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Circular RNA circ_0000043 promotes endometrial carcinoma progression by regulating miR-1271-5p/CTNND1 axis

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Abstract

Background Circular RNAs (circRNAs) are involved in a variety of biological processes, including tumorigenesis. However, the exact role and molecular mechanisms of circ_0000043 in endometrial carcinoma (EC) remain largely unknown.

Methods Quantitative real-time polymerase chain reaction (qRT-PCR) was carried out to determine the expression levels of circ_0000043, microRNA-1271-5p (miR-1271-5p) and catenin delta 1 (CTNND1). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and flow cytometry were used to measure cell proliferation, cell apoptosis and cell cycle distribution, respectively. Cell migration and invasion were assessed by transwell assay. Western blot assay was performed to examine the protein expression of matrix metalloproteinase 2 (MMP2), MMP9 and CTNND1. The interaction between miR-1271-5p and circ_0000043 or CTNND1 was predicted by starBase and confirmed by dual-luciferase reporter assay. The mice xenograft model was established to investigate the role of circ_0000043 in vivo.

Results Circ_0000043 and CTNND1 were highly expressed and miR-1271-5p was lowly expressed in EC tissues and cells. Knockdown of circ_0000043 inhibited the progression of EC by inhibiting cell proliferation, migration, invasion and tumor growth (in vivo) and promoting apoptosis. MiR-1271-5p was a direct target of circ_0000043 and its inhibition reversed the inhibitory effect of circ_0000043 knockdown on the progression of EC cells. In addition, CTNND1 was a downstream target of miR-1271-5p, and miR-1271-5p overexpression inhibited EC cell proliferation, migration and invasion and induced apoptosis by targeting CTNND1. Moreover, circ_0000043 positively regulated CTNND1 expression by sponging miR-1271-5p.

Conclusion Circ_0000043 knockdown inhibited the progression of EC by regulating miR-1271-5p/CTNND1 axis, which might provide a promising circRNA-targeted therapy for EC.

Keywords Endometrial carcinoma · Circ_0,000,043 · miR-1271-5p · CTNND1

Introduction

Endometrial carcinoma (EC) is one of the most common cancers occurring in the female genital system [1]. Although various treatment strategies have markedly improved the survival rate of patients with advanced EC, including surgery, chemotherapy, radiotherapy, and hormone therapy, the

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clinical outcome remains poor and the expected survival of recurrent EC is only 12–15 months [2–4]. Hence, understanding the molecular mechanism that regulates the progression and development of EC is critical.

Circular RNAs (circRNAs), a new group of endogenous non-coding RNAs (ncRNAs), are widely found in eukaryotic cell transcripts [5]. CircRNAs form special closed-loop structures with neither 5′ caps nor 3′ poly (A) tails and play key roles at the post-transcriptional level [6]. CircRNAs are more stable than linear RNAs and participate in the initiation and progression of many cancers, including EC [7, 8]. For example, hsa_circ_0061140 promoted the progression of EC by modulating miR-149-5p/STAT3 axis [9]. Moreover, circTNFRSF21 promoted EC progression by regulating miR-1227-MAPK13/ATF2 axis [10]. As for circRNA circ_0000043 (also known as circPUM1, chr1:31,465,236–31,468,067), it has been demonstrated to be highly expressed in EC [11]. Nevertheless, the biological role and regulatory mechanisms of circ_0000043 in EC still need to be further explored.

Large evidence has shown that circRNAs act as competitive endogenous RNA (ceRNAs) by sponging microRNAs (miRNAs) and further regulate the expression of mRNAs and the progression and development of diseases [12]. MiRNAs are a group of short ncRNAs that exert biological functions via binding to 3′untranslated regions (3′UTR) target mRNAs [13]. MiR-1271-5p was considered to be an anti-oncogene in many tumors, such as ovarian cancer [14], hepatocellular carcinoma [15], and multiple myeloma [16]. Besides, previous research indicated that miR-1271 also played an anti-cancer role in EC [17]. However, the interaction between circ_0000043 and miR-1271-5p in EC has not been reported. MiRNA regulation of catenin delta 1 (CTNND1; also known as p120-catenin) has been reported in many cancers, including EC [18, 19]. However, it is unclear whether miR-1271-5p can regulate CTNND1 in EC.

In our research, the abundance of circ_0000043, miR-1271-5p and CTNND1 in EC tissues and cells was examined. Moreover, we investigated their roles in the progression of EC by using gain- and loss-of-function experiments. Furthermore, the potential regulatory network of circ_0000043/miR-1271-5p/CTNND1 axis was also explored in EC cells. We aimed to provide a novel insight into the diagnosis and treatment of EC.

Materials and methods

Tissue samples

A total of 20 paired EC tissues and adjacent normal tissues were provided by the patients who underwent surgical resection at Penglai people's Hospital. These patients did not receive any therapy before recruitment to this research. The samples were snap-frozen in liquid nitrogen and kept in − 80 ℃ until further analysis. All enrolled patients had signed the informed consents. This research was authorized by the ethics committee of Penglai people's Hospital.

Cell culture

Two EC cell lines (HEC-1A and HHUA) and human endometrial stromal cell line (T-HESC) were bought from BeNa Culture Collection (Beijing, China). All cells were grown in RPMI-1640 medium (DMEM; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen) in a humidified air with 5% CO_2 at 37 ℃.

Cell transfection

For transfection, the small interfering RNAs (siRNA) against circ_0000043 (si-circ_0000043), mimic of miR-1271-5p (miR-1271-5p), inhibitor of miR-1271-5p (anti-miR-1271-5p), CTNND1 overexpression vector (CTNND1), and their matched controls (si-NC, miR-NC, anti-miR-NC, and vector) were obtained from Genecreat (Wuhan, China). Lentivirus-mediated short hairpin RNA (shRNA) against circ_0000043 (sh-circ_0000043) and corresponding control (sh-NC) were provided by RiboBio (Guangzhou, China). According to the recommendations, HEC-1A and HHUA cells were transfected using the Lipofectamine 3000 reagent (Invitrogen).

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

Total RNA from tissues (EC and normal) and cells (T-HESC, HEC-1A and HHUA) was extracted by TRIzol reagent (Invitrogen). The first-strand complementary DNA (cDNA) was synthesized using the Goldenstar RT6 cDNA Synthesis Kit (TSINGKE, Beijing, China) or TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Next, qRT-PCR reactions were performed in a 7500 Real-Time PCR System (Thermo Fisher Scientific) with SYBR Green PCR Master Mix (Thermo Fisher Scientific). The $2^{-\Delta\Delta Ct}$ method was employed to determine gene expression. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 acted as the internal references. The sequences of primers used in this research were listed as followed: circ_0000043 (sense 5′-3′: AGGAGGGTGATGTGATGGAC; antisense 5′-3′: TGACGTCTGCATCAATCCCA), PUM1 (sense 5′-3′: AGTGGGGGACTAGGCGTTAG; antisense 5′-3′: GTTTTCATCACTGTCTGCATCC), miR-1271-5p (sense 5′-3′: CAGCACTTGGCACCTAGCA; antisense 5′-3′: TATGGTTGTTCTCCTCTCTGTCTC), CTNND1 (sense 5′-3′: TCCAGCAAACGA

TACAGTGG; antisense 5′-3′: GAACCACCTCTGGCTGAAAT), GAPDH (sense 5′-3′: TGGGGAAGGTGAAGGTCGG; antisense 5′-3′: CTGGAAGATGGTGATGGGA), U6 (sense 5′-3′: GTGCGTGTCGTGGAGTCG; antisense 5′-3′: AACGCTTCACGAATTTGCGT).

