OncoTargets and Therapy

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ORIGINAL RESEARCH

RETRACTED ARTICLE: MicroRNA-143-3p suppresses tumorigenesis by targeting catenin-δ1 in colorectal cancer

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Background: Colorectal cancer (CRC) is the third most common malignancy in the United States. Mounting microRNAs (miRNAs) have been identified as oncogenes or tumor suppressors in various cancers including CRC.

Materials and methods: The levels of microRNA-143-3p (miR-143-3p) and catenin-δ1 (CTNND1) were determined by RT-qPCR assay. Cell proliferative ability was assessed by Cell Counting Kit-8 assay. Cell migratory and invasive capacities were measured by transwell migration and invasion assay. Luciferase reporter assay was conducted to explore whether miR-143-3p could bind with CTNND1 3′UTR. CTNND1 protein level was determined through Western blot assay. Mouse xenograft models of CRC were established to test the functions and molecular basis of miR-143-3p in the development of CRC in vivo.

Results: Low amounts of miR-143-3p were expressed in CRC tissues and cells. Functional analysis revealed that miR-143-3p overexpression suppressed cell proliferation, migration and invasion in CRC. Molecular mechanism exploration indicated that miR-143-3p directly targeted CTNND1. Moreover, enforced expression of CTNND1 contributed to cell proliferation, migration and invasion in CRC, and CTNND1 silencing exerted opposite effects. Restoration experiments disclosed that CTNND1 upregulation weakened the inhibitory effects of miR-143-3p on CRC cell proliferation, migration and invasion. Additionally, miR-143-3p inhibited the growth of HCT116-derived xenograft tumors by targeting CTNND1 in vivo.

Conclusion: miR-143-3p hampered the development and progression of CRC by targeting CTNND1 in vitro and in vivo, deepening our understanding of the functions and molecular basis of miR-143-3p in the tumorigenesis of CRC and providing some candidate prognostic markers or therapeutic targets for CRC.

Keywords: microRNA-143-3p, CTNND1, colorectal cancer, tumorigenesis

Introduction

Colorectal cancer (CRC) is the third most common malignancy in the US, with an estimated 140,250 new cases and 50,630 deaths in 2018.[1] With the advance of CRC management, the overall incidence and mortality of CRC was considerably reduced from 2000 through 2014 in the US.[2–4] The 5-year survival rate is relatively higher (70%–90%) for patients with localized and regional CRC, while it declines to 13%–14% for patients with advanced CRC during the years of 2006–2012 in the United States.[2] Thus, it is imperative to investigate the pathogenesis of CRC in order to identify more effective therapeutic targets or strategies.

MicroRNAs (miRNAs), a group of small RNA transcripts without protein-coding potential, participate in regulating various developmental and pathological processes by targeting protein-coding transcripts in animals including human.[5] Over the past decades, mounting miRNA has been reported to be closely linked with tumor

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initiation, development and metastasis in many malignancies including CRC.[6,7] For instance, microRNA-382 (miR-382) suppressed cell proliferation, migration and invasion by targeting Krueppel-like factor 12 (KLF12) and homeodomain-interacting protein kinase 3 (HIPK3) in CRC.[8] The depletion of microRNA-103 (miR-103) resulted in the reduction of cell proliferative, invasive and migratory capacities partly through targeting large tumor suppressor kinase 2 (LATS2) in CRC.[9] MicroRNA-143-3p (miR-143-3p), located on chromosome 5q32, functioned as a tumor suppressor in some neoplasms such as triple-negative breast cancer,[10] esophageal squamous cell cancer[11] and ovarian cancer.[12] Also, an earlier finding unveiled that miR-143-3p expression was reduced in CRC tissues relative to noncancerous colorectal tissues.[13] Also, the Tumor Cancer Genome Atlas database (TCGA) analysis unveiled that miR-143-3p has lower expression in CRC patients with lymphovascular invasion relative to ones without lymphovascular invasion,[13] hinting at the link between miR-143-3p and CRC tumorigenesis. However, the roles and molecular basis of miR-143-3p in the development of CRC need to be further explored.

In the literature, we found that miR-143-3p hampered proliferation, migration and invasion by reducing catenin-δ1 (CTNND1) expression in CRC in vitro and in vivo, providing some potential therapeutic targets for CRC.

Materials and methods

Clinical specimens and cell culture

A total of 37 pairs of CRC tissues (Cancer) and matched adjacent non-cancer tissues (Normal) were obtained from patients with surgical resections at our hospital. All tissues were snap-frozen in liquid nitrogen and preserved at −80°C before utilization. Our project received the approval of the Ethics Committee of Sichuan College of Xinxiang Medical University, and the written informed consents from every patient and it was conducted in accordance with the Declaration of Helsinki.

Normal human colonic epithelial cell line NCM460 was acquired from INCELL Corporation (San Antonio, TX, USA). CRC cell lines (HCT116 and SW620) were gained from American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 cells were cultured in McCoy's 5a Medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco). NCM460 and SW620 cells were maintained in DMEM medium (Gibco) supplemented with 10% FBS (Gibco). All cells were grown in a humidified atmosphere containing 5% CO_2 and 95% air at 37°C.

Reagents and cell transfection

Specific small interference RNA (siRNA) targeting CTNND1 (si-CTNND1), miR-143-3p mimic, miR-143-3p inhibitor and a scramble control (NC) were gained from GenePharma Co., Ltd (Shanghai, People's Republic of China). CTNND1 coding region was subcloned into pcDNA3.1 vector (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA)) to get CTNND1 overexpression plasmid. All transfection was performed using Lipofectamine 2000 reagent (Invitrogen) referring to the instructions of manufacturer.

