European Review for Medical and Pharmacological Sciences 2019; 23: 2366-2373

MiR-539-3p promotes the progression of epithelial ovarian cancer by targeting SPARCL1

Y.-B. GONG, X.-H. FAN

Department of Obstetrics and Gynecology, Hanchuan People's Hospital, Hanchuan, China

Abstract. – OBJECTIVE: The aim of this study was to investigate the effect of microRNA-539-3p (miR-539-3p) on the development of epithelial ovarian cancer (EOC), and to explore the possible underlying mechanism.

PATIENTS AND METHODS: A total of 40 paired EOC tissues and adjacent normal ovarian tissues were surgically resected in Hanchuan People's Hospital. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of miR-539-3p in EOC tissues and cell lines. Targeted regulatory mechanism of miR-539-3p on SPARC-like protein 1 (SPARCL1) was identified by luciferase reporter and Western blot assays. Furthermore, the effects of miR-539-3p/SPARCL1 axis on the malignant behaviors of EOC cells, including proliferation, invasion and migration abilities, were confirmed by cell counting kit-8 (CCK-8), transwell and scratch wound assays.

RESULTS: QRT-PCR showed that the expression of miR-539-3p was significantly up-regulated in EOC tissues and cell lines. SPARCL1 was a direct target of miR-539-3p in EOC cells. Overexpression of miR-539-3p significantly promoted the proliferation, migration and invasion of SKOV3 cells. Furthermore, co-transfection of miR-539-3p inhibitor and si-SPARCL1 could remarkably restore the migration and invasion abilities of SKOV3 cells.

CONCLUSIONS: MiR-539-3p acted as an oncogene in EOC by targeting SPARCL1. MiR-539-3p/SPARCL1 axis, as a target for the treatment of EOC, might become a feasible and new method of tumor treatment.

Key Words:

MicroRNA-539-3p (miR-539-3p), Epithelial ovarian cancer (EOC), SPARC-like protein 1 (SPARCL1), Proliferation, Invasion, Migration.

Introduction

Ovarian cancer (OC) is one of the three major malignant tumors of female reproductive system. OC is also a common gynecologic malignant tumor with the highest mortality rate in the world. It is estimated that there are 22,240 new OC cases in the United States in 2018, with about 14,070 deaths[1]. OC occurs in 1/2500 postmenopausal women, accounting for 5-6% of all cancer-related deaths. More than 70% of OC cases have been already in FIGO III or IV stage when they first visit the hospital. The 5-year survival of OC patients is less than 30%, showing poor prognosis[2]. Epithelial OC (EOC) is the most common type of OC. In the past 40 years, surgical techniques and chemotherapy have greatly improved, and some progress has been made in EOC therapy. There is still a lack of early effective screening methods. Most EOC patients have already been in the advanced stage when first diagnosed. Additionally, chemotherapy resistance and recurrence may occur in patients after chemotherapy, leading to high EOC mortality rate. Currently, this remains a difficulty in the medical field. Therefore, it is urgent to understand the pathogenesis of EOC and to find biomarkers and new treatments for early screening and prognosis.

Micro ribonucleic acids (miRNAs) were first discovered in *Caenorhabditis elegans* in 1993[3]. They exist in the genome of most eukaryotes, including human beings. Mature miRNA is a type of endogenous non-coding single-stranded small RNA with about 20-22 nucleotides in length. It is highly conserved in many species. The main function of miRNA is to participate in the formation of RNA silencing complexes and to act as a post-transcriptional regulator of gene expression[4]. Previous studies have showed that miRNAs exert crucial roles in many basic life activities. Under various pathological conditions, they can be abnormally expressed. The potential link between miRNAs and malignant tumor was first discovered in chronic lymphocytic leukemia[5,6]. Since then, numerous studies have revealed abnormal expressions of different miRNAs in various human malignant tumors. Over-expressed miRNAs

Corresponding Author: Xiuhua Fan, BM; e-mail: 453388345@qq.com

may play an oncogenic role by suppressing tumor suppressor genes. However, down-regulated miRNAs can also exert this effect by negatively modulating oncogenes[7-10]. Furthermore, it is surprising that about 50% of annotated human miRNAs are related to cancer. These findings suggest that miRNAs are of great significance in cancer research[11].

MicroRNA-539-3p (miR-539-3p) is an important component of the microRNA regulatory network. Recently, it has been certified that miR-539-3p exerts its critical regulatory effect on many diseases[12-14]. However, the exact role of miR-539-3p in EOC has not been fully elucidated. The aim of this study was to investigate the function of the miR-539-3p axis in the occurrence and progression of EOC. Our findings might help to provide new ideas and theoretical basis for the clinical treatment and prevention of EOC.

