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Silencing circCAMSAP1 suppresses malignant behavior of endometrial cancer by targeting microRNA-370-3p/MAPK1

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The study was conducted to figure out the function and mechanism of circular RNA circCAMSAP1 in repressing malignant behavior of endometrial carcinoma (EC) by targeting microRNA (miR)-370-3p /MAPK1. Tumor tissues and normal adjacent tissues of EC patients were harvested, and circCAMSAP1 and MAPK1 were elevated but miR-370-3p was reduced in tissues and cells of EC patients. Functional test results clarified transfection of si-circCAMSAP1 or miR-370-3p-mimic refrained cancer cell proliferation, migration and invasion, but motivated cancer cell apoptosis. Meanwhile, the amount of E-cadherin elevated and the amount of N-cadherin elevated or reduced. After co-transfection with si-circCAMSAP1 and miR-370-3p-inhibitor, miR-370-3p-inhibitor blocked si-circCAMSAP1's therapeutic impact. Furthermore, after co-transfection of pcDNA-circCAMSAP1 and si-MAPK1, si-MAPK1 turned around the malignant effect of pcDNA-circCAMSAP1. It was testified that miR-370-3p was circCAMSAP1's target, and inversely controlled *via* circCAMSAP1. Meanwhile, enhancing miR-370-3p led to repressive MAPK1, which was recognized as miR-370-3p's downstream target. All in all, the results of this study convey silencing circCAMSAP1 refrains the malignant behavior of EC by controlling miR-370-3p /MAPK1 axis.

Key words: Circular RNA CAMSAP1, MicroRNA-370-3p, MAPK1, Target binding, Endometrial carcinoma

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Abbreviations: circRNA, Circular RNA; EC, Endometrial cancer; hEEC, endometrial endothelial cells

INTRODUCTION

Endometrial cancer (EC) is an extremely familiar cancer in women, with over 380 000 new pathologies diagnosed globally every year. Recently, EC's presence has been increasing, especially in developed countries. EC ranks second among female genital cancers in China, with a mortality rate of 2.7 per 100 000 (Wilczynski *et al.,* 2020). ECs' recently new molecular classification is on grounds of the research of the Cancer Genome Atlas Research Network and assignation of them into 4 molecular subgroups is conducted. Among them, endometrioid ECs are present in all subgroups (Cancer Genome Atlas Research *et al.,* 2013), and their molecular diversity may cause more aggressive tumors in many women with type I EC. The treatment options for EC largely relie on the stage of the disease, and surgery is still the major method of early treatment, covering but not limited to pelvic lymph node cesarean section, bilateral salpingo-oophorectomy, etc. (Sonoda, 2012). Several molecular targeted therapies for EC are currently under study, like epidermal growth factor inhibitors, phosphatidylinositol 3-kinase (PI3K)/AKT inhibitors, etc. (Leslie *et al.,* 2012; Oza *et al.,* 2015). Targeted molecular identification has facilitated the diagnosis and cure of EC.

Circular RNA (circRNA) is a kind of covalent body, which is dorsally linked to the precursor mRNA and is in the form of a closed single strand (Ebbesen *et al.,* 2017). CircRNAs are not easily degraded by RNase R due to their absence of 5'-3' ends and polyadenylated tails, so they are more stable vs. linear RNAs (Suzuki *et al.,* 2014). CircCAMSAP1, a circular RNA, also known as circ_0004338, is linked with the regulation of the occurrence and development of diversified cancers (Chen *et al.,* 2021). For instance, circCAMSAP1 can motivate the biological function of hepatocellular carcinoma *in vitro*and *in vivo via* the miR-1294/GRAMD1A molecular axis (Luo *et al.,* 2021); CircCAMSAP1 can be employed as a latent therapeutic target and a biomarker for diagnosis and prognosis of colon cancer (Zhou *et al.,* 2020). However, circCAMSAP1's role and regulatory mechanism in EC remains uncertain.

By targeting and combining with mRNA in the 3' untranslated region, microRNA (miRNA) can control gene expression, cause messenger RNA (mRNA) cleavage, or lead to translational repression of target genes. Therefore, miRNA takes a momentous function in cell growth, apoptosis, migration, etc. (Bueno *et al.,* 2008). Numerous studies have clarified miRNAs can control the specificity of diversified cancers, for example, miR-411-5p can restrain the growth and metastasis of bladder cancer, and target the transport of ZnT1 protein (Finnson *et al.,* 2012); MiR-153-3p can target CFL2 to control the carcinogenesis process of breast cancer cells (Zhang *et al.,* 2021) and so on. Some researchers find miR-370-3p can down-regulate EDN1 in endometrial stromal cells, thereby depressing endometriosis' development (Zhou *et al.,* 2021); MiR-370-3p represses the cell proliferation of endometrium by regulating steroid synthesis factors (Hu *et al.,* 2019). However, the regulatory mechanism of miR-370-3p on EC is unknown.

In this paper, circCAMSAP1 was chosen as the research object to deeply figure out and study the regulatory mechanism and impacts of the circCAMSAP1/miR-370-3p/MAPK1 molecular axis on EC, offering new therapeutic targets and ideas for EC.

MATERIAL AND METHODS

Experimental subjects

Fifty EC tissue specimens and adjacent normal tissues (≥ 4 cm) were harvested from patients with primary EC

confirmed by surgery and pathology in Affiliated Hospital of Weifang Medical College from July 2014 to July 2018. The mean age of the patients was 45.1±7.2 years. In the light of the surgical and pathological staging of endometrial cancer, 8 cases of stage I, 12 cases of stage II, 15 cases of stage III, and 15 cases of stage IV were presented. Tissues were Stored in liquid nitrogen and transferring to -80°C until total RNA or protein extraction. All patients signed signature of the informed letter, and this study was reviewed and approved by the Ethics Committee of Affiliated Hospital of Weifang Medical College (No: 201302WF353L).