RT-qPCR assay

Total RNA was extracted from CRC tissues and cells using Trizol reagent (Invitrogen) following the manufacturer's protocols. Next, 1 μg RNA was sequentially treated with 1 U RNase-free DNaseI (Thermo Fisher Scientific) for 30 minutes at 37°C and 5 mM (final concentration) EDTA for 10 minutes at 65°C to remove DNA contamination. Then, cDNA first strand was synthesized from RNA using a Reverse Transcription (RT) System (Promega Corporation, Madison, WI, USA) and oligo(dT)15 primer (for CTNND1, β-actin or U6 snRNA) or specific RT primers (for miR-143-3p). Next, relative levels of miR-143-3p or CTNND1 were determined using SYBR Green PCR Master Mixes (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and specific quantitative primers with U6 snRNA or β-actin as the internal control, respectively. The RT primer for miR-143-3p is 5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAGCTA-3′.

Quantitative primers were displayed as follows: miR-143-3p, 5′-GTGAGATGAAGCACTGTAGC-3′ (forward) and 5′-GTGCAGGGTCCGAGGT-3′ (reverse); U6, 5′-CTCGCTTCGGCAGCACA-3′ (forward) and 5′-AACGCTTCACGAATTTGCGT-3′ (reverse); CTNND1, 5′-GCCATGTCTGTAGTCTCTGTGGA-3′ (forward) and 5′-TCACAGTCTTCACTACTTTCTTGACC-3′ (reverse); β-actin, 5′-CTGTCTGGCGGCACCACCAT-3′ (forward) and 5′-GCAACTAAGTCATAGTCCGC-3′ (reverse).

Western blot assay

At 48 hours after transfection, cells were lysed using ice-cold RIPA buffer (Beyotime Biotechnology, Shanghai, People's Republic of China) containing protease inhibitor cocktail (Hoffman-La Roche Ltd, Basle, Switzerland) to extract proteins. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Then, proteins were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then

transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking with 5% non-fat milk, the membranes were probed overnight at 4°C with anti-CTNND1 antibody (ab92514, 1:1,000, Abcam, Cambridge, UK) or anti-β-actin antibody (ab8227, 1:2,000, Abcam). Next, the blots were subsequently incubated for 1 hour at room temperature with Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (ab6721, 1:2,000, Abcam). At last, chemoluminescent signals were visualized using Super Signal™ West Dura Extended Duration Substrate (Thermo Fisher Scientific) and quantified via Quantity One Software Version 4.1.1 (Bio-Rad Laboratories, Hercules, CA, USA).

Cell Counting Kit-8 (CCK-8) assay

Cell proliferative ability was examined using a Cell Counting Kit-8 kit (Beyotime Biotechnology) according to the manufacturer's instructions. Generally, HCT116 and SW620 cells were plated into 96-well plates and then transfected with corresponding siRNAs, miRNAs or plasmids. At 48 hours after transfection, 10 µL of CCK-8 regent was mixed into each well of 96-well plates. After 1 hour of incubation, cell absorbance in each well was tested at a wavelength of 450 nm.

Luciferase reporter assay

Partial fragment of CTNND1 3′UTR with putative miR-143-3p binding sites was subcloned into pSICHECK-2 vector (Promega) to generate wild type CTNND1 reporter (WT). Also, mutant type CTNND1 reporter (MUT) with mutant miR-143-3p binding sites was produced using Fast Site-Directed Mutagenesis Kit (Tiangen Biotech Co., Ltd. Beijing, People's Republic of China). Then, WT or MUT reporter was transfected into HCT116 and SW620 cells together with miR-143-3p mimic, miR-143-3p inhibitor or control NC. At 48 hours upon transfection, luciferase activities were determined using a dual luciferase reporter assay kit (Promega).

Transwell migration and invasion assay

Cell migratory or invasive capacity was examined using a transwell chamber containing 8 µm pores (Corning Incorporated, Corning, NY, USA) in the absence or presence of matrigel pre-coating (Corning Incorporated), respectively. At 24 hours after transfection, HCT116 and SW620 cells were collected and resuspended in serum-free medium. Then, transfected cells (5×10^4 cells/well for migration assay and 1×10^5 cells/well for invasion assay) were plated into the upper chamber, and medium supplemented with 20% FBS (Gibco) was put into the lower chamber. At 24 hours post incubation, cells on the upper surface of membranes were erased, and cells in the lower surface were fixed, stained, photographed and counted in 15 random fields.

Mice xenograft experiments

All experimental procedures got the approval of the Institutional Animal Care and Use Committee of Xinxiang Medical University. BALB/c mice (n=8, 6–8 weeks) were obtained from Shanghai Laboratory Animal Center of Chinese Academy of Sciences (Shanghai, People's Republic of China) and bred following the national standard of the care and use of laboratory animals. For tumorigenesis experiments, HCT116 cells (5×10^6/100 µL) were subcutaneously inoculated into the right flanks of nude mice and this injection time was set as day 0. After 10 days, miR-143-3p mimic or a scramble control (NC) were injected into formed tumors every 3 days. Tumor volume was monitored every 10 days for a total of 50 days using a caliper. At the end of the experiments, the mice were sacrificed. Then, the tumors were excised, photographed and weighted. The CTNND1 level in tumors was also determined by RT-qPCR assay. The study was approved by the Institutional Animal Care and Use Committee of Sanquan College of Xinxiang Medical University. All studies were conducted in accordance with the guidelines of the National Institutes of Health for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee in Teaching and Research, Sanquan College of Xinxiang Medical University.

Statistical analysis

All experiments were conducted at least in triplicate with the results showing as mean ± SD. Significance of difference between or among groups was computed by Student's *t*-test or one-way ANOVA, respectively. Differences were considered as statistically significant when the *P*-value was below 0.05.

