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Has_circ_0008285/miR-211-5p/SIRT-1 Axis Suppress Ovarian Cancer Cells Progression

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ABSTRACT

The significant functional role of circular RNAs (circRNAs) in the progression of malignant tumors, including ovarian cancer, has been shown in various studies. In this study, we aimed to investigate the abnormal expression of hsa_circ_0008285 and its role in ovarian cancer pathogenesis. Quantitative real time polymerase chain reaction (qRT-PCR) and Western blot methods were used to detect the expression of hsa_circ_0008285 and some target genes in ovarian cancer tissues and related cell lines. To determine the functional roles of hsa_circ_0008285 in ovarian cancer, cell proliferation, apoptosis, and cell invasion assays were performed. Bioinformatics (Target scan, circ intractome) and luciferase reporter analyses were used to predict target genes. Results: In the present study, we first found that hsa_circ_0008285 was up regulated in ovarian cancer tissues and related cell lines. Bioinformatics, experimental data, and luciferase reporter analysis data showed miR-211-5p is a direct target of hsa_circ_0008285, while SIRT-1 is a direct target of miR-211-5p. Overexpression of hsa_circ_0008285 in cancer cells increased the expression of SIRT-1 and progression of cancer cells. Based on these results, inhibition of hsa_circ_0008285 expression could cause upregulation of miR-211-5p and down regulation of SIRT-1 and inhibited the proliferation and invasion of ovarian cancer cells. Conclusion: The results of the present study revealed that hsa_circ_0008285 suppressed ovarian cancer progression by regulating miR-211-5p expression to inhibit SIRT-1 expression.

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Introduction

Ovarian cancer is the deadliest gynecological malignancy and accounts for 5% of estimated cancer deaths (1–3). Females of all ages can develop ovarian carcinogenesis, but most cases are detected in postmenopausal women, and more than 75% of diagnosed cases are at an advanced stage. Late diagnosis is due to the lack of early detection screening, asymptomatic nature at early stages, and vague symptoms of late-stage disease (4,5). Despite continuous advancements in surgery and chemotherapy, the 5-year overall global survival rate of ovarian cancer is approximately 40% (6). Therefore, there is a pressing need to identify the molecular mechanisms underlying the occurrence and development of ovarian cancer for early diagnosis and targeted therapy.

Circular RNAs (circRNAs) are a class of non-coding regulatory RNAs that are mainly formed through the back-splicing of pre-mRNA (7,8). CircRNAs exhibit closed stable ring structures without free 3′ and 5′ ends, which protect them from RNA exonuclease and degradation (9). They are widely distributed in almost all eukaryotic cells and remain stable in blood, urine, saliva, and other body fluids (10). In this way, they have the potential to be used as novel biomarkers and therapeutic targets for cancer diagnosis and treatment (11).

CircRNAs mainly play a role in gene expression by sponging microRNA (12), while they can also regulate transcription and translation (13,14), facilitating interactions between two or more proteins by acting as an enzyme scaffold between them (15) and encoding oncogenic proteins (16). Several studies have suggested that circular RNAs are up regulated or down regulated and act as tumor suppressors or oncogenes in almost all types of cancers, including ovarian cancer (17,18). For example, circ-NOLC1 promotes ovarian cancer tumorigenesis and development by binding and modulating ESRP1, CDK1, and RhoA levels (19). Circular RNA 0051240 promotes cell proliferation, migration, and invasion in ovarian cancer through the miR-637/KLK4 axis (20). In contrast, circ_0078607 via sponging miR-518a-5p inhibits ovarian cancer invasion and promotes apoptosis of ovarian cancer cells (21), and circ_0061140 spong miR-761, decreases proliferation and migration and increases apoptosis of ovarian cancer through the miR-136/CBX2 axis (22).

Circ_0008285 is derived from the coding gene chromodomain y-like protein and is 667 nucleotides long. The level of circ-0008285 has been reported to increase in hepatocellular carcinoma (23), mantle cell lymphoma (24), cervical cancer (25), breast cancer (26), and multiple myeloma (27), but decrease in colon cancer (28), bladder cancer (29), and Wilms tumor (30). The mechanisms of action of circ_0008285 in ovarian cancer are not fully understood. In the present study, we characterized circRNA transcripts using RNA sequencing (RNA-seq) analyses of ribosomal RNA-depleted total RNA from nine pairs of ovarian cancer and adjacent non-cancerous tissue samples. Finally, we discovered that hsa_circ_0008285 was relatively overexpressed in ovarian cancer tissues. We subsequently expanded the sample size and detected hsa_circ_0008285 expression, and found that its expression level in ovarian cancer was significantly higher than that in adjacent non-tumorous tissues. Therefore, we further explored the underlying functions of hsa_circ_0008285 in ovarian cancer cells and speculated that overexpression of hsa_circ_0008285 could serve as a sponge of miR-211-5p to elevate SIRT-1 expression and cause high proliferation and invasion of ovarian cancer cells.

Materials and methods

Patients and clinical tissues

This project was approved by the Ethics Committee of the Qazvin University of Medical Sciences (IR.QUMS.REC.1400.475). Ovarian cancer and adjacent normal tissue samples were collected from patients diagnosed with epithelial ovarian cancer who received surgical treatment at Kowsar Hospital, Qazvin, IRAN. All the enrolled patients signed informed consent forms in compliance with the Declaration of Helsinki. None of the patients had undergone chemotherapy or radiotherapy prior to the surgery. All tissues were immersed in liquid nitrogen and preserved at −80 °C.

Cell culture

Ovarian cancer cell lines (SKOV3, A2780) and a normal ovarian cell line (OCE1) were purchased from the Institute Pastor Tehran, IRAN. The cells cultured in DMEM supplemented with 10% FBS (Gibco, GranIsland, NY, USA), 100 mg/mL penicillin, and streptomycin (Invitrogen). All the cells were maintained in an incubator with 5% CO2 and saturated humidity at 37 °C.

RNA-seq analysis and identification of circRNAs

First, small RNAs was purified according to the manufacturer's instructions. Illumina Small RNA v1.5 Sample Preparation kit (Illumina, Inc.). Sequencing was done by HiSeq 2000 (Illumina, San Diego, CA, USA). Our sequences were mapped to the respective reference genome using BOWTIE2 version 2.2.5 (GRCH 37. p13 NCBI). MaxEntScan33 was used to evaluate the splicing sites. We used BWA-mem to estimate the expression of circRNA.

RNA extraction and quantitative real time polymerase chain reaction (qRT-PCR)

RNA isolation from tissues and the indicated cell lines was performed using TRIzol reagent (Invitrogen Life Technology Co, USA). To evaluate the concentration and purity of the RNA, we used a Nano Drop w ND-1000. First-strand complementary DNA (cDNA) synthesis was performed on 500ng of treated RNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, Fermentas, Waltham, MA, USA). For the quantification of expression, qRT-PCR was performed using GAPDH as an internal control.

The reactions were incubated in a 72-well optical strip at 95 °C for 15 min (enzyme activation), followed by 95 °C for 20 s and 60 °C for 60 s (40 cycles). All reactions were performed in triplicate. After these reactions, the mean Ct was determined from triplicate PCRs. We used the $2^{-\Delta Ct}$ method to quantify gene expression.

Cell transfection

The synthetic circ-0008285 sequence was subcloned into the pcDNA3.1 vector (Invitrogen). In addition, we ordered the miR-211-5p mimic and inhibitor from the company. SIRT1 overexpressing, empty plasmid, SIRT1 short interference RNA (siRNA), and control siRNA were purchased (Exiqon, Denmark). Lipofectamine 3000 was used to transfect the cells according to the manufacturer's instructions (Invitrogen; Thermo Fisher Scientific, Inc.). Quantitative real-time PCR (qRT-PCR) was used to evaluate the transfection efficiency.

CCK-8 assay

SKOV3 and A2780 cells were harvested in a logarithmic growth phase. The cell density was adjusted to 1 × 103/ml and incubated in 96 well plates (100 μL of cell suspension per well). The 96 well plates were

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then placed in an incubator to continue the culture. Then, 10 μL of CCK-8 reagent (Dojindo, Japan) was added to the wells at 12, 24, 48, and 72 h, followed by 2 h of culture. Subsequently, the absorbance of each well was measured at 450 nm using a microplate reader. Experiments was repeated three times.

Western blotting method

Cultured SKOV3 and A2780 cells were centrifuged. Total protein was extracted from cells using lysis buffer (Beyotime Institute of Biotechnology). The total protein was quantified using a bicinchoninic acid kit (Beyotime Institute of Biotechnology). Subsequently, 40 µg of total protein was loaded per lane on a 10% SDS-PAGE gel, electroblotted onto polyvinylidene difluoride membranes (Thermo Fisher Scientific, Inc.), and blocked with 5% skimmed milk (Millipore Sigma) for 1 h at room temperature. The membranes were then incubated overnight at 4°C with the following primary antibodies: Anti-SIRT1 (1:1,000 dilution; cat. no. ab110304), Anti-Caspase-3 (1:1000 dilution; cat. no. ab 4051), Anti-Beta actin (1:1000 dilution; cat. no. ab 119716) (Abcam, USA).