Cell culture and transfection

EC cell lines (HEC-1A, HEC-1B, KLE) and endometrial endothelial cells (hEEC) (Thermo Scientific, USA) were cultured in high glucose [Dulbecco's phosphate buffer modified Eagle culture medium (Hyclone, USA) and replenishment of 10% FBS (Gibco, USA), 110 U/mL penicillin and 110 mg/mL streptomycin (Hyclone, USA)] at 37°C with 5% CO_2, selection for were conducted. Cells were sub-cultured before transfection and cells in logarithmic growth phase was selected for cell transfection. Si-circCAMSAP1, si-NC, pcDNA-circCAMSAP1, pcDNA3.1, si-MAPK1, miR-370-3p-inhibitoribitor, miR-370-3p-mimic, and mimic/inhibitor-NC were produced and validated by GeneStar (Shanghai, China). The above plasmids or oligonucleotides were transfected into HEC-1A cells using Lipofectamine 2000 (Thermofisher, USA) according to the manufacturer's instructions. The transfection efficiency was verified by RT-QPCR, and the cells were obtained for subsequent experiments.

Cell counting kit (CCK)-8 and EdU assay

The proliferation ability of EC cells was detected via CCK-8 kit and EdU kit (Enzyme-Linked Bio, Shanghai, China) in the light of the manufacturer's instructions. In the CCK-8 experiment, transfected HEC-1A cells (2×10^4 cells/well) was seeded in 96-well plates. Adding 10 μL CCK-8 solution at 24, 48, 72 and 96 h while incubation was conducted. The absorbance at 450 nm was measured with a microplate reader and the curve was drawn. In the EdU experiment, transfected HEC-1A cells (2×10^4 cells/well) was added to EdU medium, and fluorescence staining solution was added after incubation. The DNA replication activity of the fluorescently labeled mixture at 350 nm was tested applying a fluorescence microscope (Troth & Kyle, 2021).

Transwell assay

Transfected HEC-1A cells (2×10^4 cells/well) were seeded on transwell plates. The upper chamber covered serum-free DMEM (Gibco, USA), and the lower chamber covered 600 μL DMEM and 10%. FBS The upper cavity was incubated, fixed with 95% ethanol and stained. The number of cells in 5 fields was calculated randomly to analyze the migration ability of cells.. Matrigel was flattened on each transwell plate and placed. After the basement membrane gel was solidified, hydration of the basement membrane was with serum-free DMEM to analyze the cell invasion ability (Huang & Li, 2021).

Flow cytometry detection

Cells were stained with fluorescein isothiocyanate (FITC) kit (ThermoFisher, USA) according to the manufacturer's instructions. The adherent cells were harvested by trypsin; The supernatant was taken by centrifugation for the second time and mixed with the binding solution, V-FITC and propidium iodide in the kit for incubation. Part of the sample solution was added into the buffer to take effect, and then flow cytometry was performed (Zong *et al.,* 2020).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA from BMSC cells was extracted via Trizol reagent (Invitrogen, Carlsbad, CA, USA). Assessment of the quantity and quality of RNA was via adopting a NanoDrop spectrophotometer (Thermo, USA). Then, reverse transcription of the RNA was into cDNA employing PrimeScript RT Master Mix (Takara, Dalian, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 were applied as the loading controls for mRNA and miRNA separately. Primer sequences were manifested in Table 1. PCR detection was performed in the light of the instructions of Hieff® qPCR SYBR® Green Master Mix (Yisheng Bio, Shanghai, China), and the $2^{-\Delta\Delta Ct}$ method was put into effect to calculate circCAMSAP1, miR-370-3p and MAPK1 in EC cells (Huang *et al.,* 2020).

Western blot detection

Cells were lysed by Radio-Immunoprecipitation assay-covering lysis buffer (Beyotime, Shanghai, China), and the supernatant was harvested after high-speed centrifugation (12 000 rpm/min, 15 min) at 4°C. The protein concentration's detection was by bicinchoninic acid kit (Enzyme-Linked Biology, China). Isolation of proteins was by Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (120 V, 1.5 h) and electrically transferring to polyvinylidene fluoride membrane was conducted (300 mA, 1.5 h). Incubation of membranes was with primary antibody MAPK1, anti-E-Cadherin antibody, anti-N-Cadherin antibody and anti-GAPDH antibody (all 1:1000) overnight at 4°C, add horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:5000) for 1 h. Antibodies were bought from Abbott (Shanghai, China). After incubation, electrogenerated chemiluminescence luminescent solution (Yisheng Bio, Shanghai, China) was used to obtain bands and calculation of protein expression were conducted (Yang *et al.,* 2021).

Table 1. Primer sequence

Genes	Primer sequence	
MiR-370-3p	F	GCCGAGGCCTGCTGGGGTGG
	R	GCAGGGTCCGAGGTATTC
U6	F	GCTTCGGCAGCACATATACTAAAAT
	R	CGCTTCACGAATTTGCGTGTCAT
CircCAMSAP1	F	GTGTCAAGCGCTTCTCAACG
	R	GCTGGACAGGAGAAGCTTGA
MAPK1	F	TGGATTCCCTGGTTCTCTCTAAAG
	R	GGGTCTGTTTTCCGAGGATGA
GAPDH	F	CGCTCTCTGCTCCTCCTGTTC
	R	ATCCGTTGACTCCGACCTTCAC

The luciferase activity assay

HEC-1A cells were seeded in 24-well plates to reach 65% confluence. circCAMSAP1 or MAPK1 3'UTR WT and MUT (Zima Pharmaceuticals, Shanghai, China) were cloned into empty plasmids (Miaoling Bio, Wuhan, China). Lipofectamine 2000 (Thermofisher, USA) was employed for co-transfecting luciferase reporter plasmids into cells with miR-370-3p-mimic and miR-370-3p-NC. Then measurement of the luciferase activity of the samples was conducted applying a dual luciferase reporter gene analysis system (Promega, Shanghai, China).