Results

MiR-143-3p expression was notably reduced in CRC tissues and cells, and was positively associated with better prognosis of CRC patients

Firstly, the expression pattern of miR-143-3p in CRC cells and tissues was examined by RT-qPCR assay. Results showed that miR-143-3p expression was remarkably reduced in CRC cell lines (HTC116 and SW620) as compared to that in normal human colon epithelial cell line NCM460 (Figure 1A). Also, miR-143-3p was lower expressed in CRC

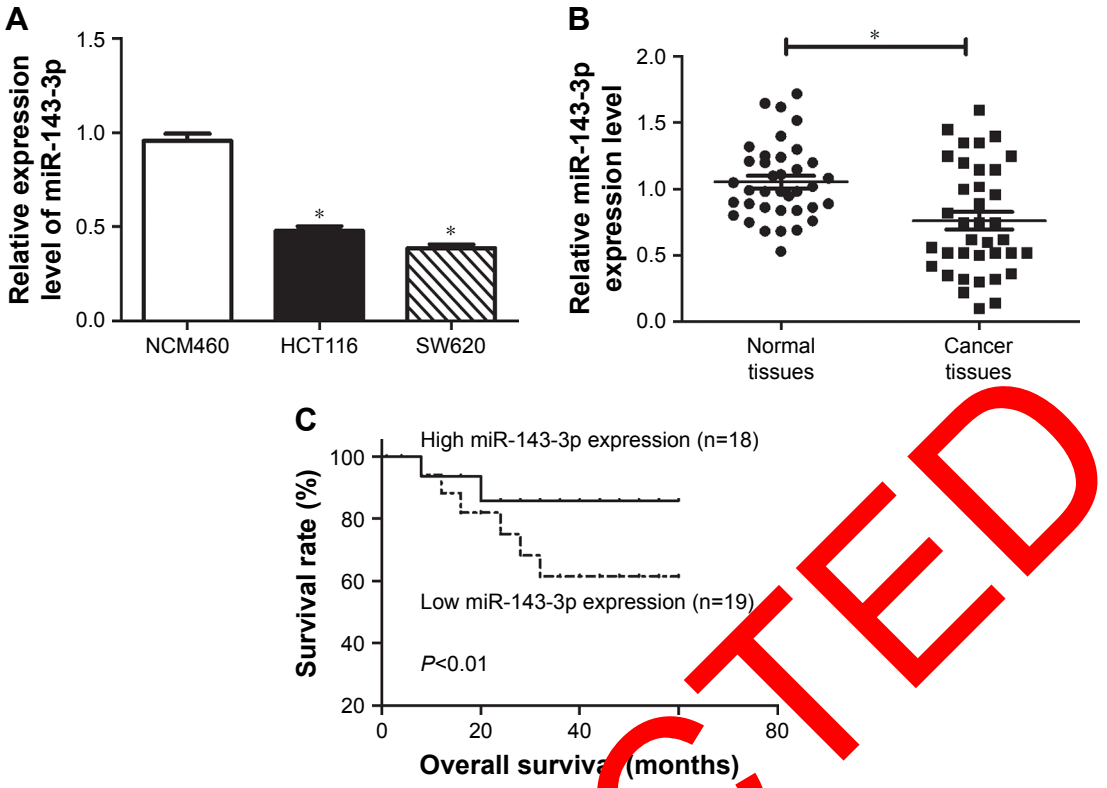

Figure 1 Expression of miR-143-3p was notably reduced in CRC tissues and cells, and was positively associated with better prognosis of CRC patients.
Notes: (**A**) RT-qPCR assay was performed to detect expression of miR-143-3p in NCM460, HCT116 and SW620 cells. (**B**) Expression of miR-143-3p in 37 pairs of CRC tissues and adjacent normal tissues was measured by RT-qPCR assay. (**C**) Kaplan–Meier survival analysis for CRC patients based on the difference of miR-143-3p level. *$P<0.05$.
Abbreviation: CRC, colorectal cancer.

tissues (n=37) compared with adjacent normal tissues (n=37) (Figure 1B). Moreover, CRC tissues (n=37) were divided into a high miR-143-3p expression group (n=18) and a low miR-143-3p expression group (n=19) by using the median value of miR-143-3p in CRC tissues as the cut-off point. Following Kaplan–Meier survival analysis revealed that CRC patients with higher miR-143-3p expression have better prognosis, as evidenced by higher survival rate and longer survival time (Figure 1C). Generally, the data indicated that miR-143-3p might be implicated in CRC progression.

MiR-143-3p overexpression inhibited cell proliferation, migration and invasion in CRC

Following RT-qPCR assay confirmed that the transfection of miR-143-3p mimic resulted in the notable increase of miR-143-3p level in HTC116 and SW620 cells compared with control (Figure 2A). Next, CCK-8 assay unveiled that the enforced expression of miR-143-3p inhibited cell proliferation relative to negative control group in HTC116 and SW620 cells (Figure 2B). Also, increased miR-143-3p induced a notable reduction in migratory ability of HTC116 and SW620 cells (Figure 2C). Additionally, the invasive capacity of HTC116 and SW620 cells was decreased in response to the upregulation of miR-143-3p (Figure 2D). Taken together, these results revealed that miR-143-3p overexpression inhibited proliferation, migration and invasion of CRC cells.

MiR-143-3p directly targeted CTNND1

Then, miRNA target prediction website (TargetScan) was used to identify potential targets of miR-143-3p in humans. Among candidate targets, CTNND1 was previously reported to be a target of miR-145 in colon cancer[14] (Figure 3A). It is well known that miR-145 is located in the same region with miR-143 in chromosome and clusters with miR-143.[15] Hence, we supposed that miR-143-3p might exert anti-tumor effects by targeting CTNND1 in CRC. To further validate this conjecture, wild type CTNND1 reporter (WT) or mutant type CTNND1 reporter (MUT) containing wild or mutant miR-143-3p binding sites were constructed, respectively. Following luciferase reporter assay we found that the introduction of miR-143-3p mimic markedly reduced luciferase activity of wild type CTNND1 reporter (Figure 3B and C). Conversely, miR-143-3p inhibitor-induced miR-143-3p depletion cause a notable increase in luciferase activity of