Dual-luciferase reporter gene assay

The 3′-UTR of SIRT1 gene containing the sequence of miR-211-5P response element (SIRT1-3′-UTR) and its corresponding mutant sequence lacking any complementarity with miR-211-5P, to be used as negative control, were amplified by PCR. They were then cloned downstream of Renilla luciferase in pGL-3 (E1771, Promega, Madison, WI) vector (Promega, USA), giving rise to SIRT1 3′-UTR pGL-3 and SIRT1-mutant- pGL-3 plasmids. Studied cell lines were seeded into 12-wells plates and co-transfected with the above plasmids along with miR-211-5P mimic, miR-211-5P inhibitor and their NCs using PEI. Meanwhile, transfection with a cloned pGL-3 plasmid either alone, or together with miR-211-5P was performed as control. Cells were also exposed to mock transfection (treatment with PEI transfection reagent alone). After 4 h, DMEM-F12 containing 1 % penicillin-streptomycin and 10 % FBS was added to each well. Subsequently, cells were harvested after 48 h and assayed with a Dual Luciferase assay kit (Promega, USA) according to the manufacturer’s instructions. Each value of luminator from the Renilla luciferase was normalized to the Firefly luciferase value.

Analysis of apoptosis

Flow cytometry was used to evaluate apoptosis. In this method, two dyes were used to distinguish apoptotic cells from necrotic cells (Annexin V-FITC and propidium iodide (PI)). Apoptotic cells were Annexin V-FITC-positive, PI-positive, and necrotic cells were Annexin V-FITC-negative, and PI-positive. To do this process, 1.5 × 105 cells/well were plated in 6-multiwell plates with or without 50 µM metformin. After 48 h, the cells were detached and analyzed by flow cytometry method. The experiment was carried out according to the Life Technologies Apoptosis Assay protocol, and the samples were analyzed by flow cytometry within 1 h using a FACS Caliber (Becton Dickinson, CA) in triplicate. Dot plot graphs were used to illustrate viable cells (lower left quadrant), early-phase apoptotic cells (lower right quadrant), late-phase apoptotic or dead cells (upper right quadrant), and necrotic cells (upper left quadrant).

Statistical analysis

Data analysis was performed using Graph Pad Prism 7 (Graph Pad PRISM V 5.04 analytical software). All data are expressed as mean ± SD. The mean differences between the two groups were analyzed by

Student's t-test, and more groups by One-way analysis of variance (ANOVA). Differences were considered statistically significant at $P < 0.05$.

Results

Expression detection results

To evaluate hsa_circ_0008285 expression in tumor tissues and cell lines, qRT-PCR analysis was performed on 35 tumor tissues, 35 adjacent normal tissues, and three cell lines. Our results demonstrated that the expression rate of hsa_circ_0008285 was markedly increased in ovarian cancer tissues (5.8±0.98) relative to their non-cancerous counterparts (2.3± 0.54) (Figure 1a).

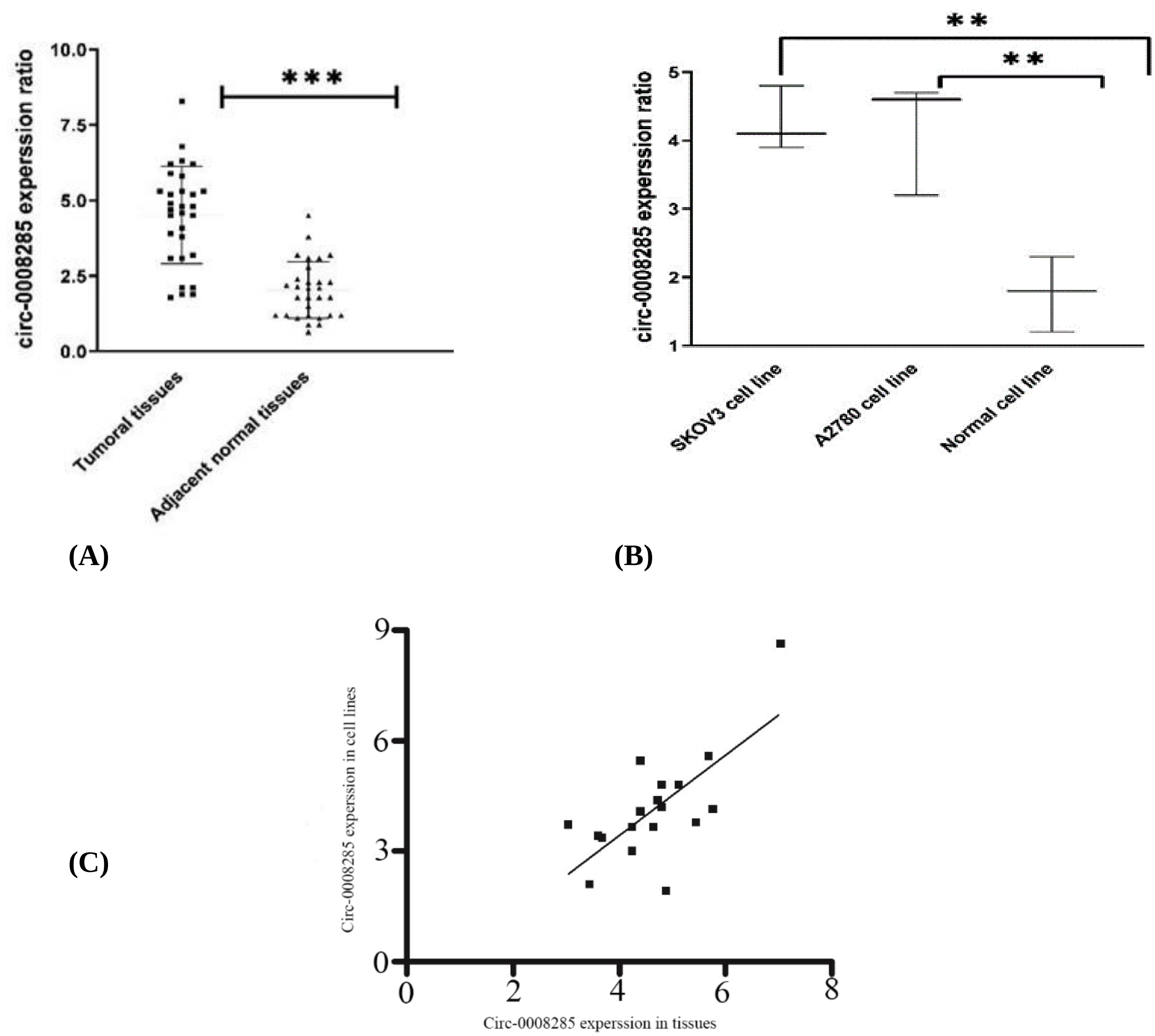


Fig. 1. Upregulation of circ-0008285 in ovarian cancer tissues and cell lines. (a) Expression ratio of circ-0008285 in 35 cancer tissues and adjacent normal tissues. (b) Expression ratio of circ-0008285 in SKOV3 and A2780 cell lines. (c) Correlation expression of circ-0008285 between tissues and cell lines ($R= 0.43$, $P<0.001$)). Error bars reprehensive mean+ Standard deviation (SD). ***$p< 0.001$.

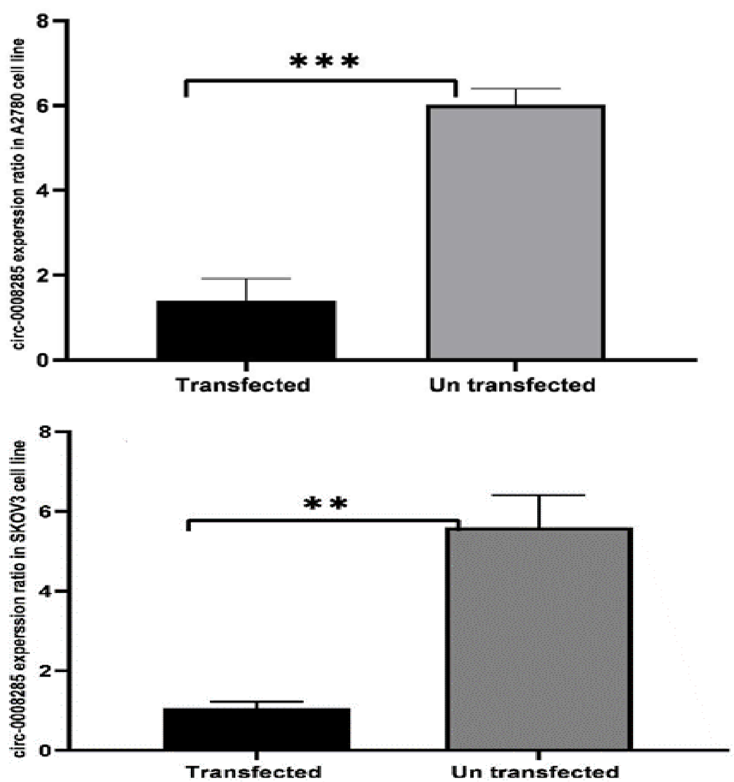
In addition, significant upregulation of hsa_circ_0008285 was seen in the SKOV3 cell line (4.26±0.43) and in the A2780 cell line (4.16± 0.65) in comparison to normal ovary cell line (OCE4) (1.76±0.45) (P=0.004) (Fig. 1b). We also detected a significant correlation between hsa_circ-0008285 expression in cancer tissues and cell lines (R= 0.43, P<0.001) (Fig. 1c).