RNA immunoprecipitation (RIP) assay

EZ-Magna RIP kit (Millipore, USA) was applied for RIP determination. Cells were lysed in RIP lysis buffers and then incubated in RIP buffers covered with magnetic beads coupled with negative controls (normal mouse IgG) or human anti-Argonaute 2 (Ago2, Millipore). Then, conducting Isolation, purification, and final detection by RT-qPCR of the co-precipitated RNA (Wang & Yang, 2021).

Statistical analysis

Data were analyzed employing PSS 24.0 software and presented as mean ± standard deviation (S.D.). A *t*-test was used for the comparison between the two groups with normal distributions, a nonparametric test was used for the comparison between the two groups with abnormal distributions. Repeating all functional tests in the research was for three times. GraphPad Prism 7.0 software was applied to draw the result pictures, and evaluation of the correlation between the expression levels and cells was by the linear correlation coefficient r. $P<0.05$ clarified statistical significance.

RESULTS

CircCAMSAP1 is elevated in EC cell lines and tissues

In contrast with adjacent normal tissues, circCAMSAP1 was clearly enhanced in EC tissues ($P<0.05$) (Fig. 1A). Compared with hEEC, circCAMSAP1 was also elevated in HEC-1A, HEC-1B and KLE cells ($P<0.05$). Among them, HEC-1A cells had the highest expression ($P<0.01$), followed by the lowest expression of KLE, and HEC-1B (Fig. 1B). Since circCAMSAP1 had the highest expression in HEC-1A cells, HEC-1A cells were chosen for subsequent experiments. These data suggested circCAMSAP1 might participate in the EC disease process.

The effect of knockdown circCAMSAP1 on the biological function of EC cells

After transfection of si-circCAMSAP1, circCAMSAP1 in cells was clearly reduced ($P<0.05$) (Fig. 2A). Cell progression results showed knocking down circCAMSAP1 reduced cell proliferation ($P<0.05$) (Fig. 2B), and EdU positive cells declined ($P<0.05$) (Fig. 2C). Meanwhile, the amount of N-cadherin declined in HEC-1A cells (both $P<0.05$) (Fig. 2D–E); Cell migration and invasion ability were descended (both $P<0.05$) (Fig. 2F–G). Moreover, the apoptosis rate was enhanced after knockdown circCAMSAP1 ($P<0.05$) (Fig. 2H). These data conveyed silencing circCAMSAP1 could refrain EC cells' advancement.

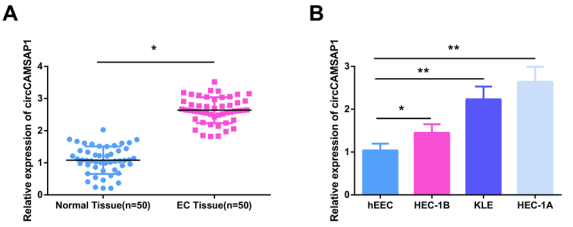

Figure 1. CircCAMSAP1 is elevated in EC cell lines and tissues
(**A, B**) CircCAMSAP1 in EC tissues and cell lines; Measurement data were clarified as mean ± S.D.; *$P<0.05$, **$P<0.01$.

CircCAMSAP1 targets miR-370-3p

Plentiful studies have clarified circRNA takes on a role in controlling diseases via competitive adsorption of downstream miRNAs (Tu *et al.,* 2021). The target genes of circCAMSAP1 were forecast employing the bioinformatics database, and miR-370-3p was found to be a candidate target gene of circCAMSAP1 (Fig. 3A). The results of luciferase activity assay illustrated the luciferase activity of HEC-1A cells were declined after co-transfection of miR-370-3p-mimic and circCAMSAP1 WT, while the co-transfection of miR-370-3p-mimic and circCAMSAP1 MUT had no clear impact on luciferase activity ($P<0.05$) (Fig. 3B). After transfection of si-circCAMSAP1, miR-370-3p in HEC-1A cells was clearly enhanced ($P<0.05$) (Fig. 3C). Meanwhile, it was also confirmed that circCAMSAP1 and miR-370-3p were abundant in the anti-Ago group (both $P<0.05$) (Fig. 3D). Moreover, miR-370-3p in EC tissues and cell lines was clearly reduced vs. adjacent normal tissues and hEEC cells (both $P<0.05$) (Fig. 3E-F); MiR-370-3p was opposite to that of circCAMSAP1 ($r = –0.7929$, $P<0.0001$) (Fig. 3G). The above results proved miR-370-3p was circCAMSAP1's target gene, and the expression patterns of the two were inversely associated.

Elevation of miR-370-3p can depress EC cell evil behavior

MiR-370-3p's biological role in EC was examined by functional acquisition experiments. After introduction of miR-370-3p-inhibitorbitor, miR-370-3p-mimic and mimic/inhibitor-NC into HEC-1A cells, miR-370-3p was measured. It turned out transfection of miR-370-3p-mimic/inhibitor elevated but reduced miR-370-3p respectively ($P<0.05$) (Fig. 4A). The results of functional experiments clarified miR-370-3p-mimic refrained the proliferation ability of HEC-1A cells, and fewer EdU positive cells exhibited (both $P<0.05$) (Fig. 4B–C). Meanwhile, the amount of E-cadherin was elevated but the amount of N-cadherin was declined (both $P<0.05$) (Fig. 4D-E), cell migration and invasion abilities were descended, but apoptosis rate was enhanced (both $P<0.05$) (Fig. 4F–H). The opposite results were gained after transfection of miR-370-3p-inhibitoribitor. These experimental results exhibited elevation of miR-370-3p could refrain EC cell biological function.