Patients and Methods

OC Cases and Cell lines

A total of 40 paired EOC tissues and adjacent normal ovarian tissues were surgically resected from EOC patients who received treatment in Hanchuan People's Hospital from 2016 to 2018. No pre-operative chemotherapy and radiotherapy was performed in all subjects. This study was approved by the Ethics Committee of Hanchuan People's Hospital. Signed written informed consents were obtained from all participants before the study.

Human ovarian serous papillary cystadenocarcinoma cell line (SKOV3) and epithelial ovarian cell line (EOC) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were cultured in the medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL Penicillin and 100 μg/mL Streptomycin, and maintained in an incubator containing 5% CO_2 at 37°C.

Cell Transfection

Cells were first inoculated into 24-well plates at a density of 1×10^4 cells/well. The medium was replaced with the serum-free medium when cell density reached 80%. MiR-539-3p inhibitor (100 mmol/L), negative control (NC) (100 mmol/L) or si-SPARC-like protein 1 (SPARCL1) (2 μg) was transfected into cells according to the instructions of Lipofectamine 2000. 6 h after transfection, the medium was replaced with fresh medium, followed by culture for subsequent experiments. Three groups were established, including: miR-NC group (negative control), miR-539-3p inhibitor (SKOV3 cells transfected with miR-539-3p inhibitor) and inhibitor + SPARCL1 (SKOV3 cells co-transfected with miR-539-3p inhibitor and si-SPARCL1).

Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) Analysis

Total RNA in tissues or cells was extracted according to the instructions of the total RNA extraction kit (TaKaRa, Otsu, Shiga, Japan). The purity and concentration of extracted RNA were then detected. Subsequently, extracted RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) using a reverse transcription kit (TaKaRa, Otsu Shiga, Japan). With cDNA as a template, fluorescence quantitative PCR was performed using the PRISM7000 quantitative PCR instrument. Specific reaction conditions were: reaction at 94°C for 10 min, 94°C for 30 s, 60°C for 30 s and 72°C for 40 s, for a total of 40 cycles, and extension at 72°C for 10 min. The relative expression level of miR-539-3p was calculated by the $2^{-\Delta\Delta Ct}$ method. U6 was used as an internal reference. Primer sequences used in this study were as follows: miR-539-3p, F: 5'-GCAGGAACGGTTCTCCACTC-3', R: 5'-GTCCCTAGGGATGTGTAAGGA-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'.

Luciferase Reporter Gene Assay

TargetScan, miRDB and microRNA websites predicted that SPARCL1 was the target gene of miR-539-3p. The binding sequence of miR-539-3p at the 3'-end of SPARCL1 was mutated using a point mutation kit (Agilent Technologies, Santa Clara, CA, USA). Subsequently, miR-539-3p mimics and miR-NC were transferred into SPARCL1 wild-type and mutant cells, respectively. After 48 h, the culture medium was removed, and the cells were washed with washing liquid. After discarding the washing liquid, 1× cell lysate was added into each well to lyse the cells. Thereafter, the cells were shaken on an oscillator for 5-10 min at room temperature. After transfer into a centrifuge tube, the cells were centrifuged at 3000 g for 5 min. The supernatant was then discarded. Finally, the luciferase activity of each sample was determined according to relevant instructions.

Western Blot (WB) Analysis

Lysis buffer was utilized to extract total protein from OEC cells. The concentration of extracted protein was determined by the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Subsequently, the protein sample was subjected to electrophoresis with 10% polyacrylamide gel and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After blocking, the membranes were incubated with primary antibodies at 4°C overnight. On the next day, the membranes were first washed with Tris Buffered Saline and Tween 20 (TBST) on a decolorizing shaker at room temperature for 4 times, with 10 min each time. After that, the membranes were incubated with the corresponding secondary antibody at room temperature for 1 h. The membranes were then washed again with TBST on a decolorizing shaker at room temperature for 5 times, with 10 min each time. Immuno-reactive bands were visualized by enhanced chemiluminescence (ECL) reaction. Finally, the gel image processing system was adopted to analyze the gray value of target bands.

Cell Proliferation

Cells in the logarithmic growth phase were collected and inoculated into 24-well plates at a density of 1×10^4 cells per well. Cell transfection was performed, and triplicate wells were set for each group. At 24, 48, 72, and 96 h after transfection, cell proliferation was detected, respectively. Briefly, 10 μL cell counting kit-8 (CCK-8) solution (the volume ratio of CCK-8 to reaction system solution = 1: 10) (Dojindo, Kumamoto, Japan) was added to each well, followed by incubation in an incubator for 2 h in the dark. Finally, the absorbance at 450 nm was measured by a microplate reader.