Promotion of apoptosis in overexpressed Circ-0008285 ovarian cancer cells

To evaluate the role of hsa_circ_0008285 in ovarian cancer cell behavior, SKOV3 and A2780 cell lines were transfected with siRNA targeting circ-0008285. qRT-PCR results indicated that hsa_circ_0008285 was effectively downregulated in transfected SKOV3 cells (2.2±0.98) in comparison to untransfected cells (5.87±0.98) (P= 0.0002) (Figure 2a), also we saw significant down regulation in A2780 transfected cell line (1.8±0.54) compared to untransfected cells (5.4±0.89) (P=0.0077) (Fig. 2b). Our CCK-8 experiment results showed under expression of hsa_circ_0008285 significantly inhibited the proliferation of ovarian cancer cells (Figure 2c, d). In addition, flow cytometry results in the SKOV3 transfected cell line showed a significant increase in the apoptosis rate of transfected cells (27%) compared to un transfected cells (%18.2) p<0.0001) (Fig. 2e), and an increase in the A2780 cell line (29.2% versus 17.8%) (p<0.0001) (Fig. 2f). Results from western blot showed in SKOV3 and A2780 cells with under expression of hsa_circ_0008285 there was increased in expression of cleaved-caspase 3 protein level dramatically (Figure 2g). Based on these results, we conclude that the down-regulation of hsa_circ_0008285 suppresses proliferation and promotes apoptosis of ovarian cancer cells.

(A)

(B)



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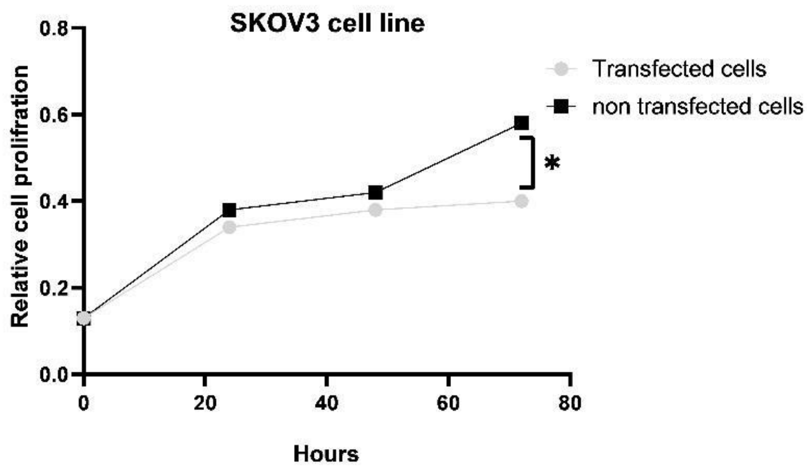
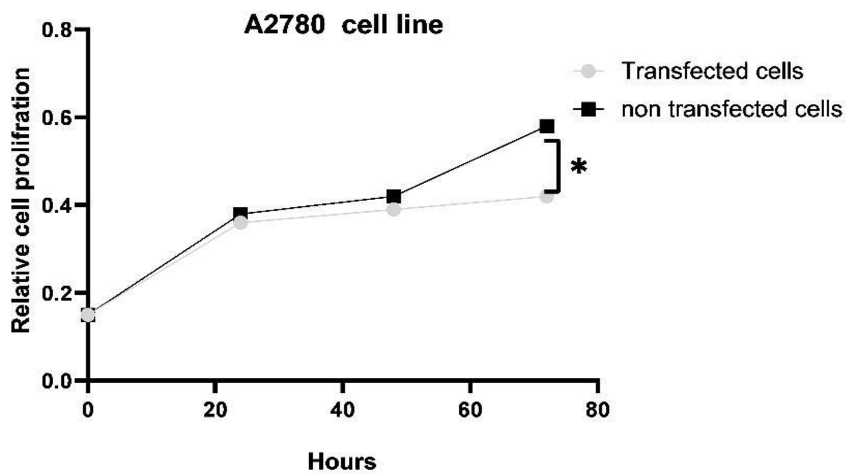
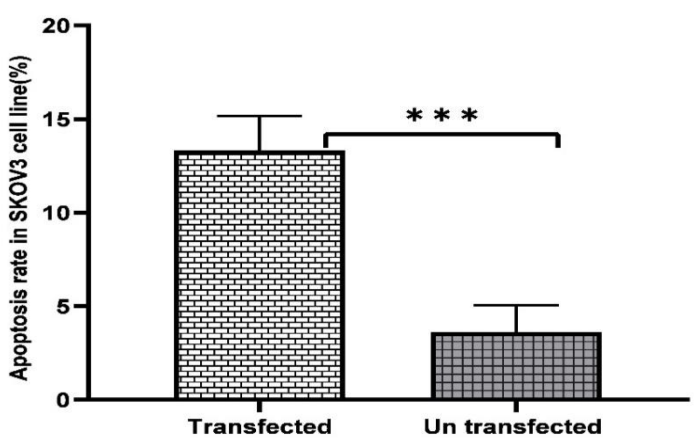
(C)

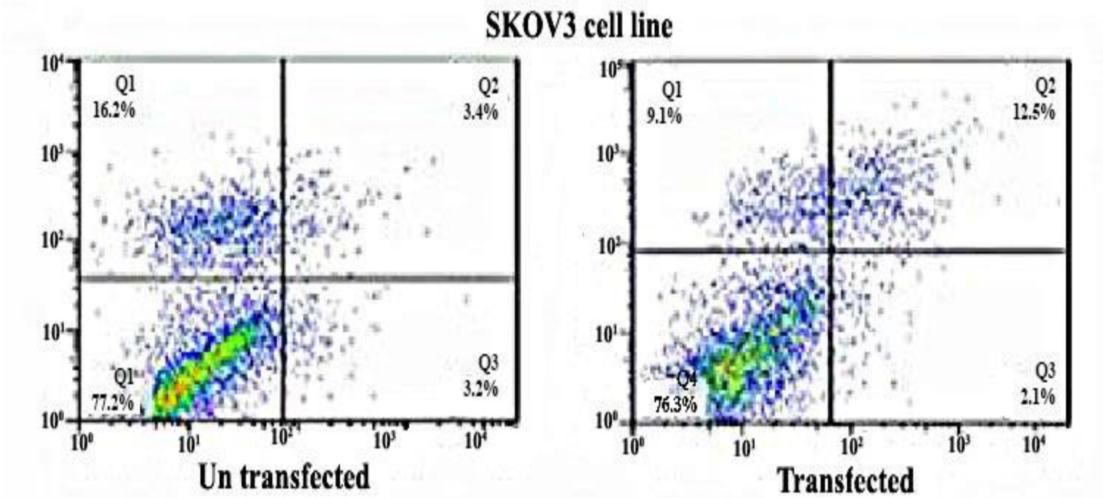

SKOV3 cell line
Transfected cells
non transfected cells
*
Relative cell prolifration
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
Hours

(D)

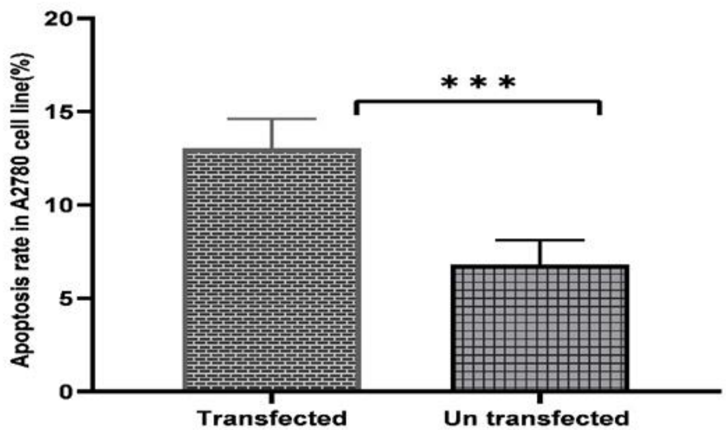

A2780 cell line
Transfected cells
non transfected cells
*
Relative cell prolifration
0.0
0.2
0.4
0.6
0.8
0
20
40
60
80
Hours