RNase R treatment

RNase R (Epicentre Technologies, Madison, WI, USA) treatment was employed for degradation linear RNA. In short, 2 μg of total RNA was incubated for 0.5 h at 37 ℃ with RNase R (3 U/μg). After that, these cells were harvested, and the abundance of circ_0000043 and PUM1 was tested using qRT-PCR.

Subcellular fractionation location

Referring to manufacturer's instructions, Nuclear/Cytosol Fractionation Kit (Biovision, San Francisco Bay, CA, USA) was utilized to isolate the RNA from cytosolic and nuclear fractions. After that, qRT-PCR analysis was conducted to analyze the extracted RNAs from nuclear and cytoplasm. U6 or GAPDH served as a control for nuclear control transcript or cytoplasmic control transcript, respectively.

Cell proliferation assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used for measuring cell proliferation. In brief, HEC-1A and HHUA cells were cultured in 96-well plates (100 μL) for 12 h and then transfected with si-circ_0000043, si-circ_0000043 + anti-miR-1271-5p, miR-1271-5p, miR-1271-5p + CTNND1, or their matched controls. At different points after transfection, 20 μL of MTT (Beyotime, Jiangsu, China) solution was placed in the wells and incubated for 3–4 h. Next, the cultured medium was removed, followed by addition of methyl sulfoxide solution (DMSO; 150 μL). Last, a microplate reader (Bio-Rad, Hercules, CA, USA) was employed to examine the optical density (OD) value at 490 nm.

Flow cytometry

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kit (Sangon Biotech, Shanghai, China) was utilized to detect apoptotic cells. At 48 h after transfection, HEC-1A and HHUA cells were harvested and resuspended in 300 μL of binding buffer, followed by staining with Annexin V-FITC (10μL) and PI (5 μL) in the darkness for 15 min. At last, flow cytometry (Partec AG, Arlesheim, Switzerland) was applied to analyze cell apoptosis. For cell cycle determination, HEC-1A and HHUA cells would be collected by centrifugation and fixed with ice-cold 70% ethanol at − 20 ℃ for 12 h. Next, the cells were collected and stained with PI (25 μg/mL, Sangon Biotech) and and 100 μg/mL RNase A (Sangon Biotech) in phosphate-buffered saline (PBS; Beyotime) for 15 min. After that, flow cytometer was applied for detecting cell cycle distribution.

Transwell assay

The transfected cells were re-seeded in top chambers (Costar, Corning, NY, USA) with non-coated membrane (for migration assay) and with Matrigel-coated membrane (BD Biosciences, San Jose, CA, USA; for invasion assay). At the same time, the bottom chambers were filled with complete medium to promote chemotaxis. Cells were incubated for 24 h at 37 ℃. Next, cells in the top chamber were removed with a cotton swab. After that, cells that migrated and invaded into the lower chamber were stained with 0.1% crystal violet (Beyotime) after fixing for 30 min in 4% paraformaldehyde (Sangon Biotech). At last, these cells were imaged and counted under a microscope (100 × magnification).

Western blot assay

Total proteins from tissues (EC and normal) and cells (T-HESC, HEC-1A and HHUA) were extracted using RIPA lysis buffer (Sangon Biotech). The protein concentration of the total cellular lysates was quantified using a BCA protein assay kit (Solarbio, Beijing, China). Next, extracted protein samples (40 μg/lane) were loaded on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE; Beyotime). Gels were electro blotted onto polyvinylidene fluoride membranes (Beyotime). Membranes were blocked with 5% nonfat milk (Sangon Biotech) for 1–2 h and then incubated with specific primary antibodies against matrix metalloproteinase 2 (MMP2; ab2462, 1:1000, Abcam, Cambridge, UK), MMP9 (ab73734, 1:1000, Abcam), CTNND1 (ab72039, 1:2000, Abcam), and GAPDH (ab37168, 1:1000, Abcam) for 12 h at 4 ℃. Afterward, these membranes were incubated using secondary antibody (ab205718, 1:5000, Abcam) and were visualized by enhanced chemiluminescence reagent (Tanon, Shanghai, China). ImageJ software was applied to assess the bands density.

Dual-luciferase reporter assay

The potential binding sites for miR-1271-5p in circ_0000043 and CTNND1 were predicted by starBase. The circ_0000043 and 3′UTR of CTNND1 sequence that contained wild-type or mutant miR-1271-5p binding sites was synthesized and individually inserted into pmirGLO luciferase reporter vector (Promega, Madison, WI, USA), named as wild-type vectors (circ_0000043 WT and CTNND1 3′UTR WT) or

mutant vectors (circ_0000043 MUT and CTNND1 3′UTR MUT). HEC-1A and HHUA cells were co-transfected with one of luciferase reporter vectors with miR-1271-5p or miR-NC for 48 h. At last, Dual-Luciferase Reporter Assay System (Promega) was used for measuring luciferase activity.

Tumor formation assay in vivo

Female BALB/c nude mice (5–6 weeks old, Huafukang, Beijing, China) were used for the in vivo tumor formation assay. HEC-1A cells transiently transfected with sh-NC (as control) or sh-circ_0000043 were injected into the right flank of nude mice (6 mice in each group). Tumor volumes were monitored and recorded every week and calculated following the equation: volume = length × width^2/2. After 5 weeks, the mice were killed; tumor specimens were weighed and collected to analyze the expression of circ_0000043, miR-1271-5p, and CTNND1. The in vivo experiments were in agreement with the Animal Care and Use Committee of Penglai people's Hospital.

Statistical analysis

All results were from three independent experiments, analyzed by Graphpad Prism version 6.0 software and displayed as the mean ± standard deviation (SD). Differences were analyzed via Student's *t* test (for 2 groups) or a one-way analysis of variance (ANOVA; for more than two groups). For all comparisons, P value < 0.05 was considered to be statistically significant.

Results

Circ_0000043 was highly expressed in EC tissues and cells

We determined the expression of circ_0000043 in EC tissues and cells by qRT-PCR. As presented in Fig. 1a, circ_0000043 expression was significantly higher in EC tissues than in normal tissues. Similarly, the expression of circ_0000043 was upregulated in EC cells (HEC-1A and HHUA) compared to that in T-HESC cells (Fig. 1b). In general, RNase R can digest linear RNA but not circRNA. As displayed in Fig. 1c, d, linear PUM1 expression was greatly reduced after digestion by RNase R, but circ_0000043 expression was not affected, suggesting the cyclic structure of circ_0000043. Next, we examined the localization of circ_0000043 in HEC-1A and HHUA cells. We found that circ_0000043 was mainly located in cytoplasm (Fig. 1e, f). These results indicated that circ_0000043 might play an important role in EC.

