European Review for Medical and Pharmacological Sciences 2021; 25: 1250-1260

MiR-34a-5p directly targeting TRIM44 affects the biological behavior of ovarian cancer cells

H.-L. LI, Y.-A. DUAN, N. ZHAO

Department of Gynecology, Dongying People's Hospital, Dongying, China

Hailing Li and Yanan Duan contributed equally as the first authors

Abstract. – OBJECTIVE: This research intended to explore the expression and molecular mechanism of miR-34a-5p and Tripartite motif-containing protein 44 (TRIM44) in ovarian cancer (OC).

PATIENTS AND METHODS: Tissue and serums of OC patients were collected, and miR-34a-5p and TRIM44 in serum and tissue were tested by Real-time quantitative PCR (qRT-PCR). In vitro cell experiment was constructed. Methyl Thiazolyl Tetrazolium (MTT), transwell, and flow cytometry were applied to test the proliferation, migration, invasion, and apoptosis. Western blot was performed to explore TRIM44 and epithelial-mesenchymal transition (EMT) proteins.

RESULTS: MiR-34a-5p was low expressed, while TRIM44 was highly expressed, which was related to Federation International of Gynecology and Obstetrics (FIGO) staging and lymph node metastasis. The receiver operating characteristic curve (ROC) revealed that the Area Under Curve (AUC) of miR-34a-5p and TRIM44 were 0.885 and 0.868, respectively. An evident increase of miR-34a-5p or decrease of TRIM44 could inhibit OC malignant behaviors, and E-cadherin increased while N-cadherin and Fibronectin decreased. The knockdown of miR-34a-5p or overexpression of TRIM44 could inhibit the malignant behavior of ovarian cancer cells, hinder the malignant behaviors, and reduce E-cadherin, while N-cadherin and Fibronectin protein was enhanced. Co-transfection found that overexpression of miR-34a-5p eliminated the biological behaviors of OC cells by TRIM44.

CONCLUSIONS: MiR-34a-5p and TRIM44 can be used as diagnostic markers for OC. MiR-34a-5p can act in biological behaviors by targeting TRIM44 in OC.

Key Words:

MiR-34a-5p, TRIM44, Ovarian cancer, Diagnosis, Cell.

Introduction

Ovarian cancer (OC) is a kind of fatal malignant tumor, and its mortality rate ranks first among gynecologic malignant tumors. It is estimated that it's mainly due to the lack of effective diagnosis and treatment methods in clinical practice[1]. OC is usually difficult to diagnose clinically. Although transvaginal ultrasound or serum markers such as CA125 can be used to diagnose OC, these methods are not sufficient to detect early OC[2]. The 5-year survival of patients with early OC can reach 70-90%, but about 70% of the patients are diagnosed as advanced stage, according to Federation International of Gynecology and Obstetrics (FIGO), and the overall 5-year survival is less than 40%[3]. The first choice for early OC is a comprehensive treatment scheme with surgery as the main treatment and radiotherapy and chemotherapy as the auxiliary treatment, but the treatment effect for advanced patients is limited[4]. Therefore, finding the diagnosis of OC and clarifying the molecular mechanism that participated in the disease are crucial to the development of effective therapies for human OC.

MicroRNA (miRNA), a non-coding RNA molecule of 22 nucleotides, can interact with target mRNA to hinder gene expression[5]. Previous researchers[6-8] revealed that miRNA could connect to multiple mRNAs and act biological effect in various cancers, thus participating in tumor proliferation, differentiation, and progression. Evidence[9] showed that miR-34a-5p in miR-34 family members played various biological functions in malignant tumors and could be directly regulated by transcription factor p53. Other researchers[10-12] have found that miR-34a is reduced in various carcinomas, including gastric carcinoma, non-small cell lung carcinoma, osteosarcoma, and OC. Therefore, miR-34a can play the effect of an inhibitory gene in tumors. Ding et al[13] reported that compared with normal ovarian epithelial cells, miR-34a-5p in human OC cells was evidently decreased, and NEAT1 negatively regulated miR-34a-5p to act as a carcinogenic role