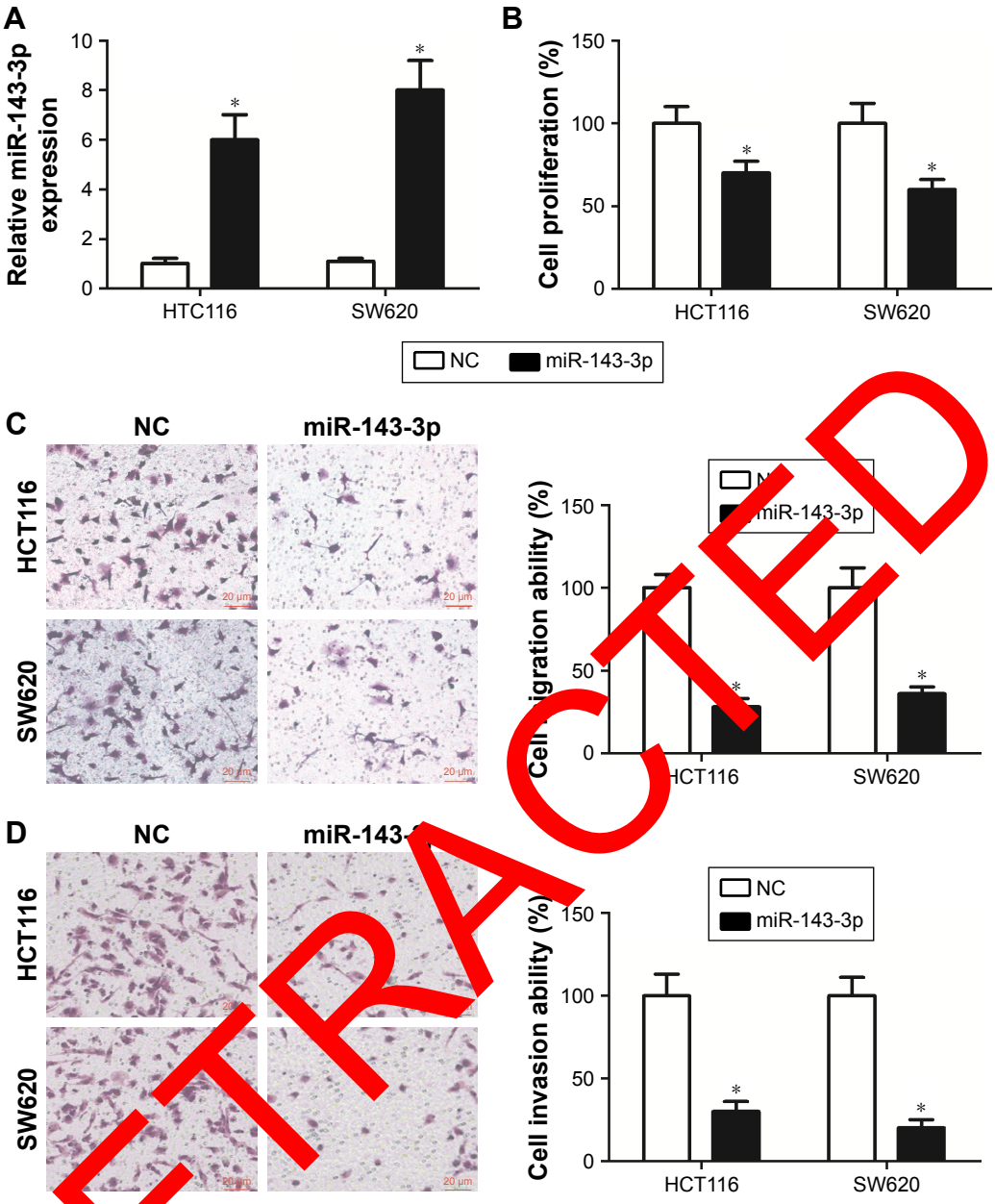

Figure 2 Overexpression of miR-143-3p inhibits proliferation, migration and invasion of CRC cells.
Notes: (**A**–**D**) HCT116 and SW620 cells were transfected with miR-143-3p mimic or its NC for 48 hours. Then, miR-143-3p level was determined by RT-qPCR assay (**A**), cell proliferation ability was assessed by CCK-8 assay (**B**), cell migratory (**C**) and invasive (**D**) capacities were examined by transwell migration and invasion assay. *$P<0.05$.
Abbreviations: CCK-8, Cell Counting Kit-8; CRC, colorectal cancer; NC, negative control.

wild type CTNND1 reporter in HTC116 and SW620 cells (Figure 3D and E). However, the introduction of miR-143-3p mimic (Figure 3B and C) or inhibitor (Figure 3D and E) had no much influence on luciferase activity of mutant type CTNND1 reporter in HTC116 and SW620 cells. In a word, these data disclosed that miR-143-3p could interact with CTNND1 3′UTR by putative binding sites. In support of these outcomes, we further demonstrated that CTNND1 protein level was obviously reduced in miR-143-3p-overexpressed HTC116 and SW620 cells, but was strikingly increased in miR-143-3p-depleted cells (Figure 3F). Additionally, CTNND1 level was inversely associated with miR-143-3p level in CRC tissues (Figure 3G). In summary, these results revealed that miR-143-3p directly targeted CTNND1 in CRC cells.