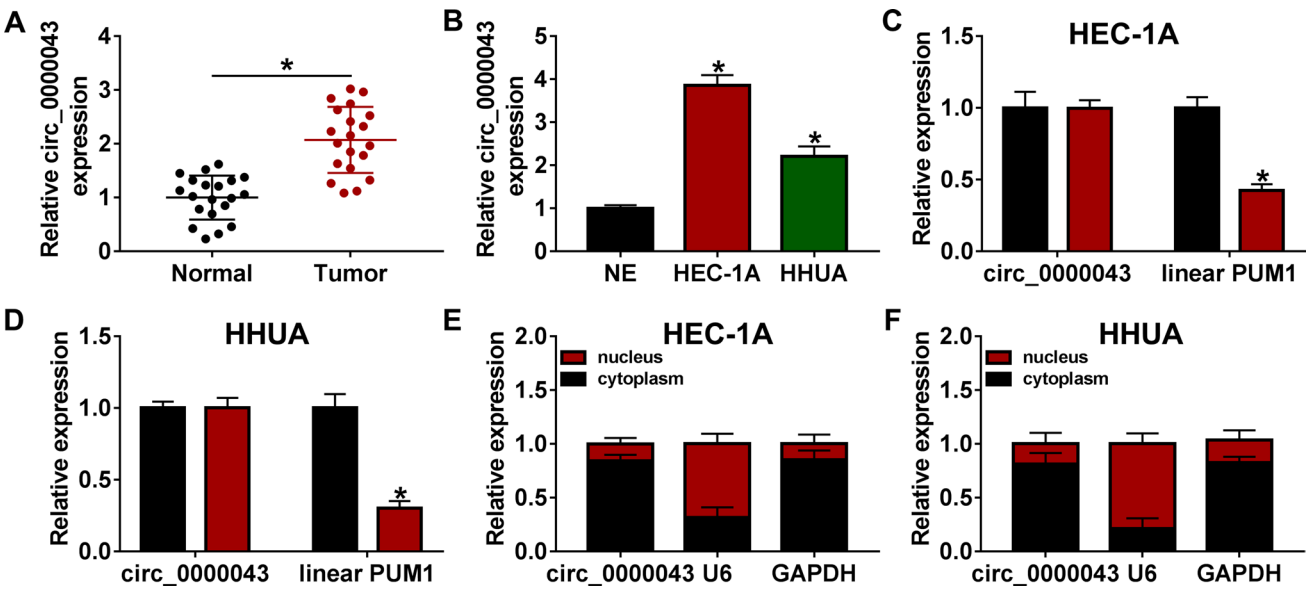

Fig. 1 Circ_0000043 was overexpressed in EC tissues and cells. **a** The expression of circ_0000043 was detected by qRT-PCR in EC tissues and normal tissues. **b** The level of circ_0000043 was examined by qRT-PCR in EC cell lines (HEC-1A and HHUA) and human endometrial stromal cell line (T-HESC). **c** and **d** The expression levels of circ_0000043 and linear PUM1 were determined after treatment of RNase R by qRT-PCR in HEC-1A and HHUA cells. **e** and **f** The subcellular location of circ_0000043 in HEC-1A and HHUA cells was determined by qRT-PCR analysis. *P < 0.05

Knockdown of circ_0000043 inhibited proliferation, migration, and invasion and promoted apoptosis in EC cells

To explore the biological roles of circ_0000043 in EC cells, circ_0000043 was knocked down in HEC-1A and HHUA cells by siRNA transfection. Results indicated that circ_0000043 was successfully knocked down in HEC-1A and HHUA cells (Fig. 2a, b). MTT and apoptosis assays showed that knockdown of circ_0000043 decreased cell proliferation and increased apoptosis in HEC-1A and HHUA cells (Fig. 2c–f). Cell cycle progression was assessed by flow cytometry. As illustrated in Fig. 2g, h, circ_0000043 silence increased the percentage of G0/G1 phase cells and decreased the percentage of S phase cells in HEC-1A and HHUA cells, indicating that the cell cycle was arrested at the G0/G1 phase. Transwell assay demonstrated that circ_0000043 interference inhibited HEC-1A and HHUA cell migration and invasion (Fig. 2i, j). Western blot assay was used to detect the metastasis-related proteins (MMP2 and MMP9). The data showed that MMP2 and MMP9 expression were both decreased in HEC-1A and HHUA cells transfected with si-circ_0000043 (Fig. 2k, l), suggesting interference of circ_0000043 inhibited EC cell metastasis. Our results indicated that circ_0000043 downregulation inhibited the progression of EC cells.