Corresponding Author: Yanan Duan, MD; e-mail: Duanyanan258@sina.com

in OC. There were also reports indicating that miR-34a-5p hindered the migration and invasion of colorectal carcinoma tumor cells, which confirmed that miR-34a-5p might play a role in inhibiting tumors[14]. Although this research concluded that miR-34a-5p widely participated in colorectal carcinoma or OC, the clinical value and molecular mechanism of miR-34a-5p in OC are unknown. For this reason, we speculated that miR-34a-5p and Tripartite motif-containing protein 44 (TRIM44) had latent target sites through TargetScan and miRBD databases. The role of the TRIM44 gene or protein in the occurrence and growth of various tumors has been studied[15,16]. Although TRIM44 can accelerate the invasion and migration of tumor cells[17], the role of TRIM44 in OC is still unknown.

In this research, we observed the clinical value and specific mechanism of miR-34a-5p in OC, in order to provide potential diagnostic and therapeutic markers for OC.

Patients and Methods

Patients

Fifty-two patients with ovarian cancer admitted to Dongying People's Hospital from February 2012 to April 2014 were selected. Inclusion criteria: patients were confirmed as ovarian cancer by postoperative pathology[18]; none received chemotherapy or radiotherapy before operation; during the operation, ovarian cancer patients' tissues and corresponding normal adjacent tissues were collected and immediately placed in liquid nitrogen and then stored at -80°C. Exclusion criteria: patients with severe liver and kidney dysfunction, infection, and other malignant tumors. In addition, 50 healthy people were included during the same period. Serum samples of ovarian cancer patients and normal healthy controls were collected. This study was approved by the Ethics Committee of Dongying People's Hospital, and all the subjects have signed informed consent.

Cell Line Culture and Transfection

Human OC cell lines (OVCAR3, SKOV3, A2780, CAOV3) were obtained from Shanghai Zishi Biotechnology Co., Ltd. (Shanghai, China, Qs101565, Qs101715, Qs100739, Qs100843) (Shanghai, China), and human ovarian surface epithelium (HOSE) was purchased from Yu Bo Biotech Co., Ltd. (Shanghai, China, YB-ATCC-8386). OC cell lines were cultivated in RPMI-1640 (Huijia Biotechnology Co., LTD., Xiamen, China, 11875101), including 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin sulfate. The cells were cultivated in 37°C incubators with 5% CO_2. Follow-up experiments were conducted when the cell density was 70-85%. MiR-34a-5p mimetics, miR-34a-5p inhibitor, miR-NC, TRIM44 overexpression sequence (sh-TRIM44), TRIM44 inhibition sequence (si-TRIM44), and si-NC were constructed and synthesized by RiboBio Co., Ltd. (Guangzhou, China). Cells were moved into Lipofectamine 3000 (T&L Biological Technology, Beijing, China, L3000015).

Real-Time Quantitative PCR (qRT-PCR)

Total RNA was obtained by the TRIzol kit (Ruan Biotechnology Co., Ltd, Shanghai, China, R523-200). Total RNA in serum was extracted using miRNeasy. The concentration, purity and integrity of RNA were measured by spectrophotometry and agarose gel electrophoresis. To obtain cDNA, we reverse transcribed total RNA into cDNA by a cDNA reverse transcription kit. PCR was conducted using the ABI 7300 system. mRNA was tested by SYBR Green PCR, and the endogenous control was GAPDH. The TaqMan miRNA was applied to detect miRNA expression, and U6 was applied as the internal user. In triplicate, genes were tested using $2^{-\Delta CT}$. The primer sequence was synthesized by RiboBio Co., Ltd. (Guangzhou, China). Primer sequence was as follows: miR-34a-5p forward: 5'-TGGCAGTGTCTTAGCTGGTTGT-3', reverse: 5'-GCGAGCACAGAATTAATACGAC-3'; TRIM44 forward: 5'-GTGGACATCCAAGAGGCAAT-3', reverse: 5'-AGCAAGCCTTCATGTGTCCT-3'; U6 forward: 5'-TGCGGGTGCTCGCTTCGGCAGC-3', reverse: 5'-GTGCAGGGTCCGAGGT-3'; GAPDH forward: 5'-AGCCACATCGCTCAGACAC-3', reverse: 5'-GCCCAATACGACCAAATCC-3'.