Depressive miR-370-3p blocks the therapeutic effect of silencing circCAMSAP1

Transfection or co-transfection of si-circCAMSAP1 and miR-370-3p-inhibitor was to study their effects and regulation on the malignant behavior of EC cells. After transfection with si-circCAMSAP1, miR-370-3p in HEC-1A cells were elevated, while after co-transfection with

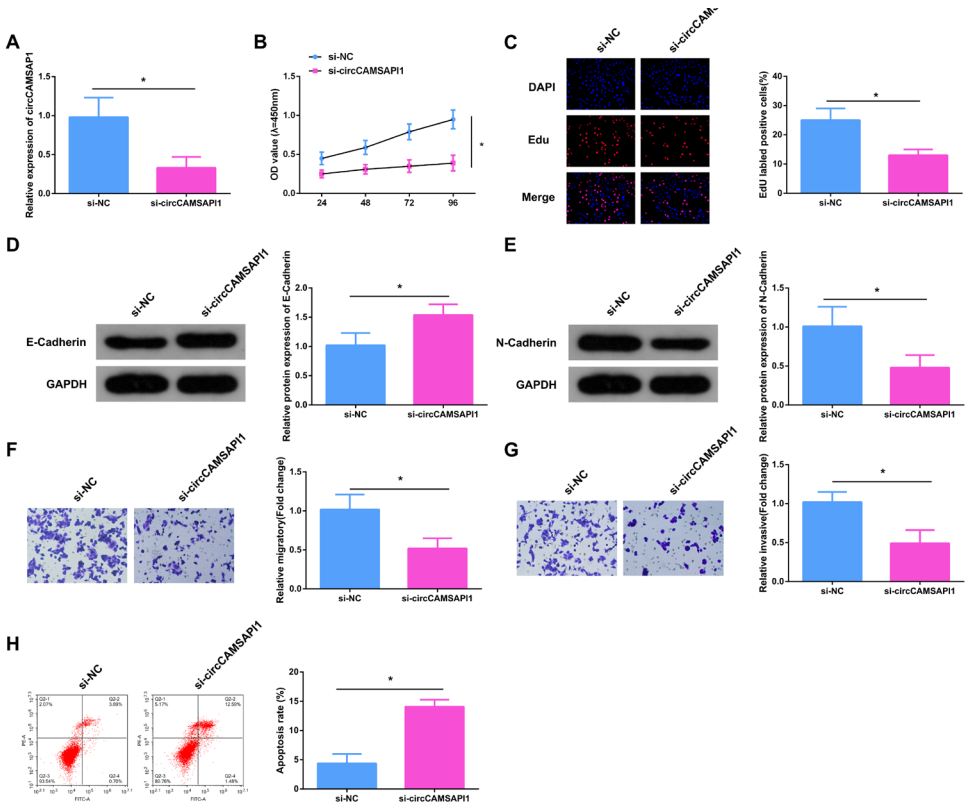

Figure 2. The effect of knockdown circCAMSAP1 on the biological function of EC cells
(**A**) RT-qPCR to test circCAMSAP1; (**B–C**) CCK-8 and EdU experiments to evaluate the effect of circCAMSAP1 down-regulation on EC cell proliferation; (**D–E**) Western blot to examine the amount of E-cadherin and N-cadherin after repressing circCAMSAP1; (**F–G**) Transwell assay to evaluate the effect of circCAMSAP1 down-regulation on EC cell migration and invasion; (**H**) EC cell apoptosis rate after circCAMSAP1 down-regulation was detected by flow cytometry; *P<0.05.

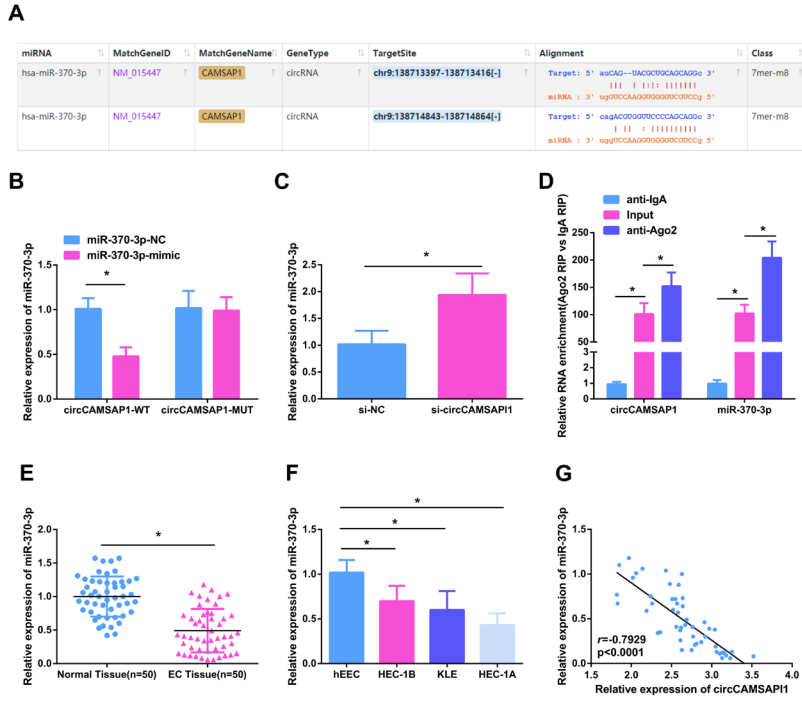

Figure 3. CircCAMSAP1 targets miR-370-3p
(**A**) Binding region of miR-370-3p and circCAMSAP1; (**B**) The luciferase activity assay to detect circCAMSAP1 and miR-370-3p's targeting; (**C**) MiR-370-3p after silencing circCAMSAP1; (**D**) CircCAMSAP1 and miR-370-3p were enriched in anti-Ago2 group; (**E–F**) MiR-370-3p in EC tissues and cells; (**G**) Correlation between circCAMSAP1 and miR-370-3p expression pattern; *P<0.05.