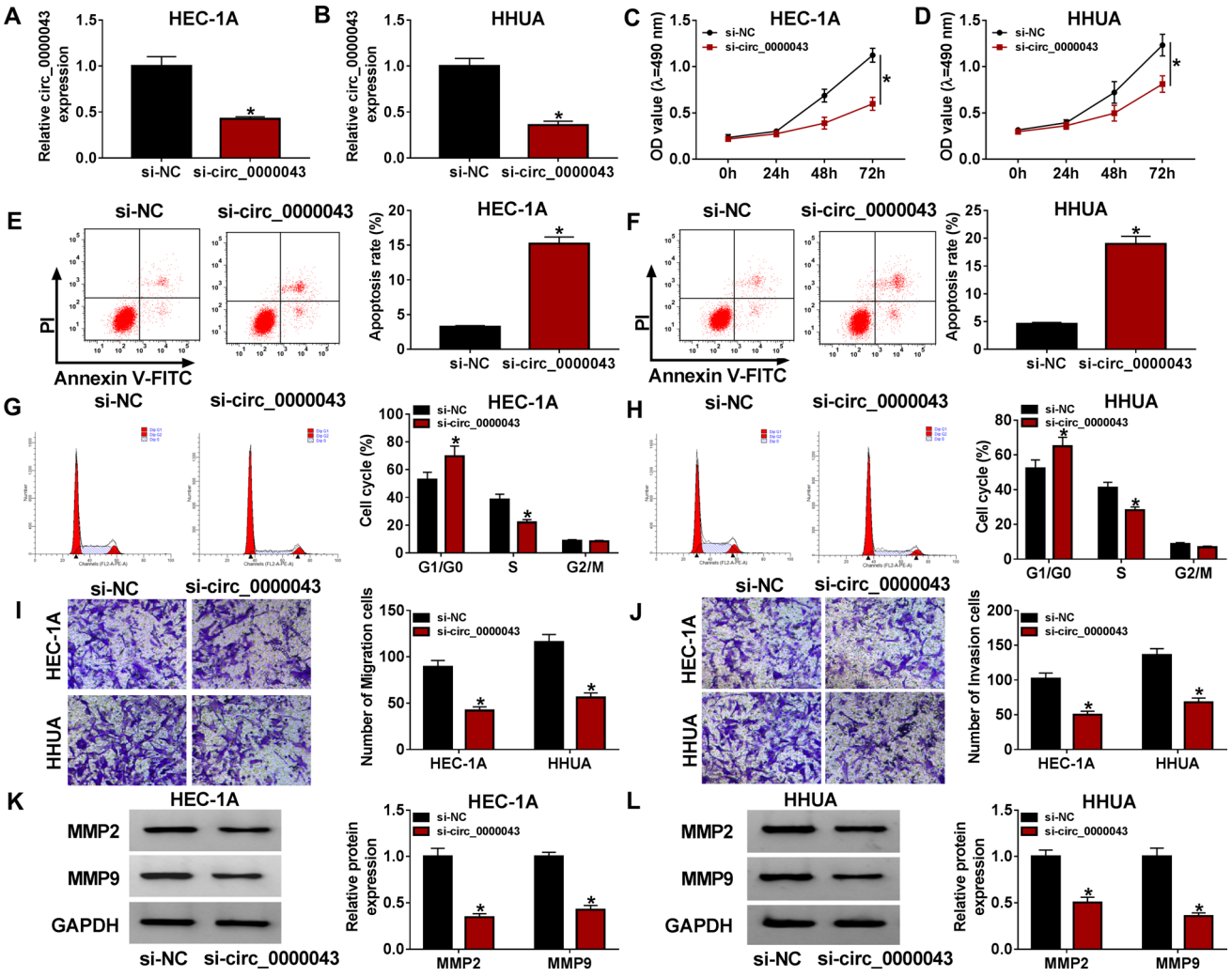

Fig. 2 Circ_0000043 downregulation suppressed the progression of EC cells. HEC-1A and HHUA cells were transfected with si-NC or si-circ_0000043. **a** and **b** Knockdown efficiency of circ_0000043 was determined by qRT-PCR. **c** and **d** MTT assay was conducted to evaluate cell proliferation. **e**–**h** Flow cytometry analysis was used to determine apoptosis rate and cell cycle distribution. **i** and **j** Transwell assay was employed to count the number of migration and invasion cells (100×). **k** and **l** The protein levels of MMP2 and MMP9 were measured by western blot assay. $^{*}P < 0.05$

Circ_0000043 functioned as a sponge of miR-1271-5p

Presently, circRNAs were widely considered as miRNA sponges to participate in the occurrence and development of cancers [20]. To investigate the underlying molecular mechanisms of circ_0000043 in the progression of EC, we screened the target miRNAs of circ_0000043 by starBase. We found that there were binding sites between circ_0000043 and miR-1271-5p (Fig. 3a). Next, dual-luciferase reporter assay was conducted to verify the targeting relationship between circ_0000043 and miR-1271-5p. The data indicated that luciferase activity of circ_0000043 WT was obviously decreased after transfection with miR-1271-5p in HEC-1A and HHUA cells (Fig. 3b, c). However, the luciferase activity of circ_0000043 MUT was unaffected after transfection with miR-1271-5p (Fig. 3b, c). Subsequently, we explored the effect of circ_0000043 on miR-1271-5p expression. As shown in Fig. 3d, e, knockdown of circ_0000043 promoted the expression of miR-1271-5p in HEC-1A and HHUA cells. Moreover, miR-1271-5p expression was downregulated in EC tissues and cells relative to normal tissues and cells (Fig. 3f, g). All these data indicated miR-1271-5p was a target of circ_0000043 in EC cells.

Circ_0000043 knockdown inhibited the progression of EC cells by upregulating miR-1271-5p

To explore whether circ_0000043 exerted its biological functions by sponging miR-1271-5p, HEC-1A and HHUA cells were transfected with si-NC, si-circ_0000043, si-circ_0000043 + anti-miR-NC, or si-circ_0000043 + anti-miR-1271-5p. The results of qRT-PCR indicated that circ_0000043 silence increased the expression of miR-1271-5p, which was reversed by downregulating miR-1271-5p in HEC-1A and HHUA cells (Fig. 3a, b). Moreover, inhibition of miR-1271-5p abated the anti-proliferation and pro-apoptosis effects caused by si-circ_0000043 in HEC-1A and HHUA cells (Fig. 3c–f). Furthermore, downregulation of miR-1271-5p abolished circ_0000043 silence-mediated promotion of G0/G1 phase cells and reduction of S phase cells (Fig. 4g–h). In addition, the inhibitory effects of circ_0000043 interference on migration and invasion were reversed by knockdown of miR-1271-5p (Fig. 4i–l). Besides, decreasing MMP2 and MMP9 expression caused

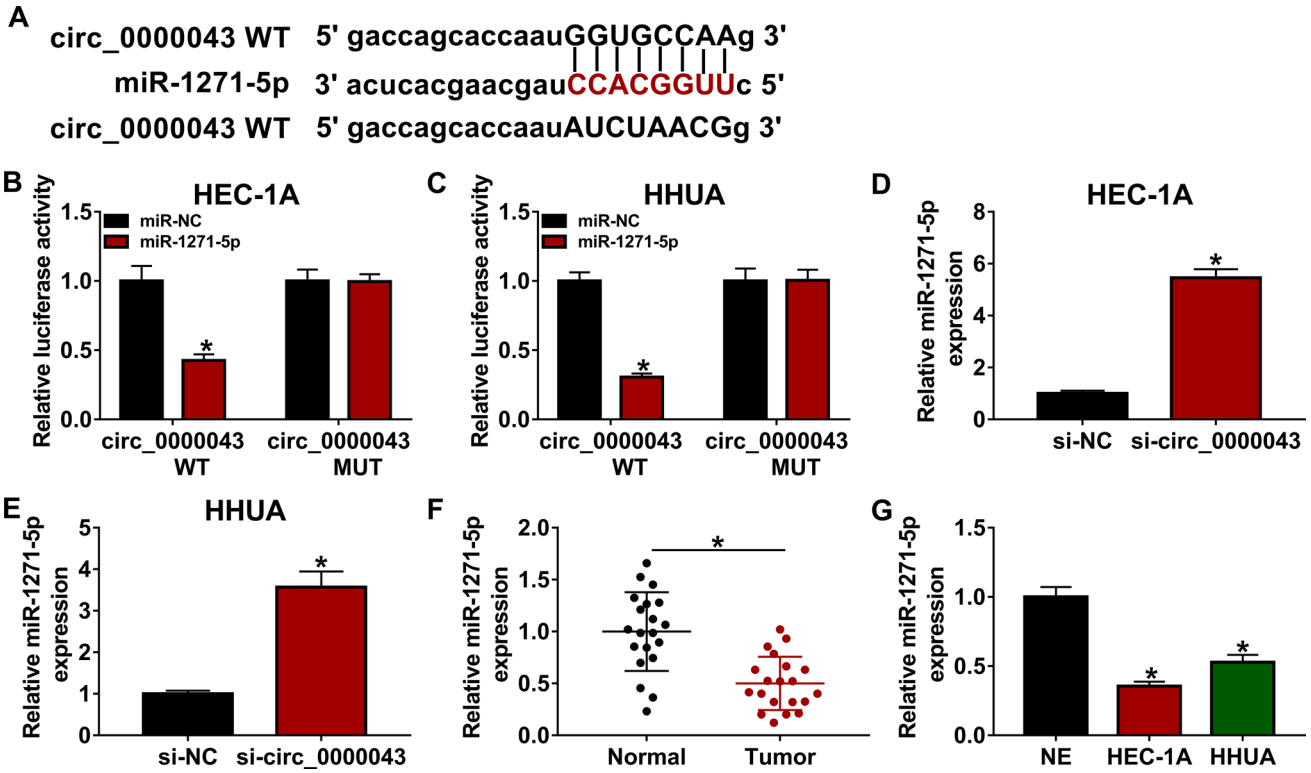

Fig. 3 Circ_0000043 directly interacted with miR-1271-5p in EC cells. **a** The complementary binding sequence of miR-1271-5p and circ_0000043 was predicted by starBase. **b** and **c** Dual-luciferase reporter assay was conducted to measure the luciferase activity in HEC-1A and HHUA cells co-transfected with circ_0000043 WT or circ_0000043 MUT and miR-1271-5p or miR-NC. **d** and **e** The abundance of miR-1271-5p was determined by qRT-PCR in HEC-1A and HHUA cells transfected with si-NC or si-circ_0000043. **f** and **g** MiR-1271-5p expression was examined by qRT-PCR in EC tissues, normal tissues, EC cells (HEC-1A and HHUA) and T-HESC cells. $^*P < 0.05$