Determination of Luciferase

According to TargetScan, the miRBD database[19] predicted the latent target of miR-34a-5p. TRIM44 3'-UTR of miR-34a-5p target site was predicted by PCR amplification. TRIM44 wild-type (WT) and mutant (MUT) 3'UTR fragments were then moved into pMIR. pMIR-WT-TRIM44, pMIR-MUT-TRIM44 and miR-34a-5p mimics or miR-NC were moved into OVCAR3 and SKOV3 cells using Lipofectamine 3000 kit. Cells were obtained 48 h after transfection, and Luciferase

activity was tested by Dual-Luciferase (Hanbio Biotechnology Co., Ltd., Shanghai, China) using the manufacturer's instructions.

Cell Proliferation Test

According to the manufacturer, Thiazolyl Blue Tetrazolium Bromide (MTT) kit (Invitrogen, Grand Island, NY, USA, V13154) was applied to test the proliferation. Cells with a density of 5×10^3 were inoculated into 96-well plate at 37°C with 5% CO_2. After incubation for 0, 24, 48, 72, 96 h, respectively, 20 μL of MTT solution was put into each well. After cultivating for 4 h, the culture solution was discarded and 200 μL dimethyl sulfoxide (DMSO) was put in for 15 min to dissolve the crystals. OD was measured at 490 mm by a Multiskan FC Microplate Reader (Thermo Fisher Scientific, Waltham, MA, USA). The experiment was repeated 3 times.

Cell Migration and Invasion Detection

Cells in the logarithmic growth stage were taken and prepared into 1×10^7 cell suspension. For cell migration, 200 μL of suspension was put in the upper chamber, and 600 μL of RPMI 1640 solution (10% FBS) was put in the lower chamber to culture overnight. Cotton swabs were used to carefully wipe off the cells on the wall of the chamber, PBS solution was used for rinsing. Then, the samples were fastened for 30 min and dyed with 0.1% crystal violet for 20 min. For cell invasion, RPMI-1640 medium and matrix gel were diluted at 1:5 and spread in the upper chamber. The following experiment was carried out according to the migration detection steps. The number of cells passing through the membrane was detected using an inverted microscope.

Apoptosis Detection

Cells were rinsed with PBS and digested with 0.25% trypsin. A total of 5 μL Annexin-V-FITC, 5 μL propidium iodide (PI) and 400 μL buffer were put into the sample and cultivated at room temperature for 15 min. Attune NxT Flow Cytometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to test samples in triplicate within 1 h.

Western Blot Experiment

RIPA lysate (P0013C) was used to lyse tissues or cells and extract total protein samples. Bio-Rad protein reagent (P0508S) was used for protein quantification. The protein lysate was resolved by SDS-polyacrylamide, and then, moved to polyvinylidene fluoride (PVDF). TRIM44 (H00054765-P01), E-cadherin (1:1000, AF0138), N-cadherin (1:1000, AF0243), Fibronectin (1:1000, AF380191-FN-02M), β-actin (1:1000, AF0003) primary antibody were put into skim milk overnight at 4°C. The membrane was rinsed, and horseradish peroxidase (HRP) labeled secondary antibody (1:1000, A0208) was put in and incubated for 1 h. Chemiluminescent reagent (P0018FM) was used to develop for 5 min, and FluorChem Q chemiluminescent gel imaging system (Biolaunching Technologies Co., Ltd., Beijing, China) was used to carry out a quantitative analysis. TRIM44 protein kit was obtained from AmyJet Scientific Co., Ltd. (Wuhan, China), and other reagents were obtained from Beyotime Biotechnology Co., Ltd. (Shanghai, China).