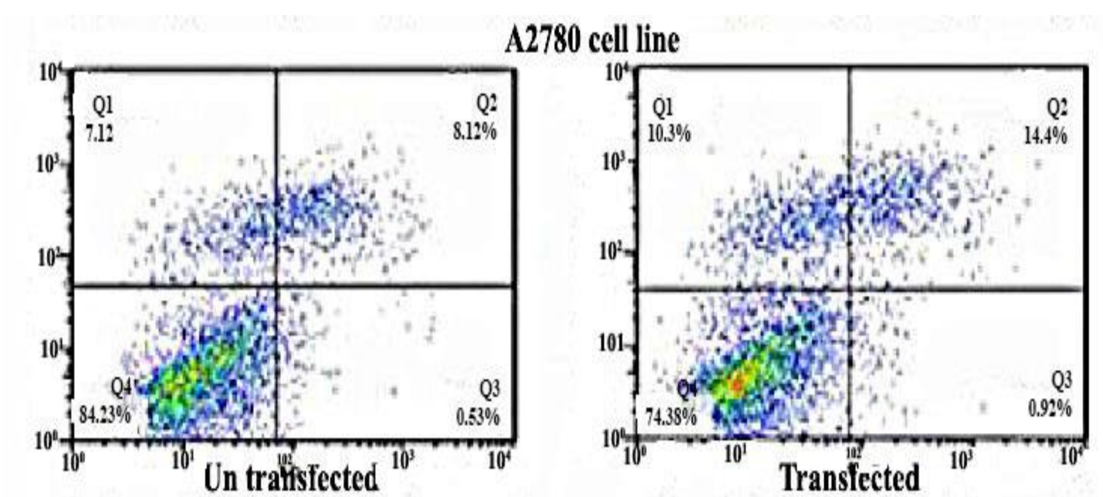
(E)


Apoptosis rate in SKOV3 cell line(%)
0
5
10
15
20
* * *
Transfected
Un transfected


SKOV3 cell line
Q1
16.2%
Q2
3.4%
Q3
3.2%
77.2%
Un transfected
Q1
9.1%
Q2
12.5%
Q4
76.3%
Q3
2.1%
Transfected

(F)


Apoptosis rate in A2780 cell line(%)
20
15
10
5
0
* * *
Transfected
Un transfected


A2780 cell line
Q1
7.12
Q2
8.12%
Q4
84.23%
Q3
0.53%
Un transfected
Q1
10.3%
Q2
14.4%
74.38%
Q3
0.92%
Transfected

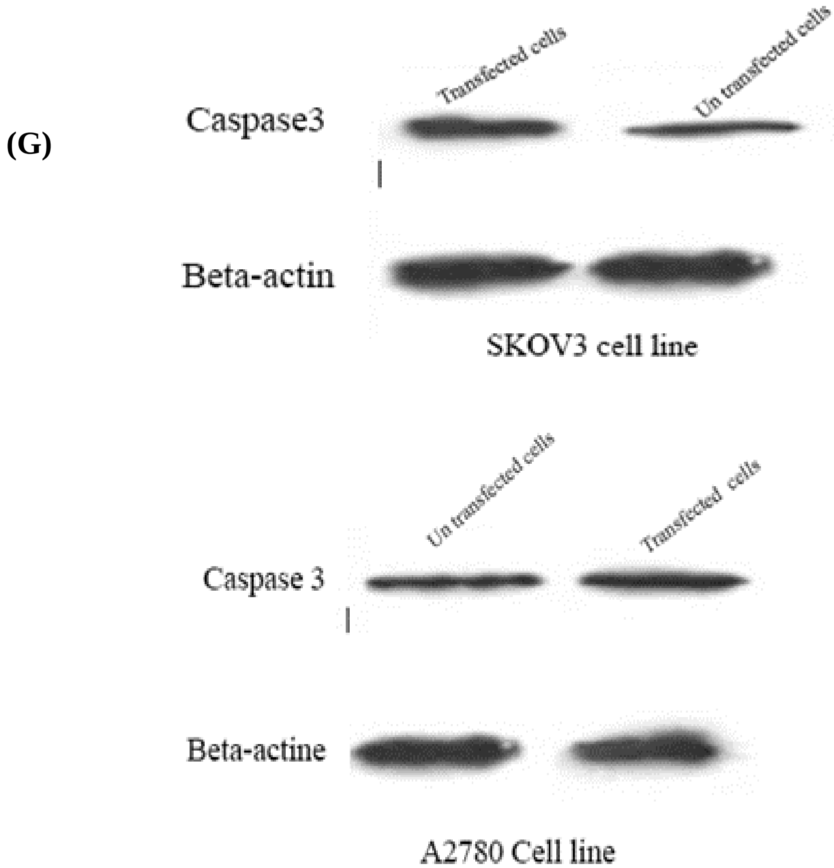


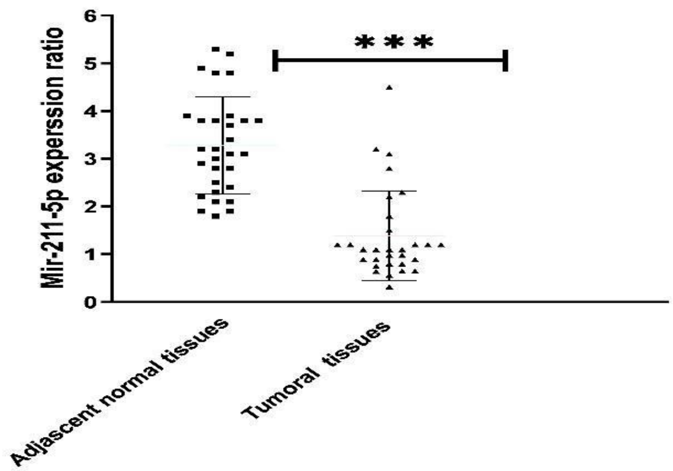
Fig. 2. Circ-0008285 downregulation suppressed cell proliferation and promoted apoptosis. (a) Expression ratio of circ-0008285 after transfection with inh- circ-0008285 in SKOV3 cell line. (b) Expression ratio of circ-0008285 after transfection with inh- circ-0008285 in A2780 cell line. (c) Down-regulation of circ-0008285 caused cell proliferation suppuration in SKOV3. (d) Down-regulation of circ-0008285 caused cell proliferation suppuration in the A2780 cell line. (e) Under expression of circ-0008285 increases the apoptosis rate of the SKOV3 cell line. (f) Under expression of circ-0008285 increased apoptosis rate of the A2780 cell line (Apoptosis was evaluated by FACS analysis, the different quadrants report the percentage of cells. Non-viable necrotic cells, upper left (Q1), late apoptotic cells, top right (Q2), early apoptotic cells, bottom right (Q3), viable cells lower left (Q4). The experiment was repeated three times and the results were always similar.) (g) Under expression of circ-0008285 increased Caspase protein expression in transfected SKOV3 and A2780 cell lines. Error bars represent the mean of three separate determinations ± SD. *p< 0.05. **p< 0.01. ***p< 0.001.

Luciferase assay results

Based on our Bioinformatics and database analysis results (circintractome), the three miRNAs with the highest binding sites for hsa_circ_0008285 were miR-211-5p, miR-519e, and miR-384. We evaluated the expression of these miRNAs in ovarian cancer patient tissues and found that miR-211-5p expression was significantly downregulated in cancer tissues (0.89±0.12) in comparison to normal adjacent tissue (3.5±0.22) (P<0.0001) (Figure 3a), and this downregulation was observed in the studied cell lines (Figure 3b) (P<0.001), whereas miR-519e and miR-384 showed no significant difference in expression between ovarian cancerous tissue and adjacent normal tissues. Primer sequences mentioned in Table 1.

[DOI: 10.22088/IJMCM.BUMS.12.4.401]

(A)



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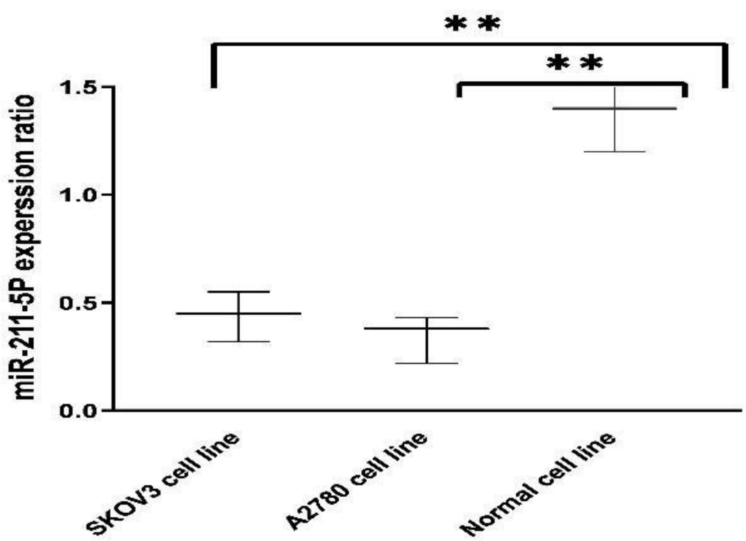