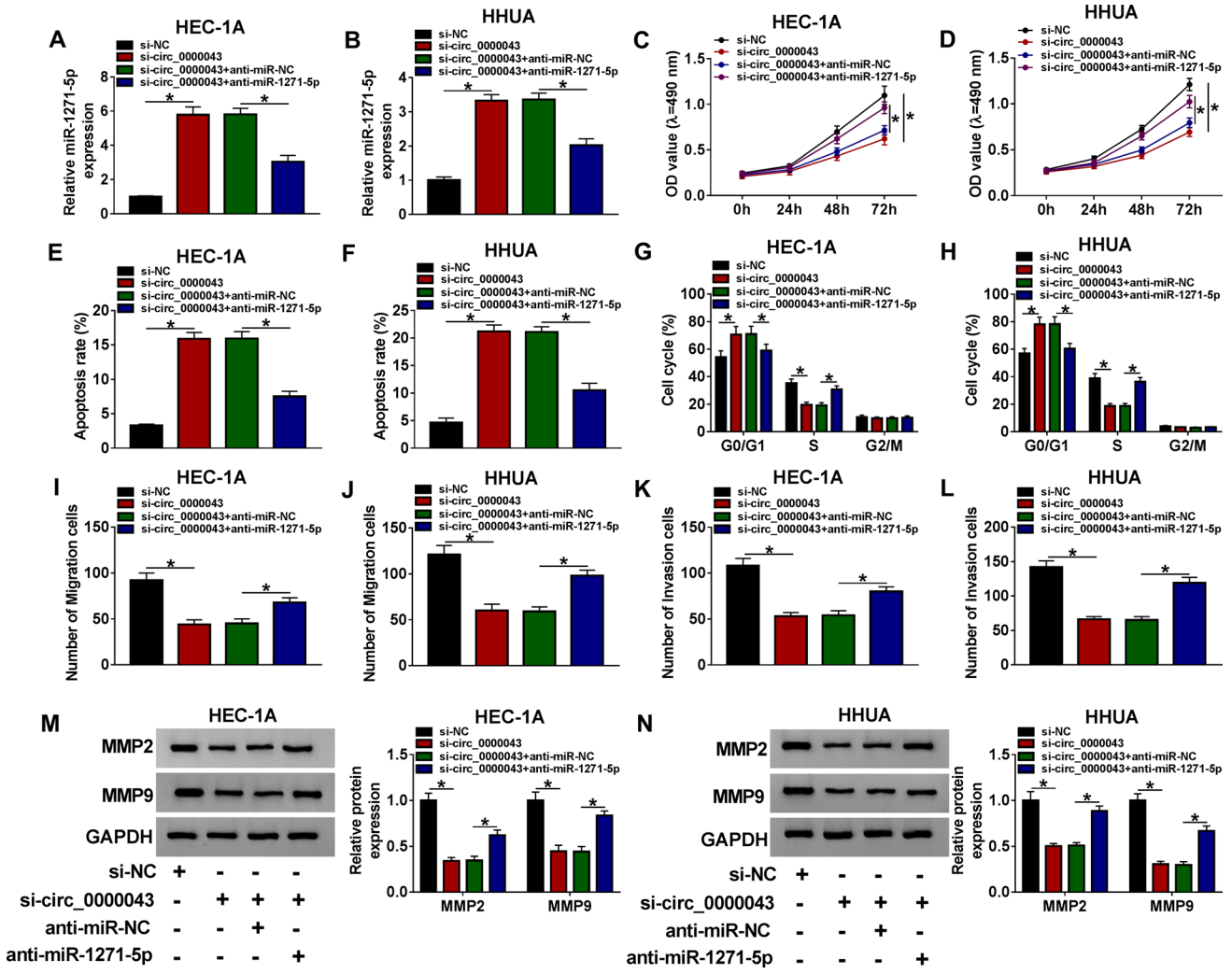

Fig. 4 Silence of circ_0000043 inhibited the progression of EC cells by upregulating miR-1271-5p. HEC-1A and HHUA cells were transfected with si-NC, si-circ_0000043, si-circ_0000043 + anti-miR-NC, or si-circ_0000043 + anti-miR-1271-5p. **a** and **b** The level of miR-1271-5p was tested by qRT-PCR. **c** and **d** Cell proliferation was assessed by MTT assay. **e**–**h** Cell apoptosis rate and cell cycle distribution were determined by flow cytometry analysis. **i**-**l** Cell migration and invasion were measured by transwell assay (100×). **m** and **n** Western blot assay was performed to analyze the protein expression of MMP2 and MMP9. *$P < 0.05$

by circ_0000043 silence were restored by downregulating miR-1271-5p (Fig. 4m, n). Collectively, these data indicated that knockdown of circ_0000043 suppressed proliferation, migration and invasion and promoted apoptosis in EC cells by sponging miR-1271-5p.

CTNND1 was a direct target of miR-1271-5p

Previous reports demonstrate that miRNAs exert their roles via binding to 3′UTR of target mRNAs [21]. Thus, we screened the potential target of miR-1271-5p by using starBase. We found that the 3′UTR of CTNND1 mRNA have potential binding sites for miR-1271-5p (Fig. 5a). Dual-luciferase reporter assay showed that miR-1271-5p overexpression markedly suppressed the luciferase activity of CTNND1 3′UTR WT, but not CTNND1 3′UTR MUT (Fig. 5b, c). In addition, we explored the effect of miR-1271-5p on CTNND1 expression. The results of qRT-PCR and western blot indicated that enforced expression of miR-1271-5p repressed the mRNA and protein expression of CTNND1 in HEC-1A and HHUA cells (Fig. 5d, e). Next, the expression of CTNND1 in EC tissues and cells was investigated. Results showed that CTNND1 mRNA and protein levels were elevated in EC tissues and cells compared with normal tissues and cells (Fig. 5f–i). These data suggested that miR-1271-5p targeted CTNND1 in EC cells.