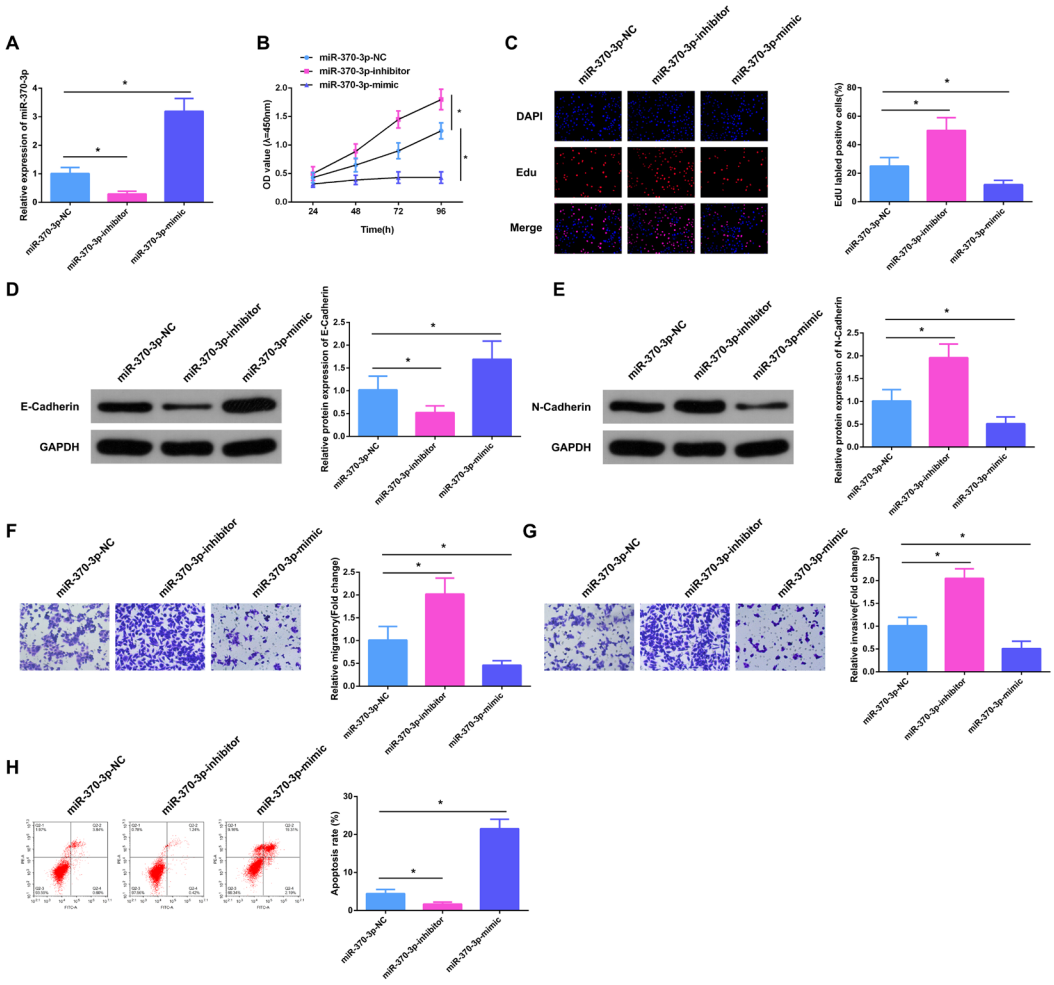

Figure 4. Elevation of miR-370-3p can depress EC cells' biological behaviors
MiR-370-3p in EC cells was regulated by transfection of miR-370-3P-mimic /inhibitior. (**A**) RT-qPCR to test miR-370-3p; (**B–C**) CCK-8 and EdU experiments to examine cell proliferation; (**D–E**) Western blot to examine the amount of E-cadherin and N-cadherin; (**F–G**) Transwell assay to check cell migration and invasion; (**H**) EC cell apoptosis was detected by flow cytometry; *P<0.05.

si-circCAMSAP1 and miR-370-3P-inhibitor, miR-370-3p in HEC-1A cells were declined (both P<0.05) (Fig. 5A). After circCAMSAP1 was silenced, the proliferation ability of HEC-1A cells (both P <0.05) (Fig. 5B), and the number of EDU-positive cells were declined (Fig. 5C), the amount of E-cadherin was elevated but the amount of N-cadherin was declined (both P<0.05) (Fig. 5D–E); The migration and invasion abilities were repressed (both P<0.05) (Fig. 5F–G), but the apoptosis rate was enhanced (P<0.05) (Fig. 5H). These effects were blocked by transfection of miR-370-3p-inhibitor. These data suggested silencing circCAMSAP1 downregulated EC and blocked the malignant behavior of miR-370-3p-inhibitor in EC cells.

MiR-370-3p targeted MAPK1

MiRNAs frequently combines with the 3 untranslated regions of mRNA and are transcribed to control miRNA expression (Jiang *et al.,* 2021). Therefore, it was predicted the target mRNA of miR-370-3p by StarBase and discovered a latent binding site between MAPK1 and miR-370-3p (Fig. 6A). It came out MAPK1 was elevated in EC tissues and cells (both P < 0.05) (Fig. 6B–C). In the light of the validation results of the bidirectional luciferase activity assay, transfection of miR-370-3p-mimic clearly restrained the reporter activity of MAPK1-WT, while miR-370-3p-inhibitorbitor elevated the reporter activity of MAPK1-WT. The reporter activity of MAPK1-MUT was almost unchanged (P<0.05) (Fig. 6D). Meanwhile, it came out miR-370-3p and MAPK1 were also abundant in the anti-Ago2 group (both P < 0.05) (Fig. 6E). Furthermore, miR-370-3p-mimic downregulated MAPK1, whereas miR-370-3p-inhibitororibitor did the opposite (both P<0.05) (Fig. 6 F–G). These results clarified miR-370-3p inversely controlled MAPK1 in EC cells. Linear analysis exhibited MAPK1 was negatively linked with miR-370-3p (r = –0.7086, P<0.0001) (Fig. 6H). The above experimental results conveyed circCAMSAP1 could target MAPK1 through miR-370-3p.