CTNND1 overexpression contributed to CRC cell proliferation, migration, and invasion

Then, RT-qPCR assay further manifested that CTNND1 expression was strikingly upregulated in CRC tissues

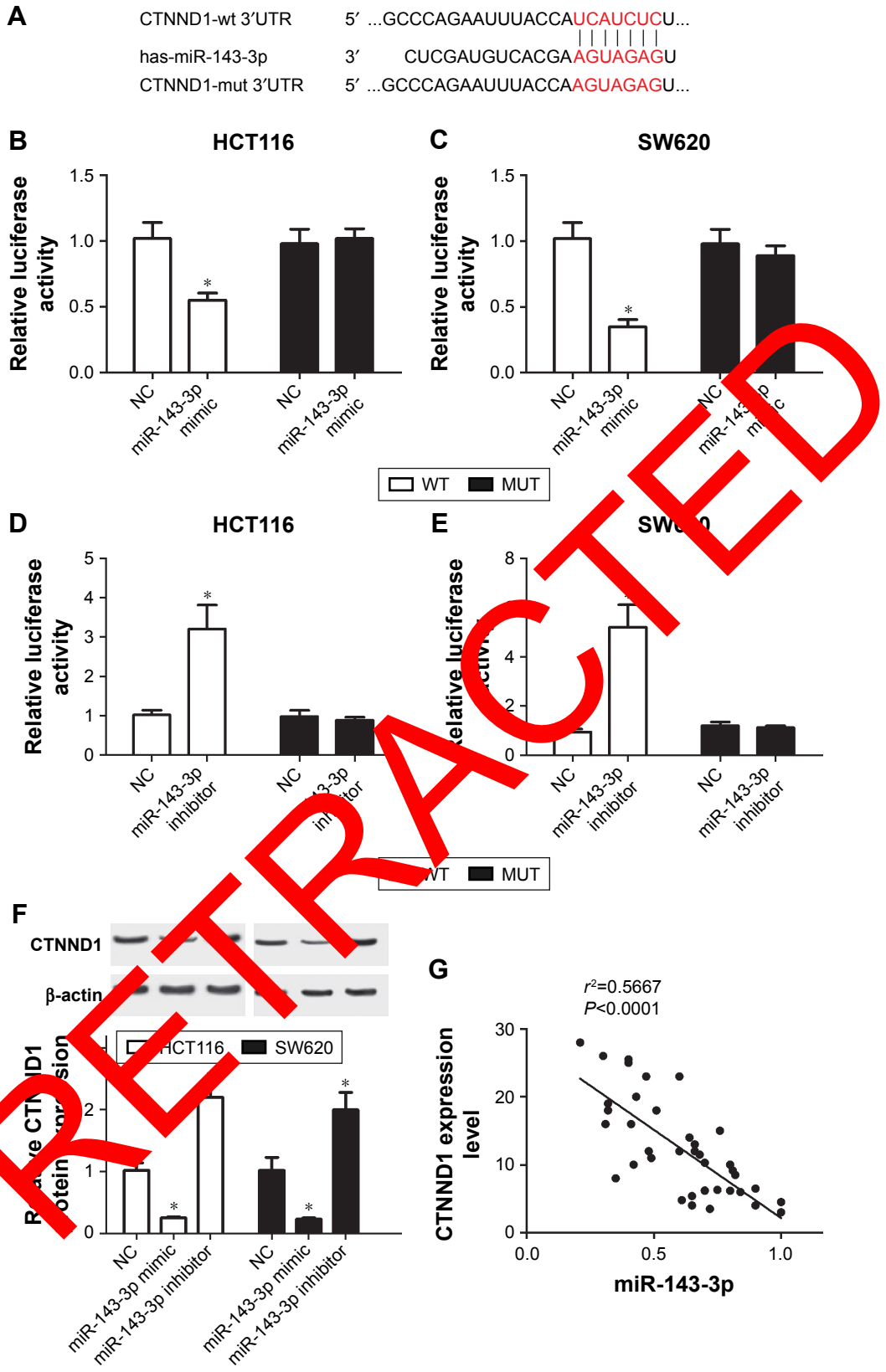

Figure 3 CTNND1 was a target of miR-143-3p.

Notes: (**A**) Predicted binding sites between miR-143-3p and CTNND1 3′UTR and mutant sites in MUT. (**B**–**E**) HTC116 and SW620 cells were co-transfected with WT or MUT and miR-143-3p mimic, miR-143-3p inhibitor or the negative control, followed by the detection of relative luciferase activity at 48 hours after transfection with renilla luciferase activity as an endogenous control. (**F**) HCT116 and SW620 cells were transfected with miR-143-3p mimic, miR-143-3p inhibitor or the negative control. CTNND1 protein level was determined by western blot assay at 48 hours post transfection. (**G**) Correlation analysis between CTNND1 mRNA level and miR-143-3p expression in 37 cases of CRC tissues. *$P<0.05$.

Abbreviations: MUT, mutant type CTNND1 reporter; WT, wild type; NC, negative control; CRC, colorectal cancer.

(n=37) as compared to that in adjacent normal tissues (n=37) (Figure 4A). To further investigate the roles of CTNND1 in CRC progression, CTNND1 overexpression plasmid (CTNND1) or siRNA (CTNND1 siRNA) were transfected into HTC116 and SW620 cells. As displayed in Figure 4B and C, the introduction of CTNND1 overexpression plasmid resulted in a conspicuous upregulation of CTNND1 expression at mRNA (Figure 4B) and protein (Figure 4C)