Cell Invasion

Transfected cells were first prepared into cell suspension with cell density of 1×10^6 cells/mL using serum-free culture medium. Then prepared cell suspension was added into the upper transwell chamber covered with an artificial basement membrane. At the same time, the culture medium containing 20% FBS was added into the lower chamber. After culture at 37°C for 24 h, the cells at the bottom of the upper chamber were stained with 0.5% crystal violet. However, the cells at the inner side of the upper chamber were removed with cotton swabs. Finally, invading cells were observed under a microscope (Nikon, Tokyo, Japan), and the number of cells was counted.

Cell Migration

Transfected cells were first prepared into cell suspension with a cell density of 1×10^6 cells/mL. Then the cells were added into 6-well plates, and cultured overnight to form monolayer cells. A transverse line was drawn on the monolayer cells with a 10 μL pipette tip. After washing with phosphate-buffered saline (PBS) for 3 times, scratching cells were removed. Finally, images were taken under a microscope to measure the scratch width after 24 h of culture.

Statistical Analysis

Prism 6.02 software (La Jolla, CA, USA) was used for all statistical analysis. Statistical analysis was performed with Student's *t*-test or *F*-test. All *p*-values were two-sided, and $p < 0.05$ were considered statistically significant.

Results

MiR-539-3p Expression Was Significantly Reduced in Both EOC Tissues and Cells

Changes in the expression of small RNAs in cancer cells usually indicate their roles. Up-regulated small RNAs act as oncogenes, while down-regulated small RNAs serve as tumor suppressor genes.

In this study, we found that miR-539-3p expression in EOC tissues was significantly higher than adjacent non-malignant tissues. Consistent with the results in clinical specimens (Figure 1A). We also found that the expression of miR-539-3p in EOC cell line (SKOV3) was significantly up-regulated when compared with ovarian epithelial cell line (OEC) (Figure 1B). Results of qRT-PCR indicated that miR-539-3p acted as an oncogene in EOC. However, the specific role of miR-539-3p in EOC still needed further experimental verification.

SPARCL1 was a Direct Target of miR-539-3p in EOC Cells

Target genes of miR-539-3p were predicted using online prediction software. It was found that SPARCL1 might be the potential target of miR-539-3p (Figure 2A). Luciferase reporter gene assay was then performed to verify whether SPARCL1 was the direct target of miR-539-3p..

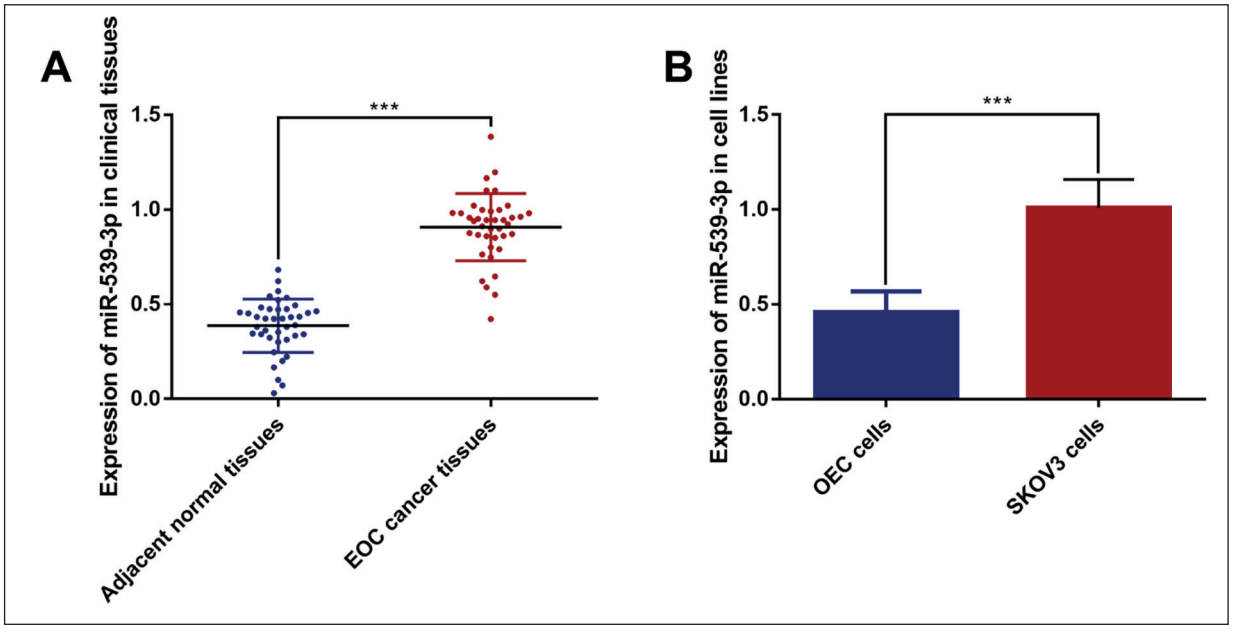

Figure 1. The expressions of miR-539-3p in EOC tissue samples and cells. ***A,*** The expression of miR-539-3p in EOC tissues and corresponding adjacent normal ovarian tissues (***$p < 0.001$). ***B,*** The expression of miR-539-3p in EOC cell line (SKOV3) and ovarian epithelial cell line (OEC) (***$p < 0.001$).