Si-MAPK1 turns around the malignant effect of pcDNA-circCAMSAP1

Functional rescue experiments were conducted to figure out the mechanism by which circCAMSAP1 controls EC development through the miR-370-3p/MAPK1 molecular axis. The experimental results manifested after transfection of pcDNA-circCAMSAP1, miR-370-3p in HEC-1A cells was reduced but MAPK1 was elevated. After co-transfection of pcDNA-circCAMSAP1 and si-MAPK1, miR-370-3p was enhanced but MAPK1 was

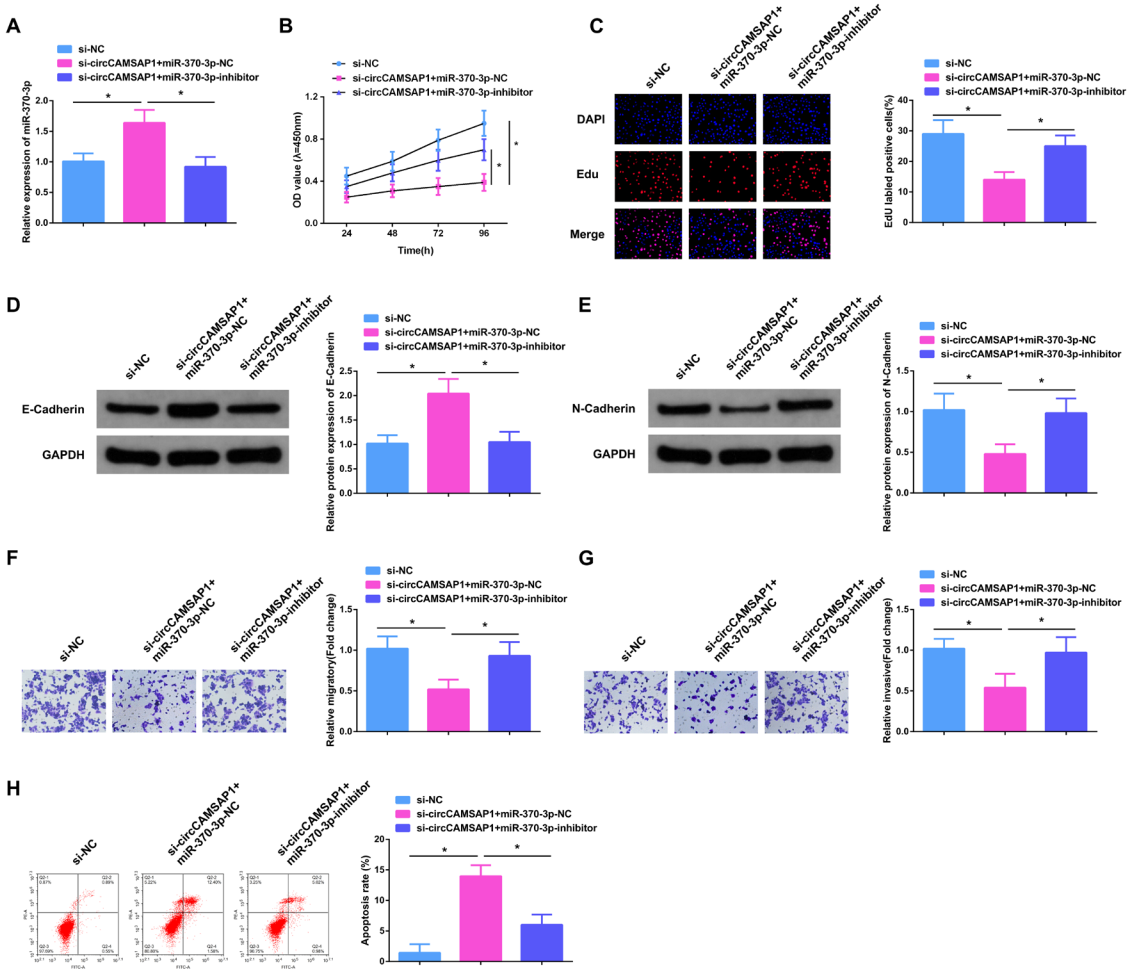

Figure 5. Depressive miR-370-3p blocks the therapeutic effect of silencing circCAMSAP1
Si-circCAMSAP1 was blocked by transfection with miR-370-3p-inhibitor. (**A**) RT-qPCR to test miR-372-3p; (**B–C**) CCK-8 and EdU experiments to examine cell proliferation; (**D–E**) Western blot to examine the amount of E-cadherin and N-cadherin; (**F–G**) Transwell assay to check cell migration and invasion; (**H**) EC cell apoptosis was detected by flow cytometry; *P<0.05.

decreased in HEC-1A cells (both P<0.05) (Fig. 7A). Results of functional experiments confirmed the proliferation ability of HEC-1A cells was enhanced after elevation of circCAMSAP1 (P<0.05) (Fig. 7B), and the number of EDU-positive cells was elevated (P<0.05) (Fig. 7C). Meanwhile, the amount of E-cadherin was reduced but the amount of N-cadherin was elevated (Fig. 7D–E), cell migration and invasion ability were strengthened (both P<0.05) (Fig. 7F–G), but apoptosis rate was declined (P<0.05) (Fig. 7H). These effects were blocked by transfection of si-MAPK1. The experimental results showed elevation of circCAMSAP1 up-regulated MAPK1 by depressing miR-370-3p.

DISCUSSION

Targeted therapy can impact tumor cell growth, apoptosis, signaling, receptor activation, etc., and is considered as a breakthrough in human cancer therapy (Padma *et al.,* 2015). Noncoding RNAs are increasingly momentous in this field as they are implicated in clinical and functional cancer development (Wang *et al.,* 2019). Although patients with early-stage EC have a relevant high survival rate, patients with advanced or recurrent disease still have poor prognosis and limited treatment options (Mitamura *et al.,* 2019). This study contrasted circCAMSAP1 in EC tissue samples and cells with normal paracancerous tissues and cells for the fist time, and found that circCAMSAP1 was elevated in EC tissues and cells, suggesting that circCAMSAP1 might have adverse effects on EC development.