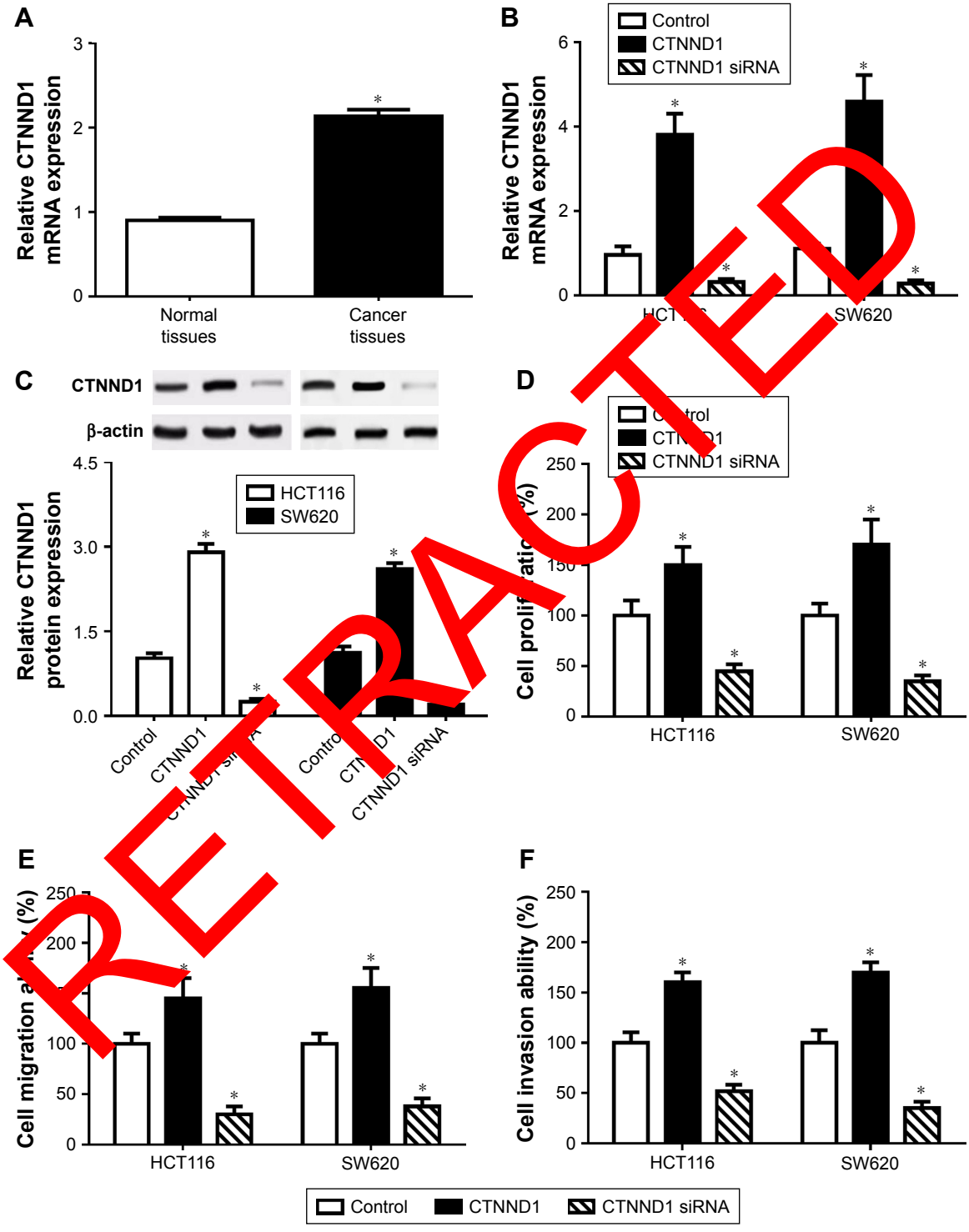

Figure 4 CTNND1 overexpression contributed to CRC cell proliferation, migration and invasion.
Notes: (**A**) RT-qPCR assay was performed to examine CTNND1 mRNA expression in 37 pairs of CRC tissues and adjacent normal tissues. (**B**–**F**) HTC116 and SW620 cells were transfected with CTNND1 overexpression plasmid or siRNA with untransfected cells as negative control (Control), followed by the determination of CTNND1 mRNA (**B**) and protein (**C**) levels, and cell proliferative (**D**), migratory (**E**) and invasive (**F**) abilities at 48 hours upon transfection. *$P<0.05$.
Abbreviation: CRC, colorectal cancer.

levels in HTC116 and SW620 cells. Inversely, CTNND1 mRNA (Figure 4B) and protein (Figure 4C) expression was noticeably reduced in HTC116 and SW620 cells following the introduction of CTNND1 siRNA. In other words, these results showed that CTNND1 overexpression plasmid or siRNA could be used for subsequent functional experiments. As depicted in Figure 4D–F, cell proliferative, migratory and invasive abilities were all enhanced in HTC116 and SW620 cells following the upregulation of CTNND1 level, but was weakened in response to CTNND1 knockdown. That was to say, enforced expression of CTNND1 contributed to CRC cell proliferation, migration and invasion, while CTNND1 knockdown exhibited opposite effects.

CTNND1 upregulation alleviated miR-143-3p-mediated anti-proliferation, anti-migration and anti-invasion effects in CRC cells

Next, Western blot assay manifested that CTNND1 overexpression notably abrogated miR-143-3p-mediated CTNND1 downregulation in HTC116 and SW620 cells (Figure 5A). Next, restoration experiments further demonstrated that upregulation of CTNND1 remarkably weakened the inhibitory effect of miR-143-3p on cell proliferation (Figure 5B), migration (Figure 5C) and invasion (Figure 5D) in HTC116 and SW620 cells.

MiR-143-3p inhibited the growth of HCT116-derived CRC xenograft tumors by targeting CTNND1

The data above prompted us to specul[illegible]t miR-143-3p might exert anti-tumor effect in C[illegible]C in vivo. To further confirm this conjecture, xenogra[illegible] models of [illegible]RC were established by injecting HC[illegible]116 cell[illegible]nto the [illegible]ght flanks of nude mice. Then, the e[illegible]ect of mi[illegible]R-143-[illegible]verexpression on the growth of CRC [illegible]nogr[illegible]t tumors was detected. As displayed in Figu[illegible]6A–C, e[illegible]pic exp[illegible]ssion of miR-143-3p resulted in a r[illegible]ta[illegible] reduction [illegible]mor weight (Figure 6B) and volume (Figure [illegible]) in vivo. Also, forced expression of miR-1[illegible]3p remarkably [illegible]hibited CTNND1 expression in