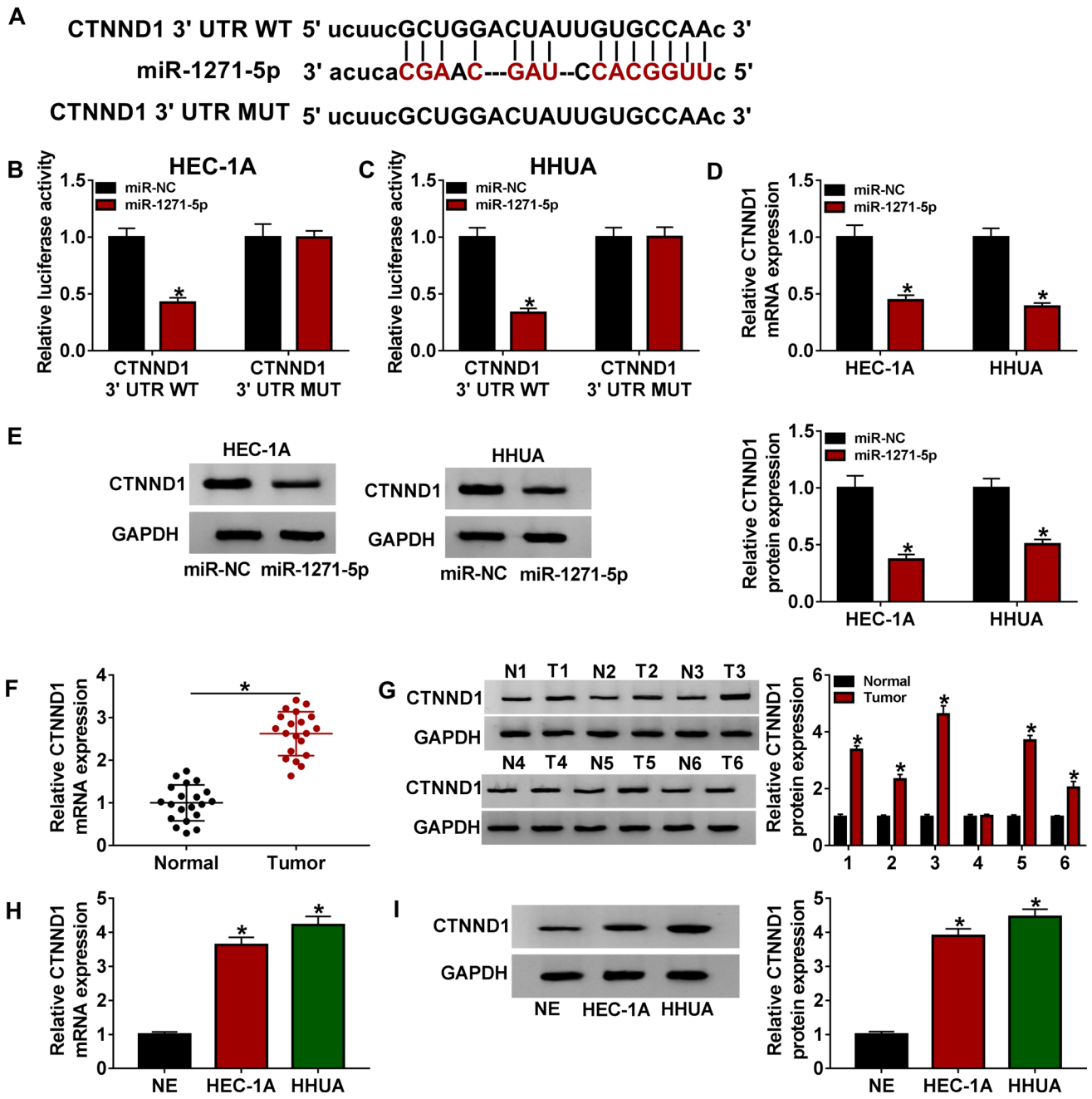

Fig. 5 MiR-1271-5p directly targeted CTNND1 in EC cells. **a** The putative binding sites between miR-1271-5p and CTNND1 3′UTR were predicted by starBase. **b** and **c** Relative luciferase activity was determined in HEC-1A and HHUA cells co-transfected with CTNND1 3′UTR WT or CTNND1 3′UTR MUT and miR-NC or miR-1271-5p. **d** and **e** QRT-PCR and western blot were used to analyze the mRNA and protein levels of CTNND1 in HEC-1A and HHUA cells transfected with miR-NC or miR-1271-5p. **f**–**i** CTNND1 mRNA and protein expression were determined by qRT-PCR and western blot analyses in EC tissues, normal tissues, EC cells (HEC-1A and HHUA) and T-HESC cells. *$P < 0.05$

Overexpression of miR-1271-5p regulated proliferation, migration, invasion, and apoptosis in EC cells by targeting CTNND1

To investigate whether CTNND1 was involved in miR-1271-5p-mediated functions, HEC-1A and HHUA cells were transfected with miR-NC, miR-1271-5p, miR-1271-5p + vector, or miR-1271-5p + CTNND1. Western blot assay indicated that overexpression of miR-1271-5p limited the protein expression of CTNND1, which was rescued by upregulating CTNND1 (Fig. 6a, b). Moreover, forced expression of miR-1271-5p suppressed cell