The results showed that miR-539-3p could significantly inhibit the luciferase activity of cells transfected with wild type SPARCL1-3'UTR plasmid. However, it had no effect on the luciferase activity of cells transfected with mutant type SPARCL1-3'UTR plasmid. These results indicated negative regulation of miR-539-3p on SPARCL1 (Figure 2B).

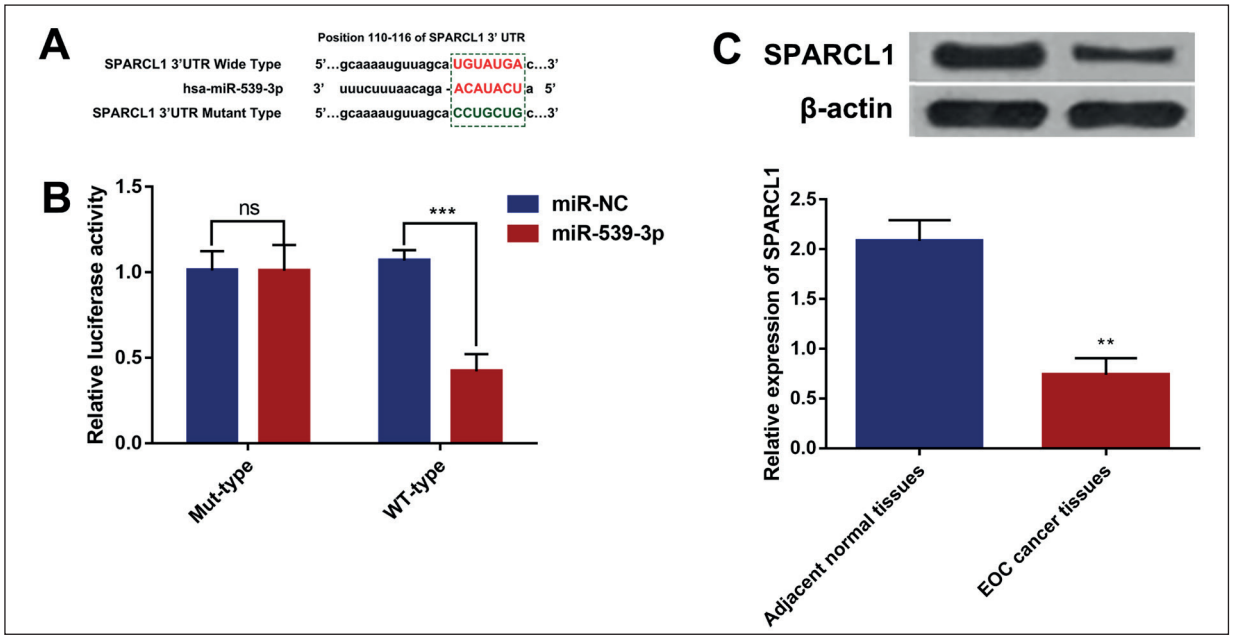

Figure 2. ***A,*** Diagram of putative miR-539-3p binding sites of SPARCL1. ***B,*** Relative activities of luciferase reporters after transfection (***$p < 0.001$)) ***C,*** The protein expressions of SPARCL1 in OC tissues and corresponding adjacent normal ovarian tissues were detected by WB assay (**$p < 0.01$).

Based on this, we examined the expression of SPARCL1 in 40 paired EOC tissues, which was consistent with Biade's experimental findings[15]. The expression of SPARCL1 in EOC tissues was significantly higher than that of adjacent normal tissues (Figure 2C).

The Regulation Mechanism of miR-539-3p in Cell Function

To further investigate the role of miR-539-3p *in vitro*, we artificially transfected miR-539-3p inhibitor into SKOV3 cells to reduce the expression of miR-539-3p. QRT-PCR detection demonstrated that miR-539-3p inhibitor transfection could significantly reduce the expression level of miR-539-3p in SKOV3 cells (Figure 3A). Subsequent WB indicated the protein expression of SPARCL1 in SKOV3 cells with low expression of miR-539-3p increased significantly. At the same time, we found that transfection of si-SPARCL1 could also counteract the effect of miR-539-3p inhibitor on the expression of SPARCL1 in SKOV3 cells as well (Figure 3B).