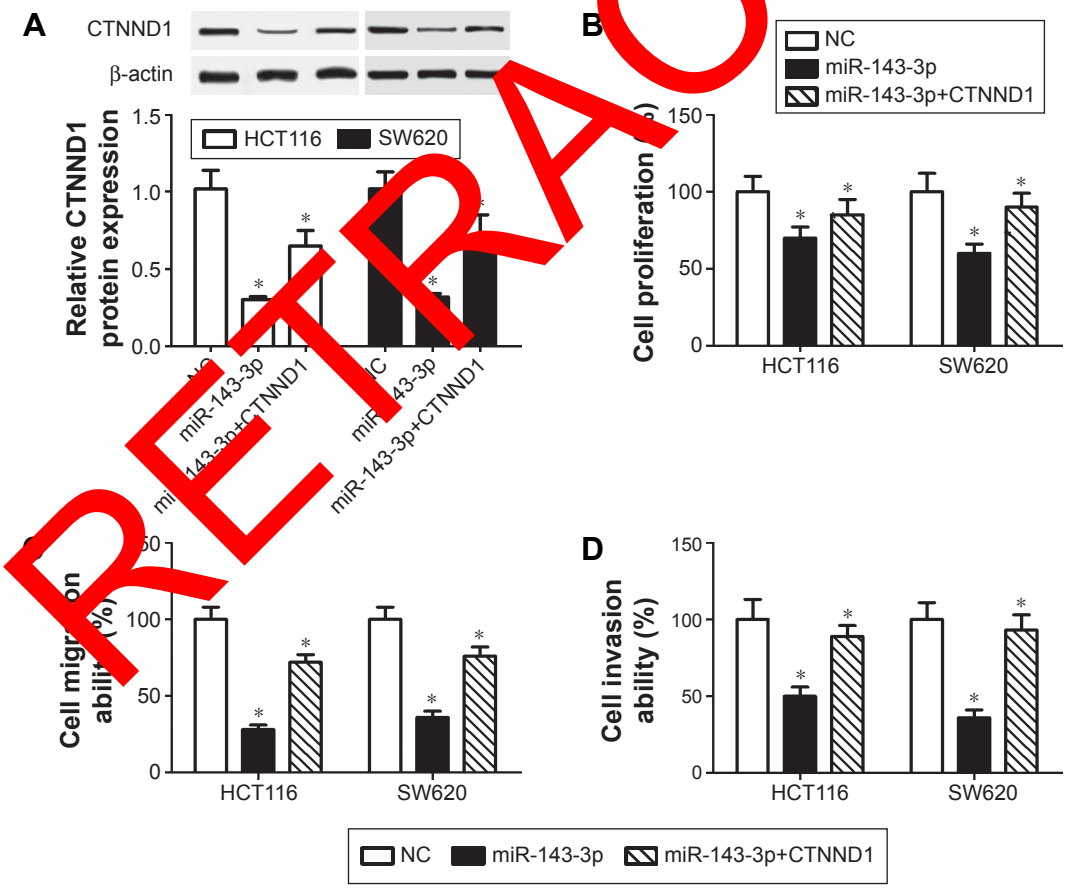

Figure 5 CTNND1 upregulation alleviated miR-143-3p-mediated anti-proliferation, anti-migration and anti-invasion effects in CRC cells.
Notes: (**A**–**D**) HTC116 and SW620 cells were transfected with miR-143-3p alone or along with CTNND1 with untransfected cells as NC. At 48 hours after transfection, CTNND1 protein level (**A**), and cell proliferative (**B**), migratory (**C**) and invasive (**D**) abilities were determined by Western blot, CCK-8, transwell migration and invasion assay, respectively. *$P<0.05$.
Abbreviations: CRC, colorectal cancer; NC, negative control.