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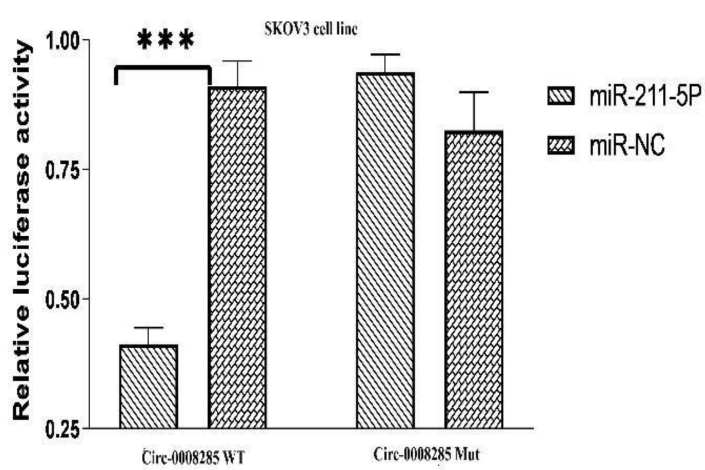

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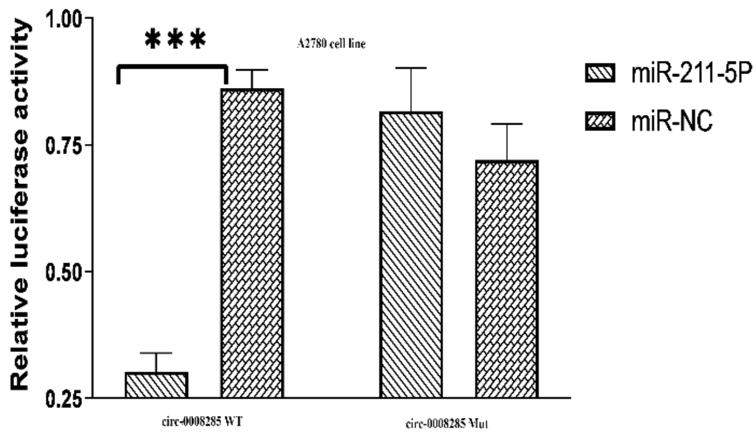
(D)



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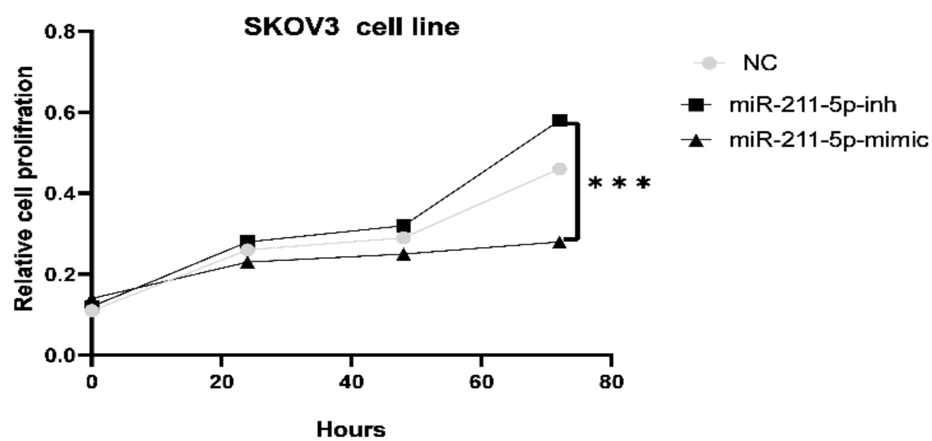

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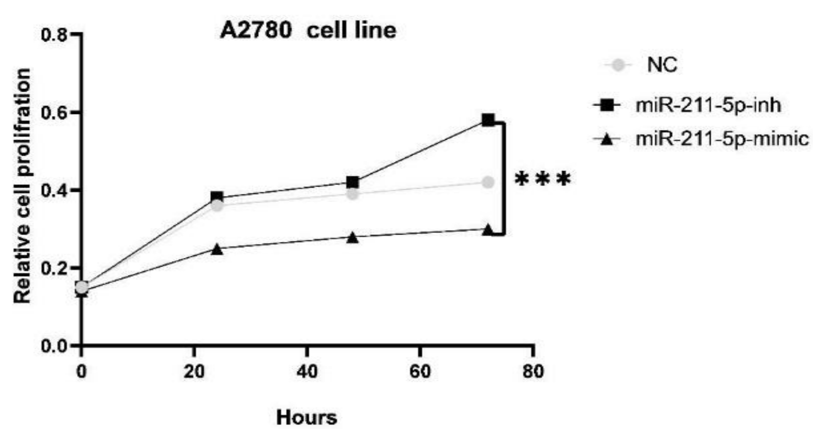

Fig. 3. Circ_0008285 is directly bound to miR-211-5p in ovarian cancer cells. (a) Relative expression levels of miR-211-5p were detected by qRT-PCR in 35 ovarian cancer tissues and paired adjacent normal tissues. (b) Relative expression levels of miR-211-5p were detected by qRT-PCR in SKOV3 and A2780 cell lines. (c) Dual-luciferase reporter assays were performed to detect the correlation between miR-211-5p and circ_0008285 in SKOV3 cell line (d) and A2780 cell line. (e) MiR-211-5p inhibition promoted the proliferation of SKOV3 and (f) A2780 cells. Error bars represent mean ± SD. *p< 0.05, **p< 0.01, ***p< 0.001.

Table 1. Primer sequences of studied genes and RNAs.

Gene	Primer Sequences
Has_circ_0008285_divergent-F	TCATAGCCTTTCCACCGA
Has_circ_0008285_divergent-R	ACAGTGCACCCGAAGTG
Has_circ_0008285_convergent-F	ACCTCTCCTAAGGCACTGGT
Has_circ_0008285_convergent-R	GGTCCAGTTTCTCAGGGCTC
MiR-211-5P	F GATGCTGTAATGGATGATATGA R ATTGGAACGATACAGAGAAGATT
SIRT-1	F TGCTGGCCTAATAGAGTGGCA R CTCAGCGCCATGGAAAATGT
GAPDH	F GTCTCCTCTGACTTCAACAGCG R ACCACCCTGTTGCTGTAGCCAA

A Dual-Luciferase Reporter Assay was performed to evaluate the interaction between circ-0008285 and miR-211-5p. The results demonstrated that miR-211-5p significantly decreased the luciferase activity of wild-type circ-0008285(WT), whereas miR-211-5p had no effect on mutant circ-0008285 (Mut) in SKOV3 and A2780 cells (P<0.0001) (Figure 3c, d). A diagrammatic sketch of the potential binding sites obtained from the bioinformatics analysis of miR-211-5p in circ_ 0008285 is shown in Table 2.

Evaluation of the regulatory actions of miR-211-5p in ovarian cancer cell lines showed that under expression of miR-211-5p dramatically promoted the proliferation of ovarian cancer cells, whereas overexpression of miR-211-5p inhibited the proliferation of ovarian cancer cells (Fig. 3e, f). Thus, the binding ability between circ-0008285 and miR-518a-5p was verified.

Table 2. The diagrammatic sketch of the potential binding sites between miR-211-5p and circ_0008285

hsa_circ_0008285 vs miRNA-211-5p	
Score	158
Energy	-15.37 kCal/mol
Position	20.41
miRNA-211-5p	3 UCCGCUUCCUACUGUUUCCCUU5 | | | | | | | | | | | |
has_circ_0008285	5 AAAGGAAAAATAAAAAAGGGAA3

MiR-211-5p negatively regulated SIRT-1 in ovarian cancer cells

The downstream target of miR-211-5p was predicted by Target Scan and SIRT-1 was selected for further assays. Bioinformatics analysis suggested that SIRT-1 mRNA harbored a putative miR-211-5p binding site in its 3′-UTR. Additionally, we found that SIRT-1 mRNA expression significantly increased in ovarian cancer tissues (3.7±0.99) compared with that in adjacent normal tissues (1.7±0.87) (P< 0.0001), (Figure 4a).

We used the Dual-Luciferase Reporter Assay to further explore whether miR-211-5p directly regulates SIRT-1 expression via interaction with its 3′-UTR. We then constructed wild-type and mutant SIRT-1 3′-

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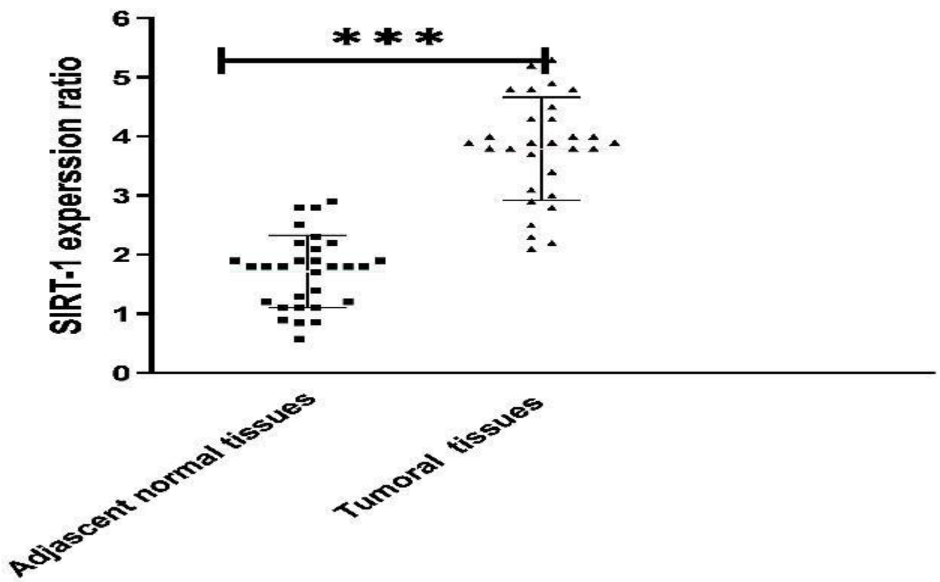
UTR reporter plasmids, SKOV3 and A2780 cells were co-transfected with miR-211-5p or scramble, and wild-type SIRT-1 or mutated SIRT-1, respectively. The results indicated that miR-211-5p significantly decreased the luciferase intensity in wild-type SIRT-1, whereas miR-211-5p could not change the luciferase intensity of the mutant SIRT-1 group (Figure 4b, c). In addition, SIRT-1 mRNA and protein expression were down regulated in miR-211-5p overexpressed ovarian cancer cell lines and up regulated in ovarian cancer cell lines with low expression miR-211-5p (Figure 4d, e). We concluded that miR-211-5p negatively regulated SIRT-1 in ovarian cancer cells.

