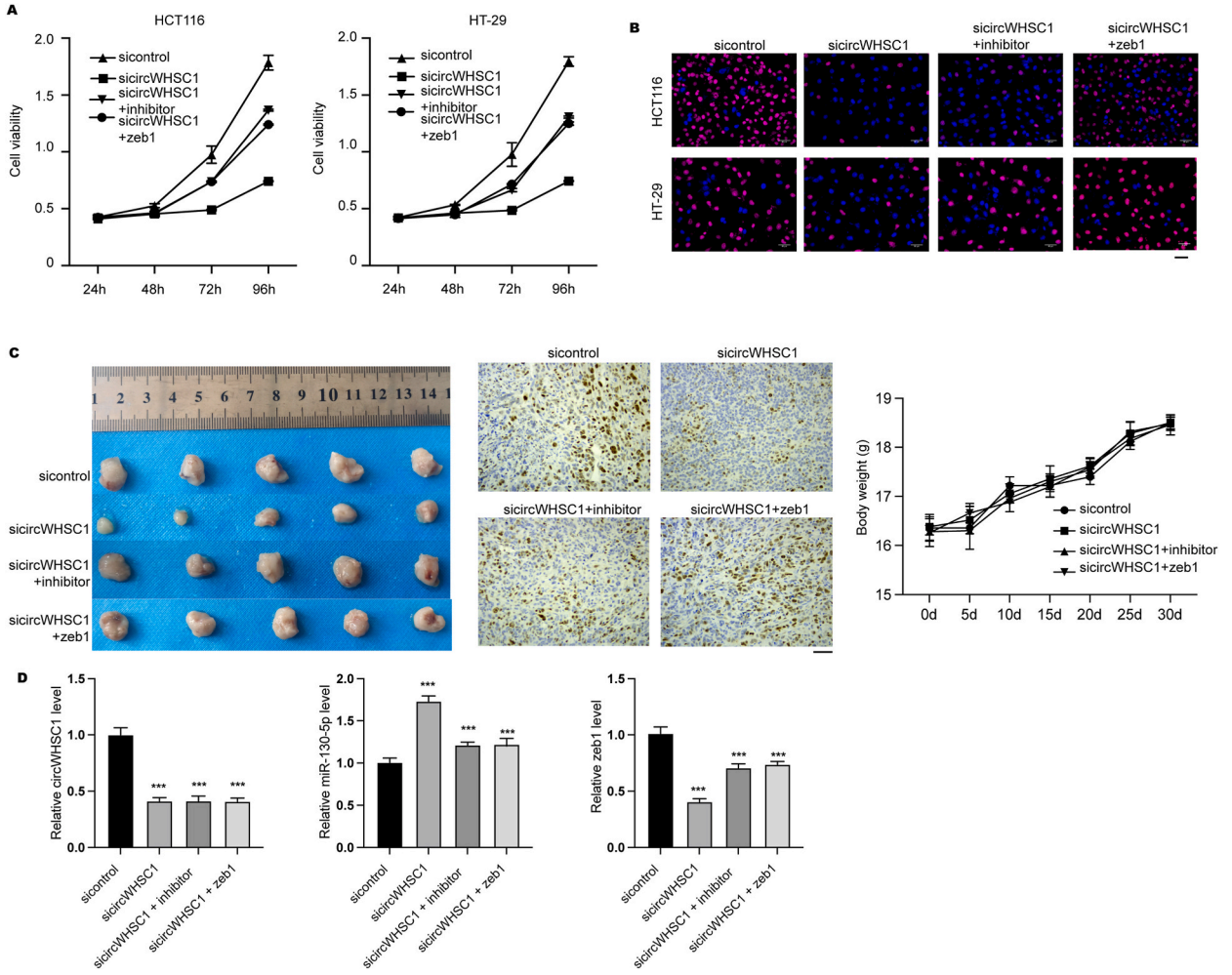

Fig. 4. circWHSC1 contributes to cell growth of colorectal cancer by miR-130a-5p/zeb1 axis *in vitro* and *in vivo*. (A and B) The HCT116 and HT-29 cells were treated with transfected with circWHSC1 siRNA and miR-130a-5p inhibitor or zeb1 overexpressing plasmids. (A) The cell viability was detected by CCK-8 assay. (B) The cell proliferation was examined by Edu assay. (C) The tumor growth and body weight of HCT116 cells was analyzed in the nude mice (n = 5). The tumor image was shown. The levels of Ki-67 were confirmed by IHC staining. (D) The RNA levels of circWHSC1, miR-130-5p and zeb1 in tumor tissues was detected by qPCR assay. $^{**}P < 0.01$, $^{***}P < 0.001$. Scale bar, 50 μm.