CCK8 assay illustrated that overexpression of miR-539-3p significantly promoted the proliferation of SKOV3 cells. The proliferation curve of miR-539-3p inhibitor group was significantly lower than that of miR-NC group. However, after the down-expression of SPARCL1 in cells through si-SPARCL1 transfection, the proliferation of SKOV3 cells tended to be active (Figure 3C).

Cell invasion and migration are important factors affecting the prognosis of cancer patients. Subsequently, the impacts of miR-539-3p on cell migration and invasion were detected by scratch wound and transwell assays. As shown in Figure 4, we could intuitively discover the limitation of miR-539-3p inhibitor in cell invasion and migration capacities. However, after co-transfection of miR-539-3p inhibitor and si-SPARCL1, the migration and invasion abilities of SKOV3 cells were restored. The above results all revealed the regulation of miR-539-3p/SPARCL1 in SKOV3 cell migration and invasion.

Discussion

OC is characterized by high malignant degree, poor prognosis and high mortality rate. It is estimated that EOC accounts for almost 90% of all OC cases. Since patients have already been in an advanced stage when diagnosed, the survival rate of these patients remains poor. Hence, there is an urgent need to further understand the molecular mechanism of EOC pathogenesis, and to find novel early diagnostic biomarkers and effective treatments for EOC.

Previous studies[16-19] have manifested that miRNAs participate in different processes of OC, such as tumor occurrence, cell cycle, apoptosis, proliferation, invasion, and metastasis, as well as progression of chemotherapy resistance. Besides, studies[20,21] have also indicated that a large number of miRNAs can be detected in early OC and are related to disease prognosis. Therefore, miRNAs are more specific and sensitive to OC diagnosis and prognosis than any other biomarkers. The

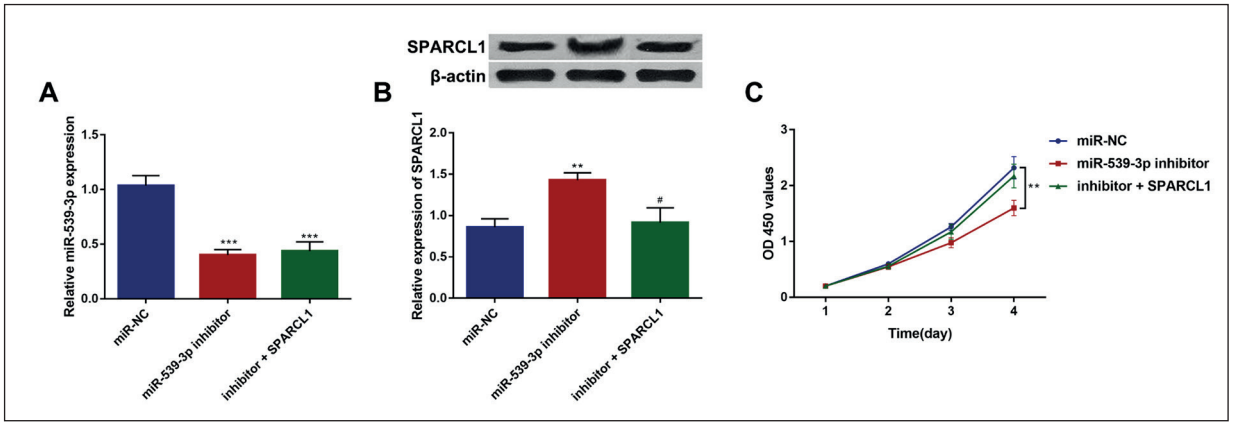

Figure 3. ***A,*** Transfection efficiency of miR-539-3p inhibitor determined by qRT-PCR (***$p < 0.001$ *vs.* miR-NC group; $^{\#\#\#}p < 0.001$ *vs.* miR-539-3p inhibitor group). ***B,*** MiR-539-3p inhibitor transfection significantly increased the protein expression of SPARCL1 (**$p < 0.01$ *vs.* miR-NC group; $^{\#}p < 0.05$ *vs.* miR-539-3p inhibitor group). ***C,*** The proliferation of EOC cells detected by CCK8 assay after different treatments (**$p < 0.01$ *vs.* miR-NC group).