Statistical Analysis

Data with normal distribution were represented by mean±standard deviation (Meas±SD), and group comparison was conducted by Student's *t*-test. One-way ANOVA was applied for comparison among multiple groups, and LSD *t*-test for intra-group comparison. The data at different time points in the group were analyzed by repeated measurement variance analysis, and Bonferroni test was used for the pair-wise comparison. The receiver operating characteristic (ROC) of the subjects were visualized, and the diagnostic value of miR-34a-5p and TRIM44 for OC was determined by calculating Area Under Curve (AUC). Pearson was applied for correlation analysis. The *p*-value less than 0.05 indicated an evident difference. SPSS 17.0 (SPSS Inc., Chicago, Il, USA) was applied for the analysis of the data, and GraphPad Prism 6.0 was used for figure visualizing.

Result

Clinical Value of MiR-34a-5p, TRIM44 in OC

MiR-34a-5p and TRIM44 in serum and tissues of OC patients were tested by qRT-PCR. Compared with normal people, miR-34a-5p in serum of OC patients was evidently reduced, while TRIM44 was highly expressed. By observing miR-34a-5p and TRIM44, it was revealed that miR-34a-5p in OC tissues was evidently reduced, while TRIM44 was evidently increased. Pearson test revealed that miR-34a-5p and TRIM44 in serum and tissue were negatively correlated. Then,

we observed the diagnostic role of miR-34a-5p, TRIM44 in OC. The AUC values of serum miR-34a-5p and TRIM44 for diagnosis of OC were 0.885 and 0.868, respectively. Further analysis of the clinicopathological features of miR-34a-5p, TRIM44 with OC patients showed that we defined the high and low two indexes by the median of miR-34a-5p (0.766) and TRIM44 (1.459), the decrease of miR-34a-5p and the increase of TRIM44 indicated higher FIGO stage and lymph node metastasis (Figure 1 and Table I).

TRIM44 – a Target of MiR-34a-5p

MiR-34a-5p, TRIM44 were evaluated by qRT-PCR. MiR-34a-5p in OC cell lines (OVCAR3, SKOV3, A2780, CAOV3) was evidently lower than that in human ovarian surface epithelium (HOSE), while TRIM44 was evidently higher. We selected OVCAR3 and SKOV3 with low miR-34a-5p for follow-up experiments. qRT-PCR was performed to verify the transfection. The results revealed that compared with miR-NC, miR-34a-5p transfected with miR-34a-5p mimics was evidently up-regulated, while miR-34a-5p inhibitor was evidently reduced. In addition, compared with si-NC, TRIM44 in cells transfected si-TRIM44 was evidently down-regulated, while TRIM44 in cells transfected sh-TRIM44 was evidently up-regulated. We suspected the latent targets of miR-34a-5p through TargetScan and