CircRNAs, highly conserved and stable non-coding RNAs, have received extensive attention owing to their great potential in cancer therapy (Barrett & Salzman, 2016). They have diversified miRNAs binding sites, which can mediate the activity of targeted miRNAs and competitively combine them, thereby depressing the transcription of downstream target genes (Mitra *et al.,* 2018). For instance, circ_0001073 targeting miR-626 can depress the occurrence and development of lung cancer (Liu *et al.,* 2021a); Circ_0004507 targeting miR-873 can upregulate drug resistance proteins, thereby motivating the development of laryngeal cancer (Yi *et al.,* 2021). In former studies, it is found that CAMSAP1 is a gene encoding mammalian astroglial cytoskeleton and neuron-associated cytoskeleton-linked proteins (Yamamoto *et al.,* 2009). Meanwhile, CAMSAP1 is expressed on mature astrocytes in the adult brain and is also considered a novel marker of cells of the astrocyte lineage (Yoshioka *et al.,* 2012). Circ_0001900 derived from exons 2 to 3 of the CAMSAP1 gene, also known as circCAMSAP1,

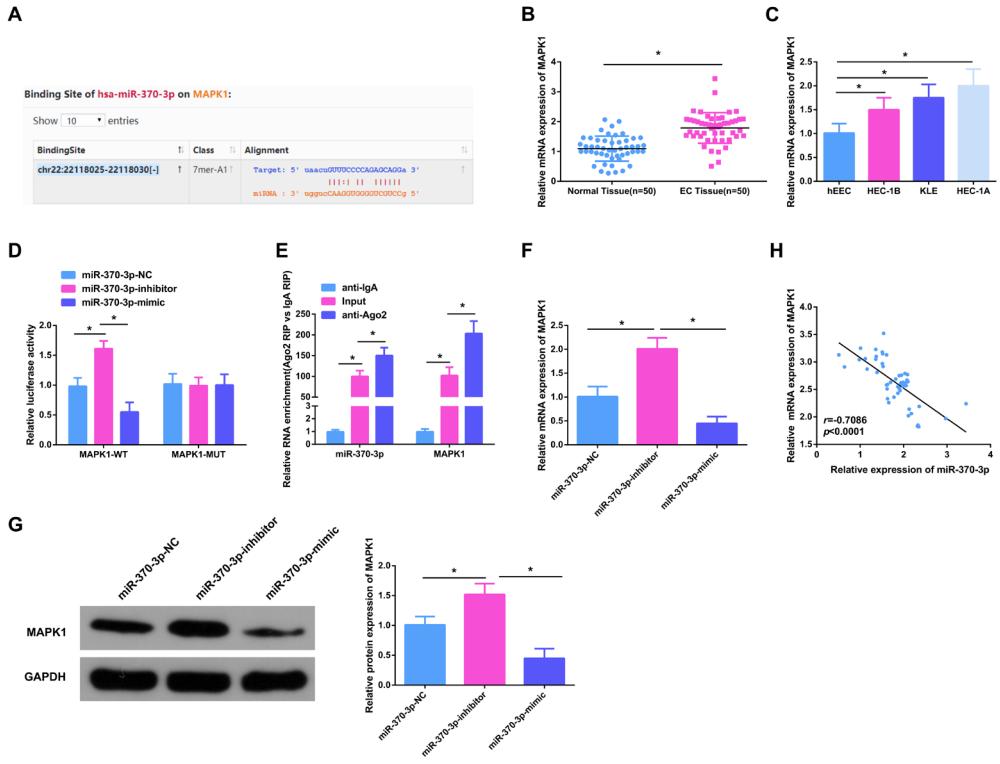

Figure 6. MiR-370-3p targeted MAPK1
(**A**) Binding region of miR-370-3p and MAPK1; (**B, C**) MAPK1 in EC tissues and cells; (**D**) The luciferase activity assay to detect miR-370-3p and MAPK1's targeting; (**E**) MiR-370-3p and MAPK1 were abundant in anti-Ago2 group; (**F–G**) RT-qPCR and western blot examination of miR-370-3p and MAPK1's targeting; (**H**) Correlation between MAPK1 and miR-370-3p; *P<0.05.

which has been clarified to have a momentous role in colorectal cancer. Circ_0004338 (circCAMSAP1 in the research) is derived from exons 1 to 7 of the CAMSAP1 gene and is spliced to form a single-stranded continuous loop structure network by connecting the 3' and 5' ends. These features convey circCAMSAP1 is formed by "direct splicing" and is stably expressed in different cell lines and tissues (Jeck *et al.,* 2014). Although circCAMSAP1 has been well studied in plentiful cancers, it has been less studied in EC. This study is the first to propose and demonstrate that circCAMSAP1 was elevated in EC, and its expression is significant. In the light of the experimental results, knockout of circCAMSAP1 can reduce the proliferation, migration, and invasion of EC cells, but motivate cancer cell apoptosis.

Recently, studies have found circRNAs can combine with miRNAs, thereby exerting tumor-promoting or tumor-suppressing effects (Han *et al.,* 2017). Using Starbase database, circCAMSAP1 and miR-370-3p were found to have binding sites. Subsequent analysis showedcircCAMSAP1 refrained miR-370-3p in EC cells. The role of miR-370 as a tumor-promoting or tumor-suppressor gene in different human tumors is controversial. For instance, miR-370 is a latent oncogene in melanoma (Wei *et al.,* 2017); up-regulation of miR-370 suppresses the tumor suppressor FOXO1 in prostate and gastric cancers and motivates cancer cell proliferation (Fan *et al.,* 2013). In contrast, miR-370 has been testified to perform as a tumor suppressor in colon and liver cancer by depressing cell proliferation and accelerating apoptosis (Shen *et al.,* 2018; Sun *et al.,* 2016). In this study, the correlation of miR-370-3p with EC was figured out for the first time, and it was found to be reduced in EC cells. Meanwhile, the results of luciferase activity assay confirmed miR-370-3p had a binding site with circCAMSAP1, and circCAMSAP1 could target miR-370-3p. The experimental results clarified elevation of miR-370-3p could depress the proliferation, migration and invasion of EC cells but accelerate apoptosis, and restraining miR-370-3p blocked the therapeutic effect of silencing circCAMSAP1. These data demonstrated miR-370-3p could downregulate EC and accelerate cancer cell apoptosis.