(A)



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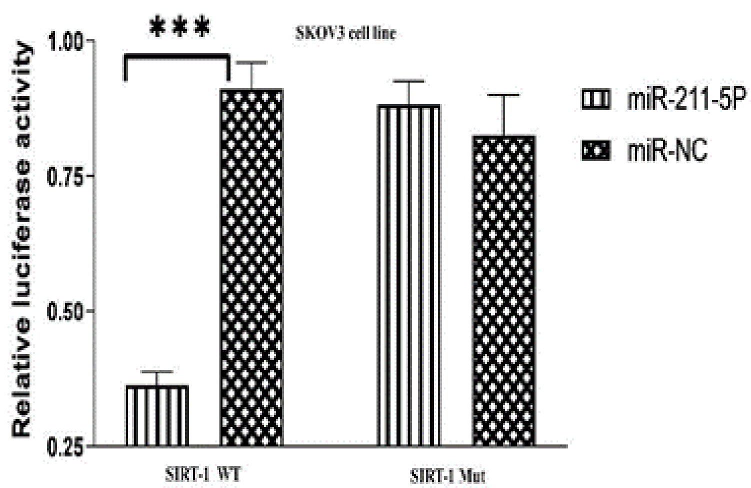

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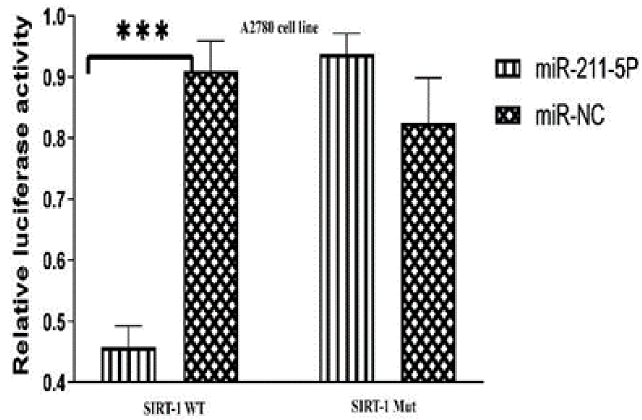
(C)



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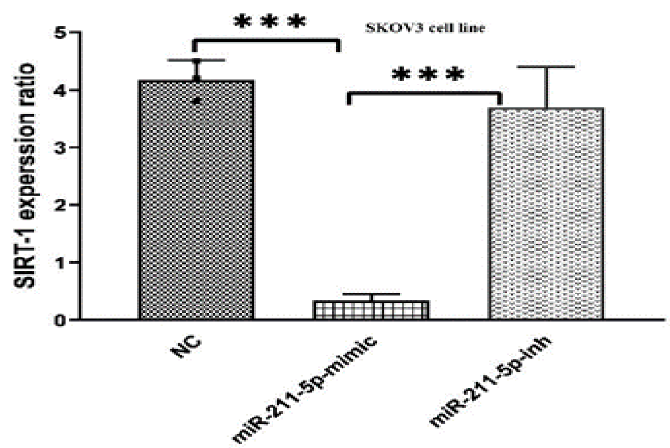


(E)

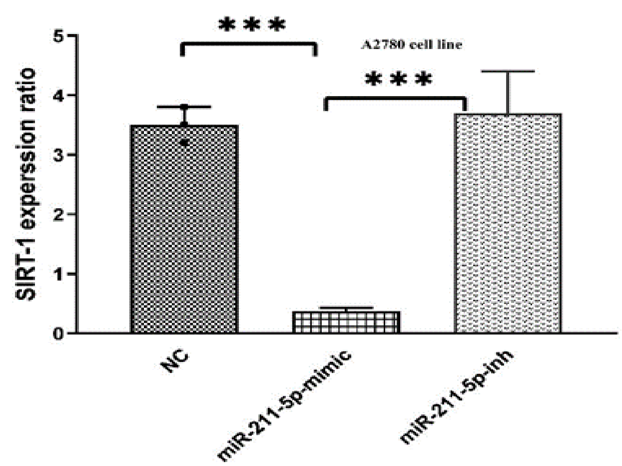
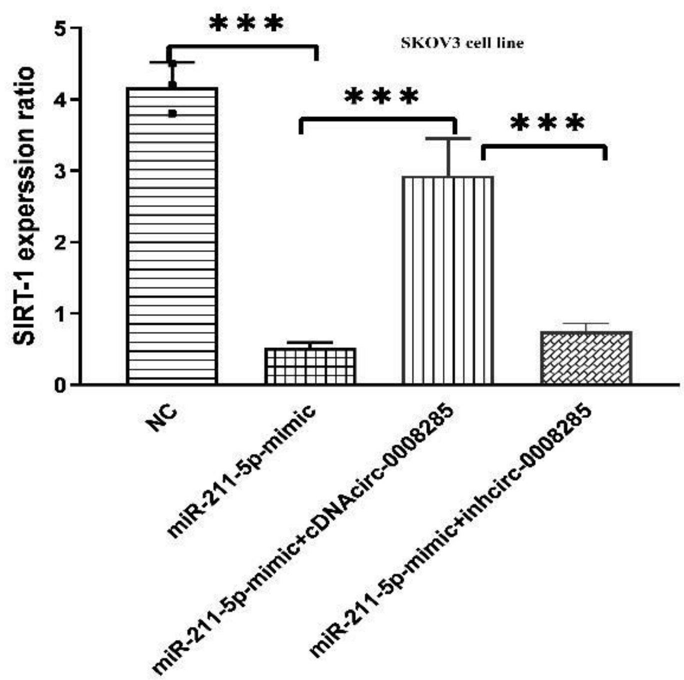


Fig. 4. MiR-211-5p negatively regulated the SIRT-1 gene in ovarian cancer cells. (a) Relative expression levels of Sirt-1 were detected by qRT-PCR in 35 ovarian cancer tissues and paired adjacent normal tissues. (b) Dual-luciferase reporter assays were performed to detect the correlation between miR-211-5p and Sirt-1 gene in SKOV3 and (c) A2780 cell line. (d) Sirt-1 expression ratio in SKOV3 cell line after miR-211-5p mimic transfection (e) and in A2780 cell line. Error bars represent mean ± SD. *p< 0.05, **p< 0.01, ***p< 0.001

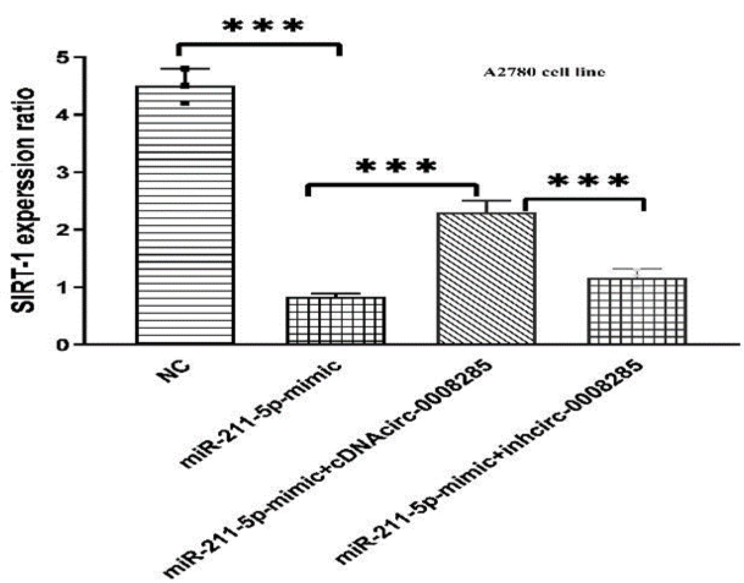
Sponging of miR-211-5p by Circ-0008285 increased SIRT-1 expression

In other sections, our real-time PCR and Western blot data showed that inhibition of hsa_circ_0008285 could decrease the expression of SIRT-1 which was upregulated with downregulation of miR-211-5p in ovarian cancer cell lines (Figure 5a, b). We assumed that hsa_circ_0008285 was involved in enhancing the expression of SIRT-1 (a downstream target of miR-211-5p) by functioning as a miR-211-5p sponge.