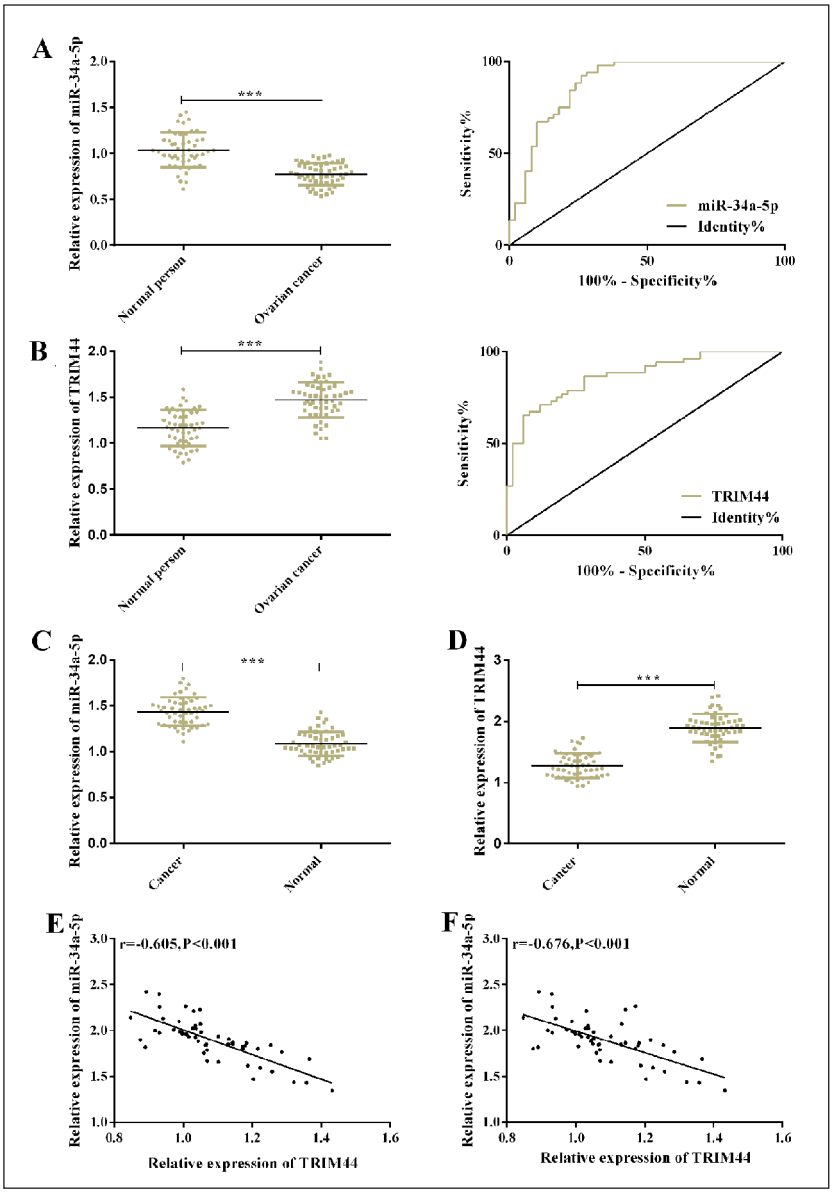

Figure 1. Clinical value of miR-34a-5p, TRIM44 in OC. **A,** ROC curve of expression and diagnosis of miR-34a-5p in serum of OC patients; **B,** ROC curve of expression and diagnosis of TRIM44 in serum of OC patients; **C,** Expression of miR-34a-5p in OC tissues; **D,** Expression of TRIM44 in OC tissues; **E,** Expression of miR-34a-5p in OC serum was negatively correlated with TRIM44; **F,** Expression of miR-34a-5p in OC tissue was negatively correlated with TRIM44. Note: *** indicates that $p<0.001$.

Table I. Relationship of serum miR-34a-5p, TRIM44 with clinicopathological features of OC patients.

Clinicopathological features	N	miR-34a-5p		χ^2	*p*	TRIM44		χ^2	*p*
		High expression (n = 26)	Low expression (n = 26)			High expression (n = 26)	Low expression (n = 26)		
Tissue type				1.238	0.266			0.310	0.578
Serous	28	12 (46.15)	16 (61.54)			15 (57.69)	13 (50.00)		
Others	24	10 (53.86)	14 (38.46)			11 (42.31)	13 (50.00)		
Age (years)				0.315	0.574			2.836	0.092
< 55	22	12 (46.15)	10 (38.46)			8 (30.77)	14 (53.85)		
≥ 55	30	14 (53.85)	16 (61.54)			18 (69.23)	12 (46.15)		
Tumor diameter (cm)				1.949	0.163			1.949	0.163
< 10	29	17 (65.38)	12 (46.15)			12 (46.15)	17 (65.38)		
≥ 10	23	9 (34.62)	14 (53.85)			14 (53.85)	9 (34.62)		
FIGO staging				7.738	0.005			4.952	0.026
I-II	24	17 (65.38)	7 (26.92)			8 (30.77)	16 (61.54)		
III-IV	28	9 (34.62)	19 (73.08)			18 (69.23)	10 (38.46)		
Lymph node metastasis				5.438	0.020			8.497	0.004
No	34	21 (80.77)	13 (50.00)			12 (46.15)	22 (84.62)		
Yes	18	5 (19.23)	13 (50.00)			14 (53.85)	4 (15.38)		
Pathological staging				0.391	0.532			0.391	0.532
G1/G2	14	8 (30.77)	6 (23.08)			6 (23.08)	8 (30.77)		
G3	38	18 (69.23)	20 (76.92)			20 (76.92)	18 (69.23)		
Abdominal dropsy				0.325	0.569			1.300	0.254
No	20	11 (42.31)	9 (34.62)			8 (30.77)	12 (46.15)		
Yes	32	15 (57.69)	17 (65.38)			18 (69.23)	14 (53.85)		