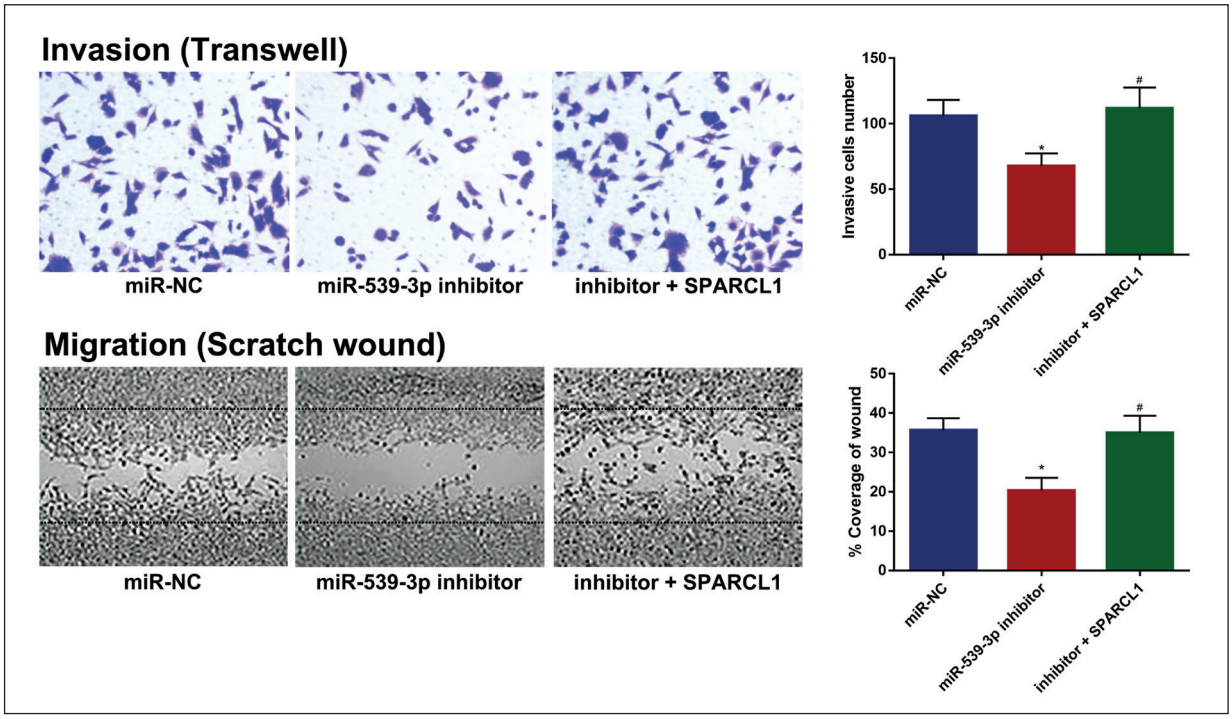

Figure 4. MiR-539-3p inhibitor suppressed the invasion and migration of EOC cells. The invasion and migration of EOC cells after transfection were analyzed using transwell assay and scratch wound assay. The results were photographed and detected by microscope (× 200) (*$p < 0.05$ *vs.* miR-NC group; #$p < 0.05$ *vs.* miR-539-3p inhibitor group).

mechanism of the occurrence and metastasis of malignant tumors can be summarized as follows. Gene damage caused by carcinogenic factors activate oncogenes or inactivate tumor suppressor genes, leading to abnormal proliferation and colony formation of cells. Through continuous evolution, sub-cloning with different biological characteristics can be formed to obtain the ability of cell invasion and migration. This may further result in the occurrence of tumor invasion and migration. Moreover, abnormal proliferation, invasion and migration are very important characteristics of malignant tumor cells.

MiRNAs are capable of modulating gene expression by binding to the 3'UTR of target mRNAs in a negative way. One miRNA can target multiple mRNAs at the same end of a cell by participating in the same cell signaling pathway or crosstalk between pathways. Additionally, the expression of a single mRNA may be regulated by a variety of miRNAs. This eventually leads to a more complicated mechanism of gene silencing mediated by miRNAs[22]. Some studies have indicated that miRNAs account for about 1-5% of the human genome, which regulate at least 30% of protein-coding genes[4]. Besides, it has been found that imbalance in the expression of miRNAs can be discovered and detected in the early stage of OC. Meanwhile, changes in miRNA expression can be detected in the development of the disease as well. These data indicate that miRNAs may become new biomarkers for early diagnosis and prognosis prediction in OC[23-26].