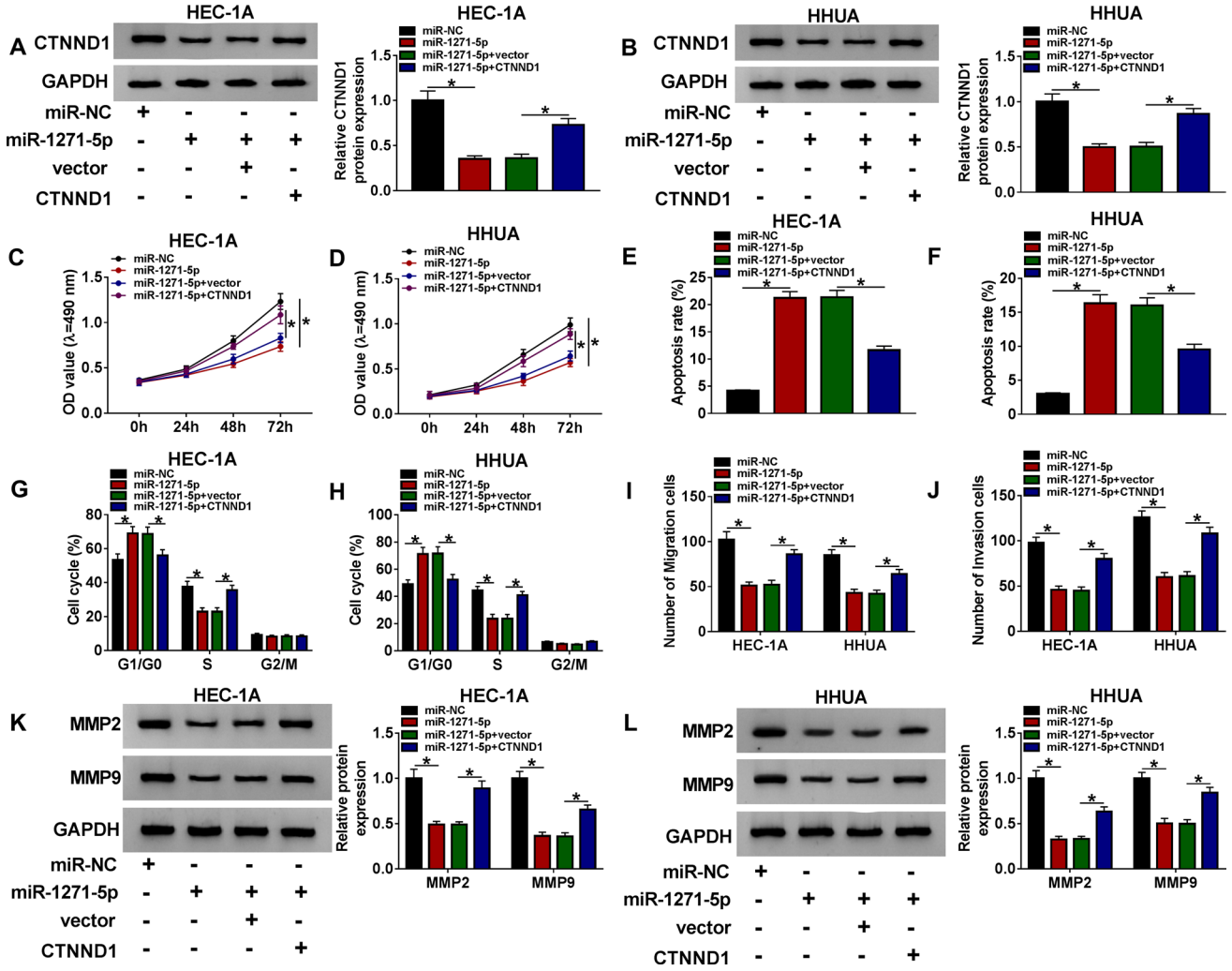

Fig. 6 Overexpression of miR-1271-5p inhibited the progression of EC cells by downregulating CTNND1. HEC-1A and HHUA cells were transfected with miR-NC, miR-1271-5p, miR-1271-5p + vector, or miR-1271-5p + CTNND1. **a** and **b** The protein expression of CTNND1 was examined by western blot assay. **c** and **d** Cell proliferation was evaluated by MTT assay. **e**–**h** Flow cytometry analysis was utilized to analyze cell apoptosis and cell cycle distribution. **i** and **j** Transwell assay was used to assess cell migration and invasion (100×). **k** and **l** The protein levels of MMP2 and MMP9 were determined by western blot analysis. *$P < 0.05$

proliferation and induced apoptosis in HEC-1A and HHUA cells, while these effects were reversed by overexpression of CTNND1 (Fig. 6c–f). In addition, miR-1271-5p upregulation arrested HEC-1A and HHUA cells in G0/G1 phase as well as suppressed cell migration and invasion, which was abated by addition of CTNND1 (Fig. 6g–J). Furthermore, overexpression of miR-1271-5p inhibited the protein levels of MMP2 and MMP9, whereas this effect was abolished by transfection with CTNND1 (Fig. 6k, l). Taken together, these findings demonstrated that miR-1271-5p exerted its anti-cancer role in EC cells by downregulating CTNND1.

Circ_0000043 regulated CTNND1 expression by sponging miR-1271-5p in EC cells

Next, we confirmed whether circ_0000043 regulated CTNND1 expression by acting as a molecular sponge of miR-1271-5p. Results of qRT-PCR and western blot showed that interference of circ_0000043 inhibited the mRNA and protein expression of CTNND1 in HEC-1A and HHUA cells, which was restored by silence of miR-1271-5p (Fig. 7a–d). All these results demonstrated that circ_0000043 acted as a molecular sponge of miR-1271-5p to modulate the expression of CTNND1.

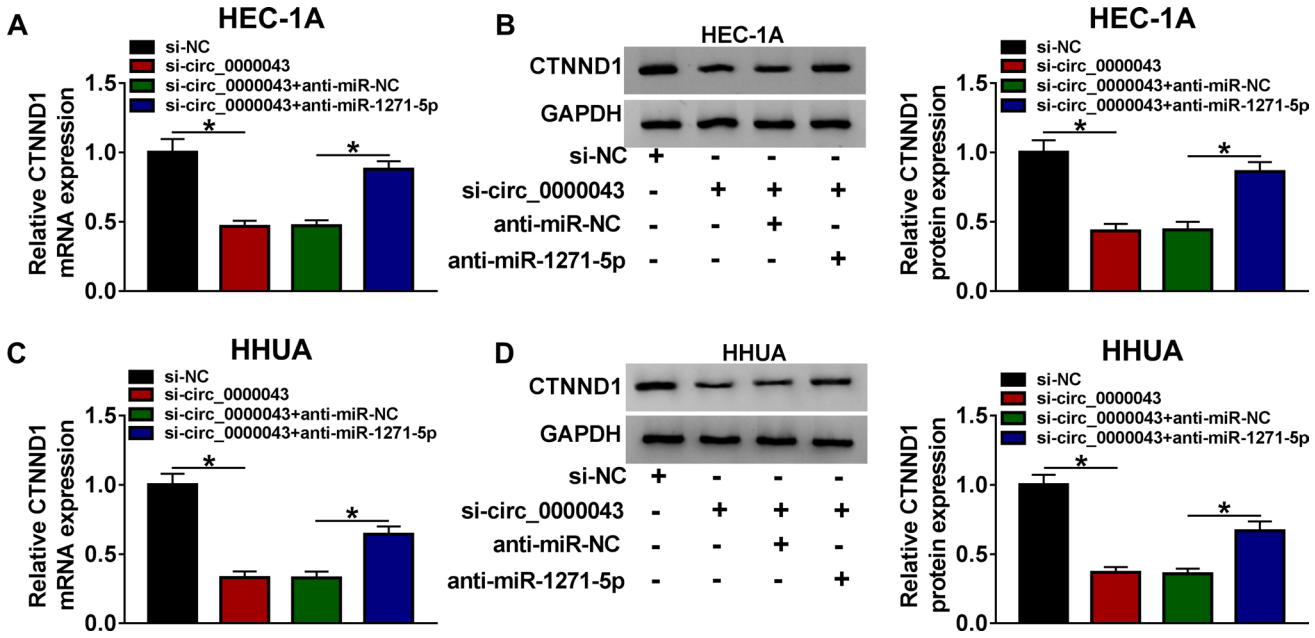

Fig. 7 Circ_0000043 modulated CTNND1 expression via sponging miR-1271-5p in EC cells. (**a**–**d**) CTNND1 mRNA and protein levels were analyzed by qRT-PCR and western blot analyses in HEC-1A and HHUA cells transfected with si-NC, si-circ_0000043, si-circ_0000043 + anti-miR-NC, or si-circ_0000043 + anti-miR-1271-5p. *$P < 0.05$

Interference of circ_0000043 repressed tumor growth through regulating miR-1271-5p and CTNND1 expression

Next, we explored the effect of circ_0000043 on EC in vivo, sh-NC or sh-circ_0000043-transfected HEC-1A cells were injected into the flank of nude mice. Knockdown of circ_0000043 significantly decreased tumor volume and tumor weight (Fig. 8a, b). Moreover, circ_0000043 inhibition decreased the expression of circ_0000043 and promoted the expression of miR-1271-5p in excised tumor tissues (Fig. 8c, d). Western blot analysis revealed that