miRBD. The results revealed that TRIM44 3'-UTR and miR-34a-5p had targeted binding sites. By constructing miR-34a-5p luciferase reports of pMIR-WT-TRIM44 and pMIR-MUT-TRIM44, the results revealed that the over-expression of miR-34a-5p evidently inhibited the Luciferase activity of pMIR-WT-TRIM44 in cells but did not affect the Luciferase activity of pMIR-MUT-TRIM44. Western blot experiments showed that compared with miR-NC, TRIM44 transfected with miR-34a-5p mimics was evidently reduced, while TRIM44 transfected with miR-34a-5p inhibitor was evidently increased (Figure 2).

MiR-34a-5p Acts in the Biological Behavior of OC Cells

We intended to determine the role of miR-34a-5p on the biologic behaviors and EMT of OVCAR3 and SKOV3. MTT analysis showed that miR-34a-5p mimics can hinder the proliferation, while miR-34a-5p inhibitor can promote the proliferation. Transwell experiment showed that miR-34a-5p mimics can inhibit the migration and invasion, while miR-34a-5p inhibitor can promote cell migration and invasion. Flow cytometry showed that miR-34a-5p mimics could induce apoptosis, while miR-34a-5p inhibitor ev-

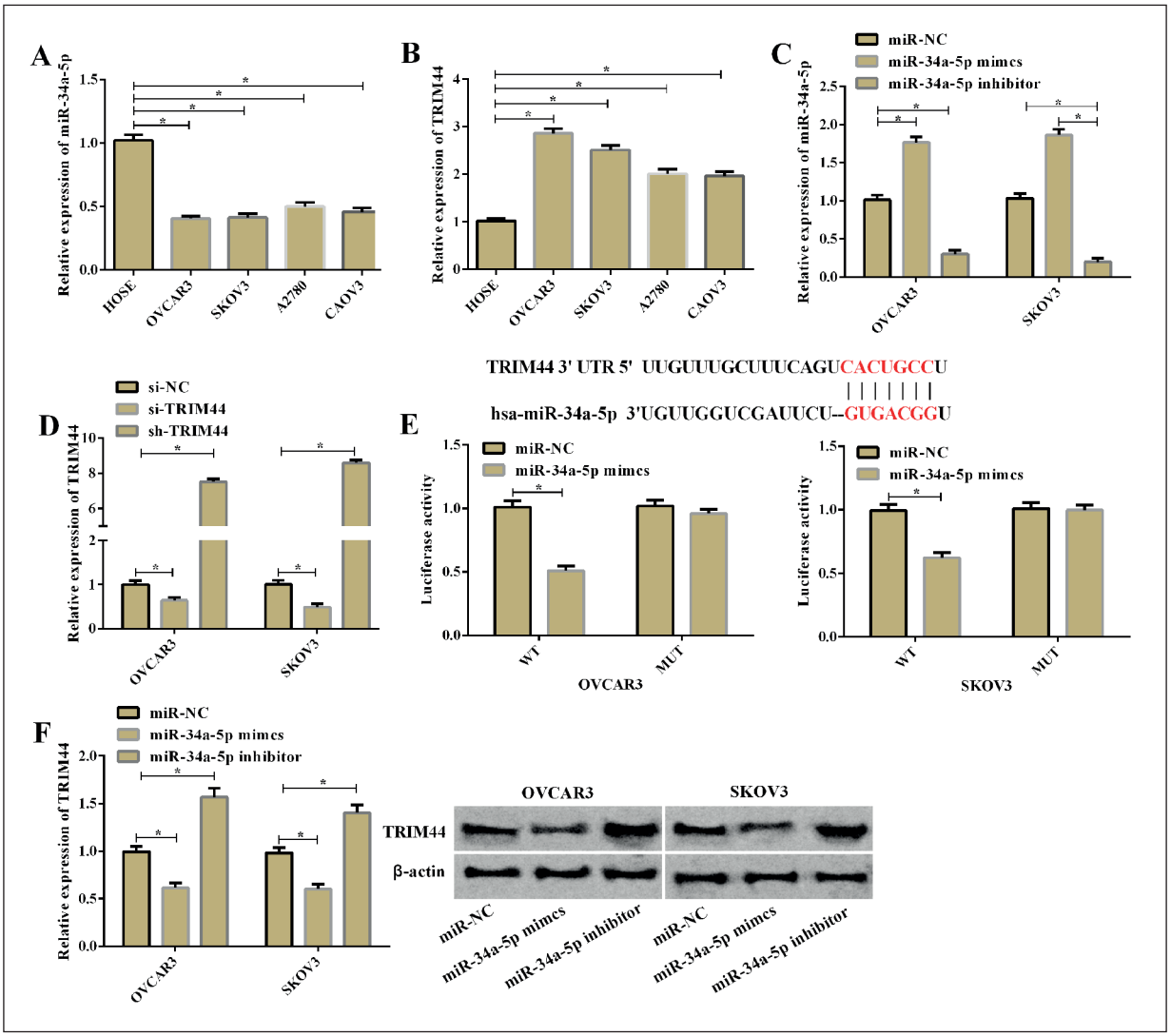

Figure 2. TRIM44 is the target of miR-34a-5p. **A,** qRT-PCR results showed that miR-34a-5p was down-regulated in OC cells; **B,** qRT-PCR results showed that TRIM44 was up-regulated in OC cells; **C,** qRT-PCR verified the expression of miR-34a-5p in cells transfected with miR-NC, miR-34a-5p