SPARCL1 is a member of the SPARC family that can regulate adhesion, inhibit tumor growth and reduce angiogenesis[27]. It exerts a key role in biological growth and maintenance of physiological functions. Besides, the expression of SPARCL1 is significantly down-regulated in most tumors, playing an anti-cancer role[28-31]. However, its expression increases markedly in uterine leiomyoma. This may be due to the influence of tumor micro-environment on SPARCL1 expression and function[32]. Scholars[33,34] have demonstrated that the expression of SPARCL1 is absent in normal liver tissues. However, it is highly expressed in hepatocellular carcinoma, acting as a tumor suppressor gene. The above findings suggest that the expression of SPARCL1 is closely related to malignant degree, metastasis and prognosis of the tumor. Furthermore, Biade's study[15] has found that the expression of SPARCL1 in ovarian malignant

tumors is significantly lower than that of ovarian benign tumors. All the above results indicate that SPARCL1 can be used as a reliable target for OC therapy.

In our research, we highlighted the regulation of miR-539-3p in EOC cells for the first time. By targeting to inhibit SPARCL1 expression, miR-539-3p significantly enhanced the proliferation, invasion and migration ability of EOC cells. After down-regulation of miR-539-3p *in vitro*, cellular functions of EOC cells were significantly inhibited. However, after simultaneously down-regulating the expressions of miR-539-3p and SPARCL1 in EOC cells, the malignant behaviors of EOC cells reawakened again. Our findings indicated that miR-539-3p/SPARCL1 might become a feasible and new target for EOC treatment.

Conclusions

We indicated that miR-539-3p acted as an oncogene in EOC by targeting SPARCL1. Furthermore, miR-539-3p/SPARCL1 axis might become a novel target for the treatment of EOC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Zhou H, Zhao H, Liu H, Xu X, Dong X, Zhao E. Influence of carboplatin on the proliferation and apoptosis of ovarian cancer cells through mTOR/p70s6k signaling pathway. J BUON 2018; 23: 1732-1738.
2) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin 2018; 68: 7-30.
3) Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993; 75: 843-854.
4) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
5) Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, Shimizu M, Cimmino A, Zupo S, Dono M, Dell'Aquila ML, Alder H, Rassenti L, Kipps TJ, Bullrich F, Negrini M, Croce CM. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A 2004; 101: 11755-11760.
6) Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, Aldler H, Rattan S, Keating M, Rai K, Rassenti L, Kipps T, Negrini M, Bullrich F, Croce CM. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc Natl Acad Sci U S A 2002; 99: 15524-15529.
7) Prahm KP, Novotny GW, Hogdall C, Hogdall E. Current status on microRNAs as biomarkers for ovarian cancer. APMIS 2016; 124: 337-355.
8) Hemmatzadeh M, Mohammadi H, Jadidi-Niaragh F, Asghari F, Yousefi M. The role of oncomirs in the pathogenesis and treatment of breast cancer. Biomed Pharmacother 2016; 78: 129-139.
9) Hemmatzadeh M, Mohammadi H, Karimi M, Musavishenas MH, Baradaran B. Differential role of microRNAs in the pathogenesis and treatment of esophageal cancer. Biomed Pharmacother 2016; 82: 509-519.
10) Jiang C, Chen X, Alattar M, Wei J, Liu H. MicroRNAs in tumorigenesis, metastasis, diagnosis and prognosis of gastric cancer. Cancer Gene Ther 2015; 22: 291-301.
11) Pal MK, Jaiswar SP, Dwivedi VN, Tripathi AK, Dwivedi A, Sankhwar P. MicroRNA: a new and promising potential biomarker for diagnosis and prognosis of ovarian cancer. Cancer Biol Med 2015; 12: 328-341.
12) Gu L, Sun W. MiR-539 inhibits thyroid cancer cell migration and invasion by directly targeting CARMA1. Biochem Biophys Res Commun 2015; 464: 1128-1133.
13) Zhang H, Li S, Yang X, Qiao B, Zhang Z, Xu Y. miR-539 inhibits prostate cancer progression by directly targeting SPAG5. J Exp Clin Cancer Res 2016; 35: 60.
14) Ding M, Shen W, Hu Y. The Role of miR-539 in the anterior cingulate cortex in chronic neuropathic pain. Pain Med 2017; 18: 2433-2442.
15) Biade S, Marinucci M, Schick J, Roberts D, Workman G, Sage EH, O'Dwyer PJ, Livolsi VA, Johnson SW. Gene expression profiling of human ovarian tumours. Br J Cancer 2006; 95: 1092-1100.
16) Jiang JH, Lv QY, Yi YX, Liao J, Wang XW, Zhang W. MicroRNA-200a promotes proliferation and invasion of ovarian cancer cells by targeting PTEN. Eur Rev Med Pharmacol Sci 2018; 22: 6260-6267.
17) Bendoraite A, Knouf EC, Garg KS, Parkin RK, Kroh EM, O'Briant KC, Ventura AP, Godwin AK, Karlan BY, Drescher CW, Urban N, Knudsen BS, Tewari M. Regulation of miR-200 family microRNAs and ZEB transcription factors in ovarian cancer: evidence supporting a mesothelial-to-epithelial transition. Gynecol Oncol 2010; 116: 117-125.
18) Zhou X, Zhao F, Wang ZN, Song YX, Chang H, Chiang Y, Xu HM. Altered expression of miR-152 and miR-148a in ovarian cancer is related to cell proliferation. Oncol Rep 2012; 27: 447-454.
19) Li J, Liang S, Yu H, Zhang J, Ma D, Lu X. An inhibitory effect of miR-22 on cell migration and invasion in ovarian cancer. Gynecol Oncol 2010; 119: 543-548.
20) Wan WN, Zhang YQ, Wang XM, Liu YJ, Zhang YX, Que YH, Zhao WJ, Li P. Down-regulated miR-22 as predictive biomarkers for prognosis of epithelial ovarian cancer. Diagn Pathol 2014; 9: 178.