(A)



(B)



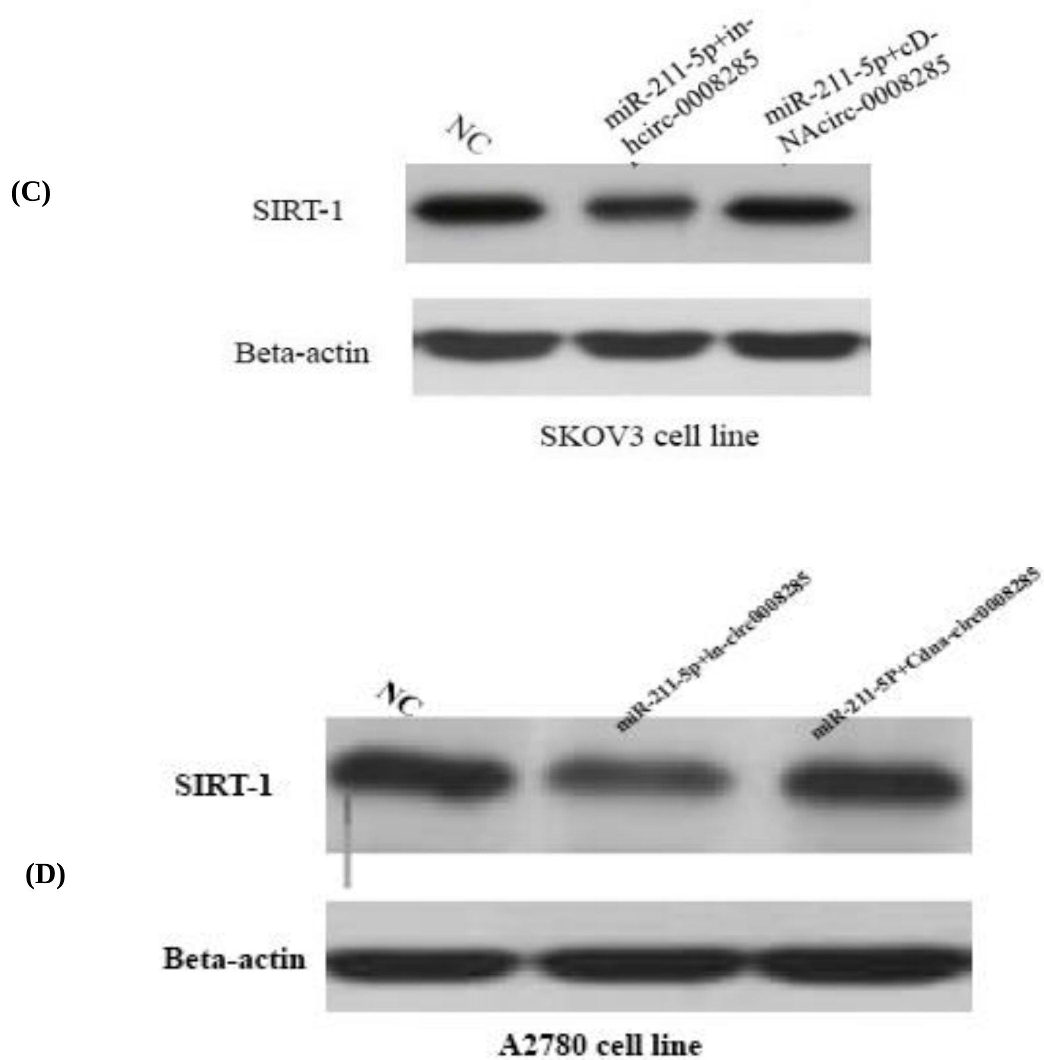


Fig. 5. Circ_0008285 enhanced SIRT-1 expression via sponging miR-211-5p in ovarian cancer cells. (a,b). The mRNA level of SIRT-1 in SKOV3 and A2780 cells after transfection of mir-211-5p mimic with cDNA and inhibitor of circ-0008285. (c,d). The protein level of SIRT-1 in SKOV3 and A2780 cells after transfection. Error bars represent the mean of three separate determinations ± standard deviation (SD). **p< 0.01. ***p< 0.001.

Circ_0078607 decrease cells proliferation of ovarian cancer cells via regulating miR-211-5p/SIRT-1 signaling

The CKK-8 assay showed that down regulation of hsa_circ_0008285 could inhibit the proliferation of ovarian cancer cells (Figure 6 a, b). Our results also showed that hsa_circ_0008285 could inhibit the invasion of ovarian cancer cells (Figure 6 c, d). These data confirmed that hsa_circ_0008285 could affect the function of miR-211-5p in suppressing ovarian cancer progression.

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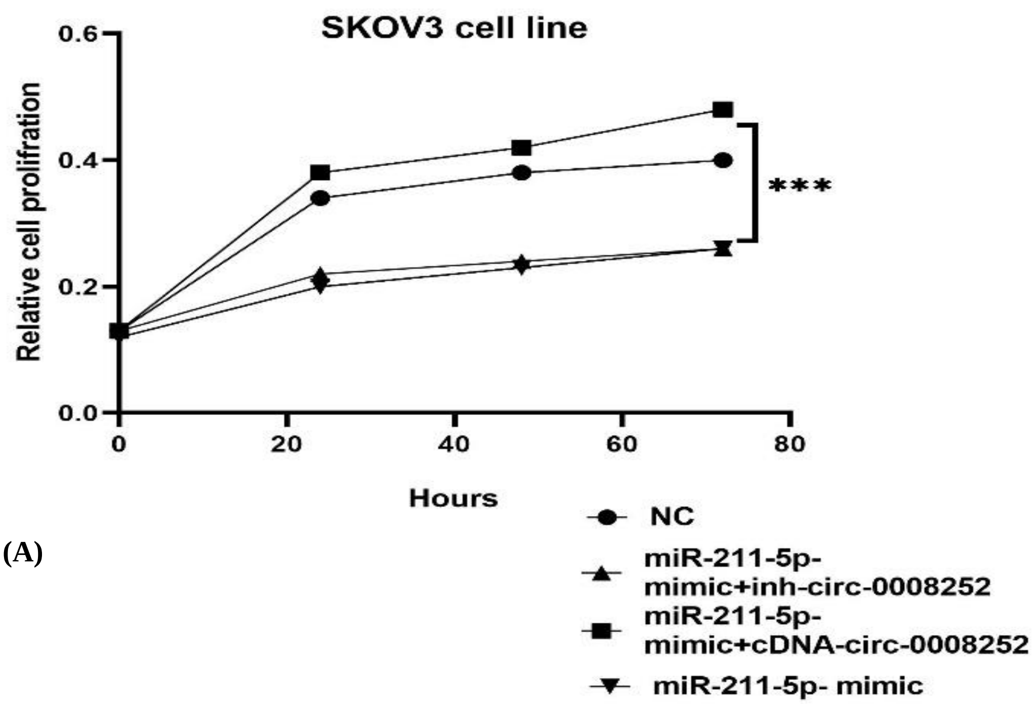
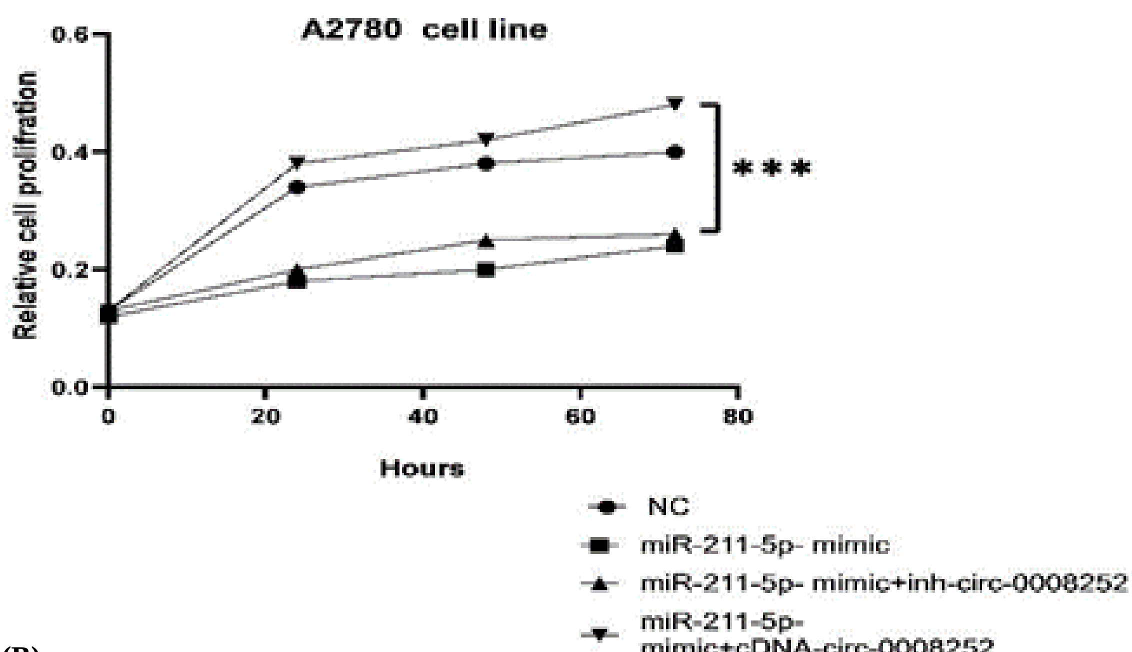