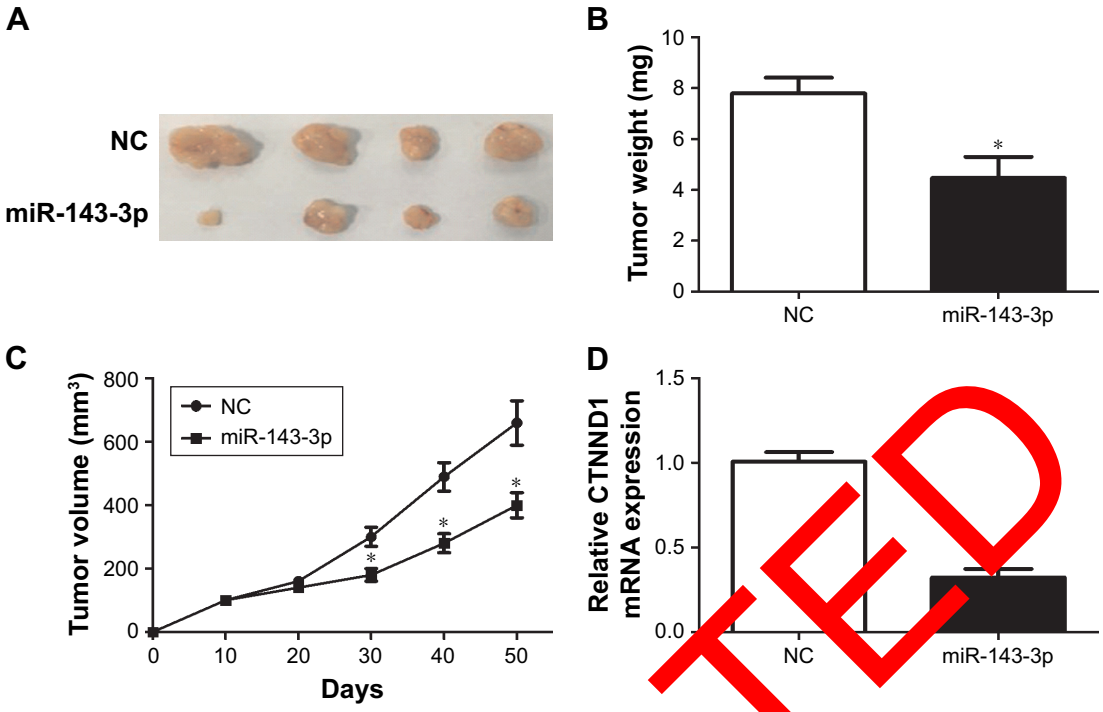

Figure 6 miR-143-3p inhibited the growth of HCT116-derived CRC xenograft tumors by targeting CTNND1.
Notes: HCT116 cells were subcutaneously inoculated into the right flanks of nude mice. After 10 days, miR-143-3p mimic or a scramble control were injected into formed tumors every 5 days for a total of 50 days. (**A**, **B**) At the end of the experiments, the tumors were resected, photographed (**A**) and weighed (**B**). (**C**) Tumor volume was monitored every 10 days for a total of 50 days. (**D**) CTNND1 mRNA level in tumors was determined by RT-qPCR assay at the end of the experiments. *$P<0.05$.
Abbreviations: CRC, colorectal cancer; NC, negative control.

CRC xenograft tumors (Figure 6D). In a word, these data indicated that miR-143-3p curbed CRC xenograft tumor growth through inhibiting CTNND1 expression in vivo.

Discussion

CRC is a major public health problem, bringing about massive economic burden for individuals and society worldwide.[16,17] Recently, accumulating miRNA has been reported to be potential biomarker or therapeutic targets for CRC prediction, diagnosis and treatment by virtue of their specific expression and central roles in the tumorigenesis of CRC.[18,19]

MiR-143 containing miR-143-3p and miR-143-5p has been reported to be downregulated in CRC cell lines and tissues.[20] Moreover, prior studies showed that miR-143 suppressed CRC progression by regulating different targets.[21,23,24] For example, miR-143 inhibited proliferation, migration and invasion through targeting metastasis-associated in colon cancer-1 (MACC1) in CRC cells (SW620 and SW480).[21] However, a study pointed out that miR-143 overexpression inhibited cell invasion, but did not influence cell proliferation in CRC cell lines (HCT116 and SW480).[25] In the present study, we further confirmed that miR-143-3p expression was notably reduced in CRC tissues and cell lines (HCT116 and SW620). Moreover, CRC patients with higher miR-143-3p expression had a better prognosis. Following functional analyses disclosed that miR-143-3p upregulation hampered proliferation, migration and invasion in HCT116 and SW620 cells.

Then, bioinformatical analysis, luciferase reporter assay and Western blot assay further evinced that miR-143-3p directly targeted CTNND1 in CRC cells. Also, CTNND1 level was inversely associated with miR-143-3p level in CRC tissues.

CTNND1, also named as p120-catenin, belongs to a protein subfamily with armadillo repeats (ARMs).[26] Over the past decades, CTNND1 has been identified as a vital regulator of adherens junction maturation and stabilization.[27,28] Moreover, CTNND1 can function as an oncogene or a tumor suppressor in cancers by regulating various signaling pathways such as MAPK, ROCK and Wnt pathway.[28,29] For instance, CTNND1 upregulation promoted proliferation and metastasis of hepatocellular cancer cells partly through activating Wnt/β-catenin signaling in vitro and in vivo.[30] However, Schackmann et al[31] pointed out that CTNND1 deletion facilitated metastasis of breast cancer by activating growth factor receptor signaling and inducing anoikis resistance. Moreover, an earlier study pointed out that the downregulation of CTNND1 in CRC was linked with higher pathogenic status and unfavorable clinical outcome.[32] Additionally, Greco et al[33] pointed out that the knockdown of CTNND1

resulted in a Co-029-dependent cell motility acceleration in colon cancer, hinting the anti-tumor effect of CTNND1 in colon cancer. However, Yamada et al[14] pointed out that CTNND1 knockdown inhibited proliferation, migration and invasion by disturbing nuclear translocation of β-catenin in DLD-1 and WiDr cells, while DLD-1 and WiDr cells are APC mutant CRC cell lines.[34] Previous studies also showed that β-catenin was highly expressed in the cytoplasm and nucleus of HCT116 and DLD1 cells.[35] Also, SW620 is a APC mutant CRC cell line, while the inactivation of APC can result in the constitutive activation of Wnt/β-catenin pathway.[36–38] Hence, we supposed that miR-143-3p/CTNND1 might exert their function partly through regulating Wnt pathway in HCT116 and SW620 cells. However, the effect of miR-143-3p and CTNND1 on CRC cells that lack Wnt signaling needs to be further investigated. MiR-143-3p and CTNND1 might regulate these CRC cell developments by other signaling pathways.

Our study further showed that CTNND1 expression was notably increased in CRC tissues. Additionally, enforced expression of CTNND1 facilitated cell proliferation, migration and invasion in CRC. Conversely, the knockdown of CTNND1 resulted in the reduction of cell proliferative, migratory and invasive capacities in CRC.

Restoration experiments also manifested that CTNND1 upregulation reversed miR-143-3p-mediated anti-proliferation, anti-migration and anti-invasion effects in CRC cells. Also, mice experiments further demonstrated that miR-143-3p hampered CRC xenograft tumor growth by reducing CTNND1 expression in vivo.

Conclusion

In conclusion, our study manifested that miR-143-3p hindered the development of CRC via targeting CTNND1 in vitro and in vivo, providing a compelling argument for the significance of miR-143-3p and CTNND1 in the tumorigenesis of CRC, highlighting the potential values of miR-143-3p and CTNND1 in prediction, diagnosis, and treatment of CRC.

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Author contributions

Xiaohua Ding, Jiying Du, Kai Mao and Xiaoyu Wang performed the experiments. Xiaoyu Wang and Yuange Ding conceived and designed this work. Fanping Wang and Xiaohua Ding prepared the manuscript. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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