21) Zhou QH, Zhao YM, Jia LL, Zhang Y. Mir-595 is a significant indicator of poor patient prognosis in epithelial ovarian cancer. Eur Rev Med Pharmacol Sci 2017; 21: 4278-4282.

22) Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. Prediction of mammalian microRNA targets. Cell 2003; 115: 787-798.

23) Dahiya N, Sherman-Baust CA, Wang TL, Davidson B, Shih I, Zhang Y, Wood WR, Becker KG, Morin PJ. MicroRNA expression and identification of putative miRNA targets in ovarian cancer. PLoS One 2008; 3: e2436.

24) Mitamura T, Watari H, Wang L, Kanno H, Hassan MK, Miyazaki M, Katoh Y, Kimura T, Tanino M, Nishihara H, Tanaka S, Sakuragi N. Downregulation of miRNA-31 induces taxane resistance in ovarian cancer cells through increase of receptor tyrosine kinase MET. Oncogenesis 2013; 2: e40.

25) Kobayashi M, Salomon C, Tapia J, Illanes SE, Mitchell MD, Rice GE. Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200. J Transl Med 2014; 12: 4.

26) Gu Y, Zhang M, Peng F, Fang L, Zhang Y, Liang H, Zhou W, Ao L, Guo Z. The BRCA1/2-directed miRNA signature predicts a good prognosis in ovarian cancer patients with wild-type BRCA1/2. Oncotarget 2015; 6: 2397-2406.

27) Girard JP, Springer TA. Cloning from purified high endothelial venule cells of hevin, a close relative of the antiadhesive extracellular matrix protein SPARC. Immunity 1995; 2: 113-123.

28) Hu H, Zhang H, Ge W, Liu X, Loera S, Chu P, Chen H, Peng J, Zhou L, Yu S, Yuan Y, Zhang S, Lai L, Yen Y, Zheng S. Secreted protein acidic and rich in cysteines-like 1 suppresses aggressiveness and predicts better survival in colorectal cancers. Clin Cancer Res 2012; 18: 5438-5448.

29) Xiang Y, Qiu Q, Jiang M, Jin R, Lehmann BD, Strand DW, Jovanovic B, DeGraff DJ, Zheng Y, Yousif DA, Simmons CQ, Case TC, Yi J, Cates JM, Virostko J, He X, Jin X, Hayward SW, Matusik RJ, George AJ Jr, Yi Y. SPARCL1 suppresses metastasis in prostate cancer. Mol Oncol 2013; 7: 1019-1030.

30) Cao F, Wang K, Zhu R, Hu YW, Fang WZ, Ding HZ. Clinicopathological significance of reduced SPARCL1 expression in human breast cancer. Asian Pac J Cancer Prev 2013; 14: 195-200.

31) Li P, Qian J, Yu G, Chen Y, Liu K, Li J, Wang J. Down-regulated SPARCL1 is associated with clinical significance in human gastric cancer. J Surg Oncol 2012; 105: 31-37.

32) Mencalha AL, Levinsphul A, Deterling LC, Pizzatti L, Abdelhay E. SPARC-like1 mRNA is overexpressed in human uterine leiomyoma. Mol Med Rep 2008; 1: 571-574.

33) Lau CP, Poon RT, Cheung ST, Yu WC, Fan ST. SPARC and Hevin expression correlate with tumour angiogenesis in hepatocellular carcinoma. J Pathol 2006; 210: 459-468.

34) Esposito I, Kayed H, Keleg S, Giese T, Sage EH, Schirmacher P, Friess H, Kleeff J. Tumor-suppressor function of SPARC-like protein 1/Hevin in pancreatic cancer. Neoplasia 2007; 9: 8-17