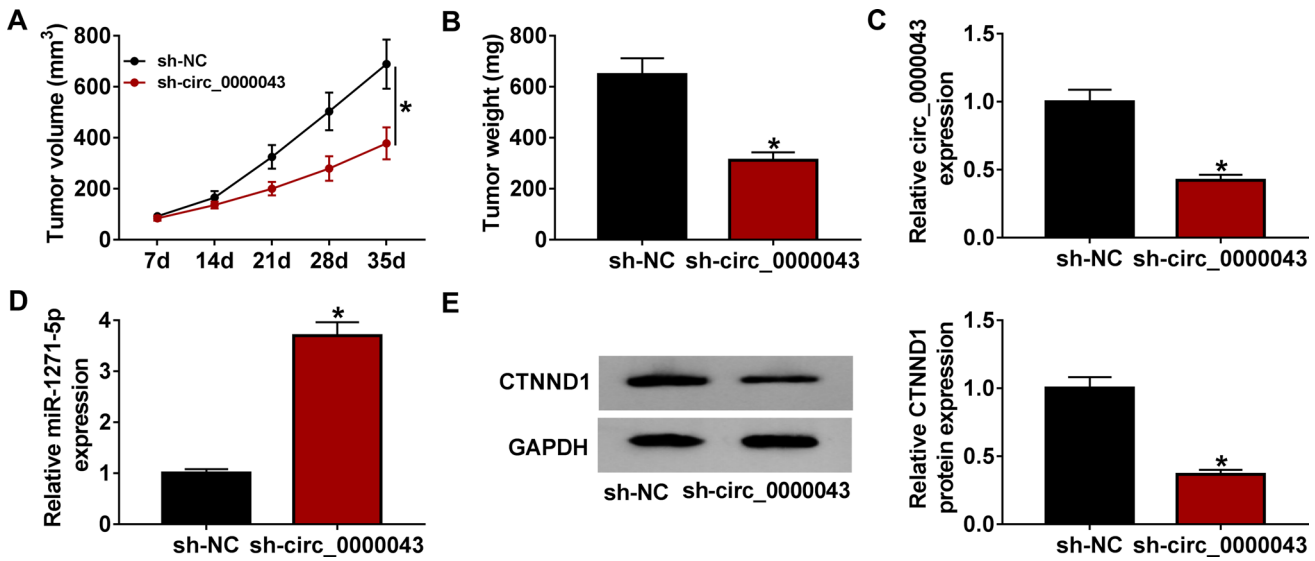

Fig. 8 Circ_0000043 knockdown repressed tumor growth by upregulating miR-1271–5 and downregulating CTNND1. Transfected (sh-NC or sh-circ_0000043) HEC-1A cells were introduced into nude mice to establish mice xenograft model. **a** and **b** Tumor volume and weight were examined. **c** and **d** QRT-PCR was carried out to test the expression of circ_0000043 and miR-1271-5p in tumor tissues. **e** The protein expression of CTNND1 was determined by western blot assay in tumor tissues. *$P < 0.05$

circ_0000043 silence reduced the protein level of CTNND1 in tumor tissues (Fig. 8e). Our findings indicated that circ_0000043 limited tumor growth via upregulating miR-1271-5p and downregulating CTNND1.

Discussion

EC is one of the most common and aggressive cancers among women [22]. Recently, emerging evidence has indicated that circRNAs are involved in the regulation of multiple physiological activities and diseases, especially cancer [23, 24]. In our research, results demonstrated that circ_0000043 was upregulated in EC, and its downregulation repressed the progression of EC by regulating miR-1271-5p/CTNND1 axis.

Due to the abundance and stability of circRNA in plasma and tissues, it has become a hotspot in the field of ncRNA [25]. Recent studies showed that dysregulation of circ_0000043 played pivotal roles in some cancers. For example, Guan et al. showed that circ_0000043 was upregulated in ovarian cancer tissues and promoted the progression of ovarian cancer via sponging miR-615-5p and miR-6753-5p [26]. Chen et al. proved that circ_0000043 was upregulated in both lung adenocarcinoma cells and tissues, and acted as an oncogene to promote lung adenocarcinoma progression by regulating miR-326 [27]. More importantly, Zong et al. pointed out that circ_0000043 was expressed at significantly higher level in EC tissue samples and its upregulation accelerated EC cell growth and metastasis via targeting the miR-136/NOTCH3 pathway [11]. In accordance with these findings, we observed that circ_0000043 was also overexpressed in EC tissue samples and cell lines. Besides, circ_0000043 silence inhibited EC cell proliferation and metastasis whilst promoted apoptosis, and also suppressed tumor growth in vivo. Our results suggested that circ_0000043 might be an oncogene in EC.

Recent studies found that cirRNAs could act as ceRNAs to interact with miRNAs, releasing expression of downstream target mRNAs and participating in a variety of biological processes [6, 28]. To explore whether circ_0000043 acted as a miRNA sponge, the potential target miRNAs of circ_0000043 were predicted by starBase. The results demonstrated that circ_0000043 served as a sponge for miR-1271-5p in EC cells. Emerging evidence suggested that miR-1271-5p played key roles in the regulation of tumor progression. For example, miR-1271-5p inhibited the progression of ovarian cancer by regulating E2F5 and inhibiting mTOR signaling pathway [14]. Moreover, miR-1271-5p repressed cell growth and enhanced radiosensitivity in hepatocellular carcinoma via regulating CDK1 [29]. Furthermore, miR-1271 was reported to be expressed at a low level in human EC tissues and cells, and miR-1271 upregulation inhibited EC cell proliferation and facilitated apoptosis via targeting CDK1 [17]. In our research, we observed that miR-1271-5p level was reduced in EC tissues and cells. Rescue experiments indicated miR-1271-5p inhibition reversed the repressive impact of circ_0000043 silence on EC progression. These results disclosed that circ_0000043 exerted its biological roles in EC by sponging miR-1271-5p.

MiRNAs are known to participate in many physiological and pathological processes through modulating the expression of target mRNAs [30]. Through bioinformatics tool (starBase) and dual-luciferase reporter analysis, we proved that CTNND1 could directly bind to miR-1271-5p. CTNND1 is defined as a component of the adherens junction complex and acts as a potential therapeutic target for cancers [31, 32]. Ding et al. found that CTNND1 was overexpressed in colorectal cancer, and enforced expression of CTNND1 promoted the progression of colorectal cancer [33]. Shen et al. revealed that CTNND1 abundance was upregulated in EC tissues and its overexpression abolished the suppressive effects of circ_0002577 interference on EC cell proliferation and invasion [19]. Consistent with this research, our data suggested that CTNND1 expression was elevated in EC tissues and cells. Moreover, CTNND1 overexpression abated the anti-tumor role of miR-1271-5p in EC cells. Besides, circ_0000043 positively regulated CTNND1 expression via sponging miR-1271-5p. Thus, the present research indicated that circ_0000043/miR-1271-5p/CTNND1 axis might play critical roles in EC progression.

In conclusion, these results showed that circ_0000043 and CTNND1 were overexpressed and CTNND1 was lowly expressed in EC. Downregulation of circ_0000043 inhibited the progression of EC via modulating miR-1271-5p/CTNND1 axis. Our research is the first to reveal the possible associations among circ_0000043, miR-1271-5p and CTNND1 in EC cells. The circ_0000043/miR-1271-5p/CTNND1 axis provides a new insights for EC treatment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no financial or non-financial conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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