(A)



(B)

[DOI: 10.22088/IJMCM.BUMS.12.4.401]

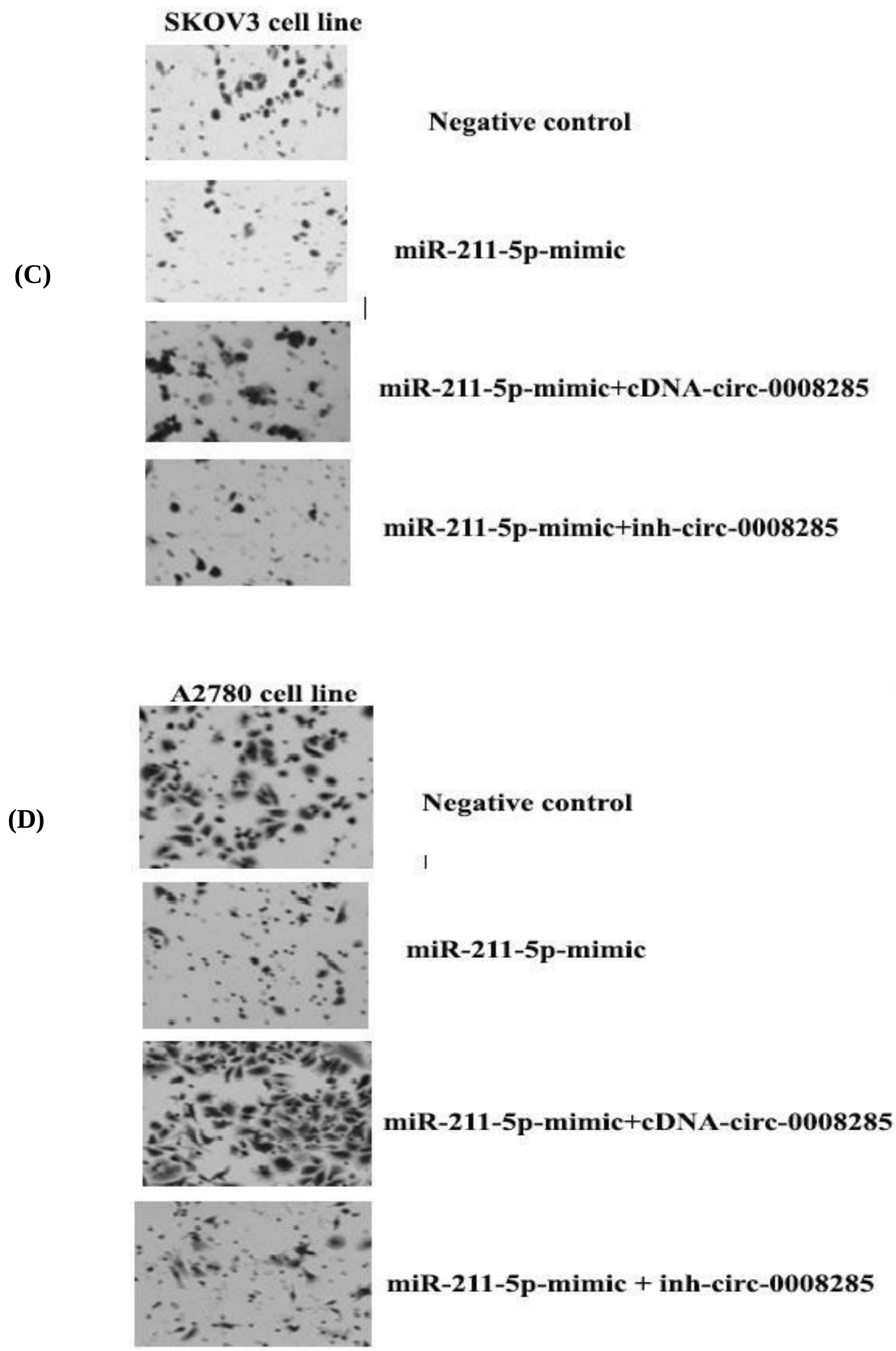


Fig. 6. Circ_0008285 repressed proliferation of ovarian cancer cells via regulating miR-211-5p/SIRT-1 signaling. (a,b) CCK-8 assay was performed to analyze the proliferation of SKOV3 and A2780 cells under different transfections. (c,d) The trans well assay was performed to detect the invasion of SKOV3 and A2780 cells under different transfections. Error bars represent the mean of three separate determinations ± standard deviation (SD). *p< 0.05, **p< 0.01, *** p< 0.001, **** p< 0.0001.

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Discussion

Ovary cancer manifests itself in old age and is one of the women's cancers with a poor prognosis (31). Considering the targeted therapy of cancers that have been made in the treatment of cancers, including ovarian cancer, identifying the molecular pathways involved in the pathogenesis of this disease plays an important role in its treatment (32). The role of circRNAs as a recently identified type of non-coding RNAs in ovarian cancer is also significant (33). In our study, we used the HiSeq 2000 (Illumina, San Diego, CA) sequencing method for 6 pairs of ovarian cancer and adjacent non-cancerous tissue samples. Mapping on the reverence genome was done by Top Hat (GRCH37.p13 NCBI). Circ_0008285 was one of the most differentially expressed circRNA and has significant upregulation in tumoral tissues and confirmation of this upregulation was done on the 35 pairs of tumor and normal tissues of ovary for the first time. We also with function assay showed that under expression of circ_0008285 significantly suppressed ovarian cancer cells proliferation and invasion as well as promoted apoptosis of ovarian cancer cells. These results suggested that hsa_circ_0008285 might act as a new oncogenic circRNA in ovarian cancer progression.

Several studies have shown one of the main roles of circRNAs is the sponging of miRNAs and via this function participate in circRNA, miRNA, and target gene networks for regulating cancer pathogenesis (34–37). Based on circtranscriptome and circbase data miR-211-5p is one of the targets of circ-0008285. About the role of miR-211-5p in the pathogenesis of different cancers several studies were done. Wu et al., 2022 showed that miR-211-5p suppressed the invasion, migration, proliferation, and progression of CRC cells through sponging SPARC-related growth factor pathways (38). Wang et al., 2020 reported miR-211-5p may play a role as a tumor suppressor and prognostic marker in bladder cancer (39). Qin et al., 2020 showed targeting miR-211-5p and the downstream gene ACSL4 will possibly provide novel insight and represents a promising approach to future therapy of HCC patients (40). About the role of this miRNA in the pathogenesis of ovarian cancer Li et al., 2020 reported CircNRIP1 silencing could inhibit the PTX resistance of ovarian cancer via regulating the miR-211-5p/HOXC8 axis, showing that circNRIP1 might be a potential target for ovarian cancer resistance treatment (41). In line with those above studies, our results showed that miR-211-5p was downregulated in ovarian cancer cells and _circ_0008285 interacted with miR-211-5p as a competing endogenous RNA to inhibit their action in ovarian cancer. Based on the above data till here, we concluded that has_circ_0008285 might exert an oncogenic role by sponging miR-211-5p in ovarian cancer progression.

SIRT1 is over-expressed in a majority of ovarian cancers (42, 43) implying the role of SIRTs in ovarian tumorigenesis. High expression of SIRT1 is also associated with malignant transformation of human ovarian tissue and with ovarian carcinoma with poor prognosis (44). Therefore, SIRT1 inhibition appears to be a good strategy to overcome cancer drug resistance and improve therapy. In the newest article, the role of this gene in ovary function and polycystic ovary syndrome (PCOS) has been shown (45). In our study, SIRT-1 was forecasted to be the direct target of miR-211-5p by bioinformatics analysis. RT-PCR showed that SIRT-1 expression was significantly increased in ovarian cancer tissues and was negatively correlated with miR-211-5p expression. In addition, this study demonstrates that down regulation of SIRT-1 could inhibit the proliferation and invasion of ovarian cancer cells and increase the apoptosis of cancer cells. This phenomenon was the same as the effect of circ-0008285 in ovarian cancer cells. We further demonstrated

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that miR-211-5p mimics down regulated SIRT-1 mRNA and protein expression while miR-211-5p inhibitor upregulated SIRT-1 mRNA and protein expression in ovarian cancer cells. In addition, the down-regulation of circ-0008285 could significantly down regulated the expression of SIRT-1 which was over-expressed by miR-211-5p inhibition. So, we speculated that circ-0008285 acted as a sponge of miR-211-5p and regulated the expression of SIRT-1 in this way. Thus, the circ-0008285 / miR-211-5p / SIRT-1 network may facilitate a novel aspect of the treatment of patients with ovarian cancer. However, further investigation should be made on nude mice to verify our conclusion in vivo.

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