mimics, miR-34a-5p inhibitor; **D,** qRT-PCR verified the expression of TRIM44 in cells transfected with si-NC, sh-TRIM44 and si-TRIM44; **E,** Double luciferase report verified the relationship between miR-34a-5p and TRIM44; **F,** Western blot was used to detect the expression of TRIM44 in cells transfected with miR-NC, miR-34a-5p mimics and miR-34a-5m inhibitor. Note: * indicates that $p<0.05$.

idently hindered apoptosis. Western blot experiment showed that E-cadherin was enhanced while N-cadherin and Fibronectin were reduced in cells transfected with miR-34a-5pmiccs. After transfecting miR-34a-5p inhibitor, E-cadherin was reduced, while N-cadherin and Fibronectin were increased (Figure 3).

TRIM44 Acts in the Biological Behavior of OC Cells

Then, by detecting the biological function of TRIM44 on OVCAR3 and SKOV3 cells, it was revealed that compared with transfected si-NC, transfected si-TRIM44 can inhibit the proliferation, migration and invasion, and can induce the apoptosis. E-cadherin in cells was enhanced, while N-cadherin and Fibronectin were reduced. Transfection of sh-TRIM44 could accelerate the proliferation, migration and invasion, inhibit the apoptosis, reduce E-cadherin protein, and enhance N-cadherin and Fibronectin (Figure 4).

Co-Transfection of MiR-34a-5p and TRIM44 Affects the Biological Behavior of OC Cells

In the above studies, miR-34a-5p could adjust the biological function of OC cells by targeting