3.3. CircWHSC1 targets miR-130a-5p to promote zeb1 expression in colorectal cancer cells

Next, we further explored the downstream mechanism by which circWHSC1 contributes to colorectal cancer progression. The bioinformatic prediction identified the potential interaction of miR-130a-5p with circWHSC1 and zeb1 (Fig. 3A). The upregulation of miR-130a-5p by miR-130a-5p mimic was validated in HCT-116 and HT-29 cells (Fig. 3B). The treatment of miR-130a-5p mimic suppressed the luciferase activity of wild-type circWHSC1 and zeb1 3′UTR in HCT-116 and HT-29 cells (Fig. 3C and E). The knockdown of circWHSC1 enhanced the expression of miR-130a-5p in the cells (Fig. 3D). Moreover, the results from RIP experiment indicated that circWHSC1 interacted with wild type sequence of miR-130a-5p (Fig. 3F). Meanwhile, miR-130a-5p inhibited the expression of zeb1 in HCT-116 and HT-29 cells (Fig. 3G). Significantly, the expression of zeb1 was repressed by the depletion of circWHSC1 and miR-130a-5p inhibitor could restore the expression in the cells (Fig. 3H and I), suggesting that circWHSC1 targets miR-130a-5p to promote zeb1 expression in colorectal cancer cells.

3.4. CircWHSC1 contributes to cell growth of colorectal cancer by miR-130a-5p/zeb1 axis in vitro and in vivo

Then, we validated whether circWHSC1 promotes colorectal cancer cell proliferation by miR-130a-5p/zeb1 signaling. We found that the depletion of circWHSC1 remarkably reduced the cell viability and Edu-positive HCT-116 and HT-29 cells, and the miR-130a-5p inhibitor or zeb1 overexpression could restore the phenotypes (Fig. 4A and B). Furthermore, the tumor growth of HCT-116 cells in nude mice was attenuated by circWHSC1 knockdown, while miR-130a-5p depletion or zeb1 overexpression reversed the effect in the model (Fig. 4C). The sicircWHSC1 suppressed the expression of circWHSC1 and zeb1 and elevated the level of miR-130-5p in tumor tissues, whereas inhibition of miR-130-5p and overexpression of zeb1 could recover the expression of zeb1 (Fig. 4D).

4. Discussion

In this research, we explored the crucial biological impact of circWHSC1 on colorectal cancer *in vitro* and *in vivo*. It has been reported that circWHSC1 contributes to progression of non-small cell lung cancer by microRNA-296-3p/AKT serine/threonine kinase 3 signaling [15]. CircWHSC1 promotes progression of cervical cancer by targeting miR-532-3p/LTBP2 [23]. CircWHSC1 regulates miR-646/NPM1 axis to enhance endometrial cancer development [16]. In breast cancer, circWHSC1 increases glycolysis through regulating the miR-212-5p/AKT3 axis [13]. CircWHSC1 plays an oncogene role in hepatocellular carcinoma [12] and induces progression of ovarian cancer by targeting miR-145/miR-1182/MUC1/signaling [11]. These previous studies indicate that circWHSC1 plays an important role in cancer development, but the effect of circWHSC1 on colorectal cancer is still unclear. In this study, we observed that circWHSC1 was elevated in colorectal cancer cells compared with normal colon epithelial cells. CircWHSC1 depletion repressed the viability of colorectal cancer cells. The number of colonies and Edu-positive colorectal cancer cells were decreased by the depletion of circWHSC1, respectively. Knockdown of circWHSC1 promoted the apoptosis of colorectal cancer cells. The tumor growth of colorectal cancer cells in nude mice was attenuated by circWHSC1 silencing. Meanwhile, the invasion and migration ability of colorectal cancer cells was suppressed by circWHSC1 depletion. Our data suggest that circWHSC1 promotes cell proliferation of colorectal cancer *in vitro* and *in vivo*. The clinical significance of circWHSC1 in colorectal cancer should be evaluated in future investigations.

Our mechanism study showed that circWHSC1 targets miR-130a-5p to promote zeb1 expression in colorectal cancer cells. The depletion of circWHSC1 remarkably reduced the cell viability and Edu-positive colorectal cancer cells, and the miR-130a-5p inhibitor or zeb1 overexpression could restore the phenotypes. Furthermore, the tumor growth of colorectal cancer cells in nude mice was attenuated by circWHSC1 knockdown, while miR-130a-5p depletion or zeb1 overexpression reversed the effect in the model. It has been reported that miR-130a-5p inhibits malignantly phenotypes in multiple cancer types [24,25]. Nevertheless, the function of miR-130a-5p in colorectal cancer remain obscure. Meanwhile, the previous studies have identified the oncogene potential of zeb1 in colorectal cancer [26,27]. Our finding provides new insight into the mechanism by which circWHSC1 promotes colorectal cancer progression by miR-130a-5p/zeb1 axis. However, ectopic overexpression experiments are necessary to profoundly confirm the effects of circWHSC1, and whether circWHSC1 affects other cell behaviors such as nutrient metabolism is still unclear. And other potential mechanisms underlying circWHSC1-modulated CRC development are unclear. It is necessary to perform further studies on circWHSC1 in CRC model.

In conclusion, we discovered that circular RNA circWHSC1 facilitated colorectal cancer cell proliferation by targeting miR-130a-5p/zeb1 signaling *in vitro* and *in vivo*. Our findings presented circWHSC1 as a novel target for CRC pathogenesis and identified the potential mechanisms, which provided novel insight into CRC development and exploration of effective treatment.

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Author contribution statement

Lei Shi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Wen Li: Conceived and designed the experiments; Wrote the paper.
Yuanshun Zhao; Xu Liu: Performed the experiments; Analyzed and interpreted the data.

Jingyao Qian: Performed the experiments.
Xiao Yang: Performed the experiments; Wrote the paper.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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