The MAPK signaling cascade is an intracellular series of serine/threonine protein kinases that take on key roles in diversified cellular functions (Xiong *et al.,* 2021). Studies have shown the p38-MAPK signaling pathway participates in signal transduction, and is initiated by extracellular signals to initiate cascade transduction. Phospho-p38-MAPK forms double phosphorylation through threonine and tyrosine binding sites, and is transferred to the nucleus. Depending on the corresponding target, this can motivate hepatic stellate cell activation (Yang *et al.,* 2021). Meanwhile, MAPK can also control various cellular activities, such as proliferation, differentiation, apoptosis, etc. (Yi *et al.,* 2021). Some studies have suggested MAPK signaling is a promising target for cancer therapeutic intervention (Zhang *et al.,* 2021). As a member of the MAPK family, MAPK1 is a extensively used oncogene that can be activated or expressed, and is elevated in various types of human cancers (Zhao *et al.,* 2021). At present, MAPK1 has been confirmed to combine with diversified miRNAs to regulate downstream signals to achieve cell biological functions (Liu *et al.,* 2021b). This study originally demonstrated miR-370-3p could bind MAPK1 to regulate EC. In the light of the target gene prediction and the results of the bidirectional luciferase activity assay, this study confirmed MAPK1 was miR-370-3p's target gene, and miR-370-3p could

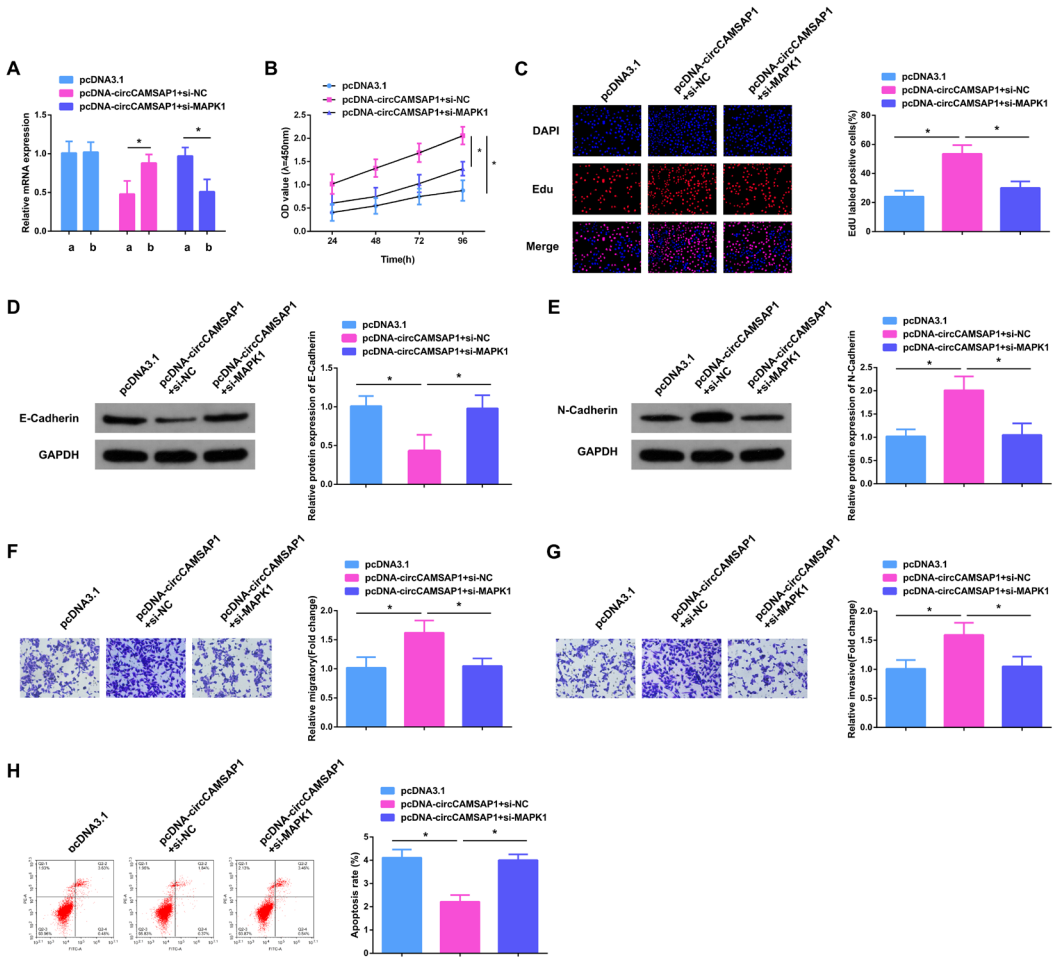

Figure 7. Si-MAPK1 turns around the malignant effect of pcDNA-circCAMSAP1
PcDNA 3.1-circCAMSAP1 was reversed by transfection of si-MAPK1. (**A**) RT-qPCR to test miR-370-3p (**a**) and MAPK1 (**b**); (**B–C**) CCK-8 and EdU experiments to examine cell proliferation; (**D–E**) Western blot to examine the amount of E-cadherin and N-cadherin; (**F–G**) Transwell assay to check cell migration and invasion; (**H**) EC cell apoptosis was detected by flow cytometry; *P<0.05.

negatively regulate MAPK1. MAPK1 was elevated. The present study also found silencing MAPK1 could reverse the malignant effects of overexpressing circCAMSAP1 on cells. These experimental results suggested MAPK1 could regulate EC and upregulate disease.

CONCLUSION

In summary, silencing circCAMSAP1 can restrain EC cells' biological functions by targeting the miR-370-3p/MAPK1 molecular axis. The circCAMSAP1/miR-370-3p/MAPK1 axis can become a new marker for EC diagnosis and treatment, and offer a theoretical basis for more research later. However, the research still takes on several limitations. In the research, it was only figured out the circCAMSAP1/miR-370-3p/MAPK1 signaling pathway, more mechanistic signaling pathways, and the possibility of targeting miR-370-3p or MAPK1 to treat EC require to be explored, and further investigation and research are needed.

Declarations

Conflict of interest statement. There are no conflicts to declare.

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