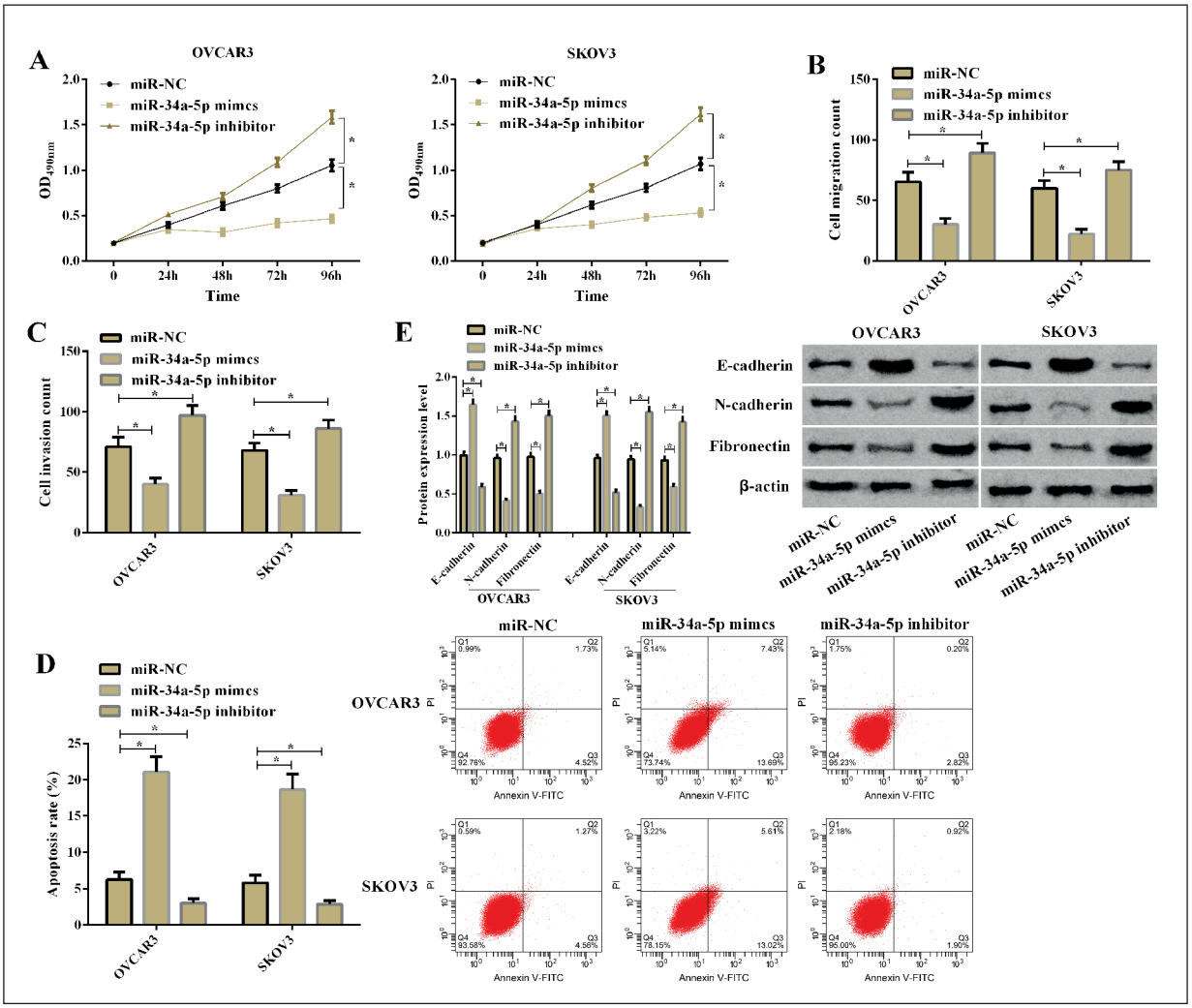

Figure 3. MiR-34a-5p plays a role in biological behavior of OC cells. **A,** MTT analysis of transfected miR-NC, miR-34a-5pmics, miR-34a-5mpinitor-OVCAR3, SKOV3 cells; **B,** Determination of migration of OVCAR3 and SKOV3 cells after transfection of miR-NC, miR-34a-5pmics, miR-34a-5p inhibitor; **C,** Determination of invasion of OVCAR3 and SKOV3 cells after transfection of miR-NC, miR-34a-5pmics, miR-34a-5p inhibitor; **D,** Determination of apoptosis rate and apoptosis figure of OVCAR3 and SKOV3 cells transfected with miR-NC, miR-34a-5pmics, miR-34a-5p inhibitor. **E,** Expression of E-cadherin, N-cadherin, Fibronectin protein and its protein bands in OVCAR3, SKOV3 cells after transfection of miR-NC, miR-34a-5pmics, miR-34a-5p1 inhibitor. Note: *indicates that $p<0.05$.

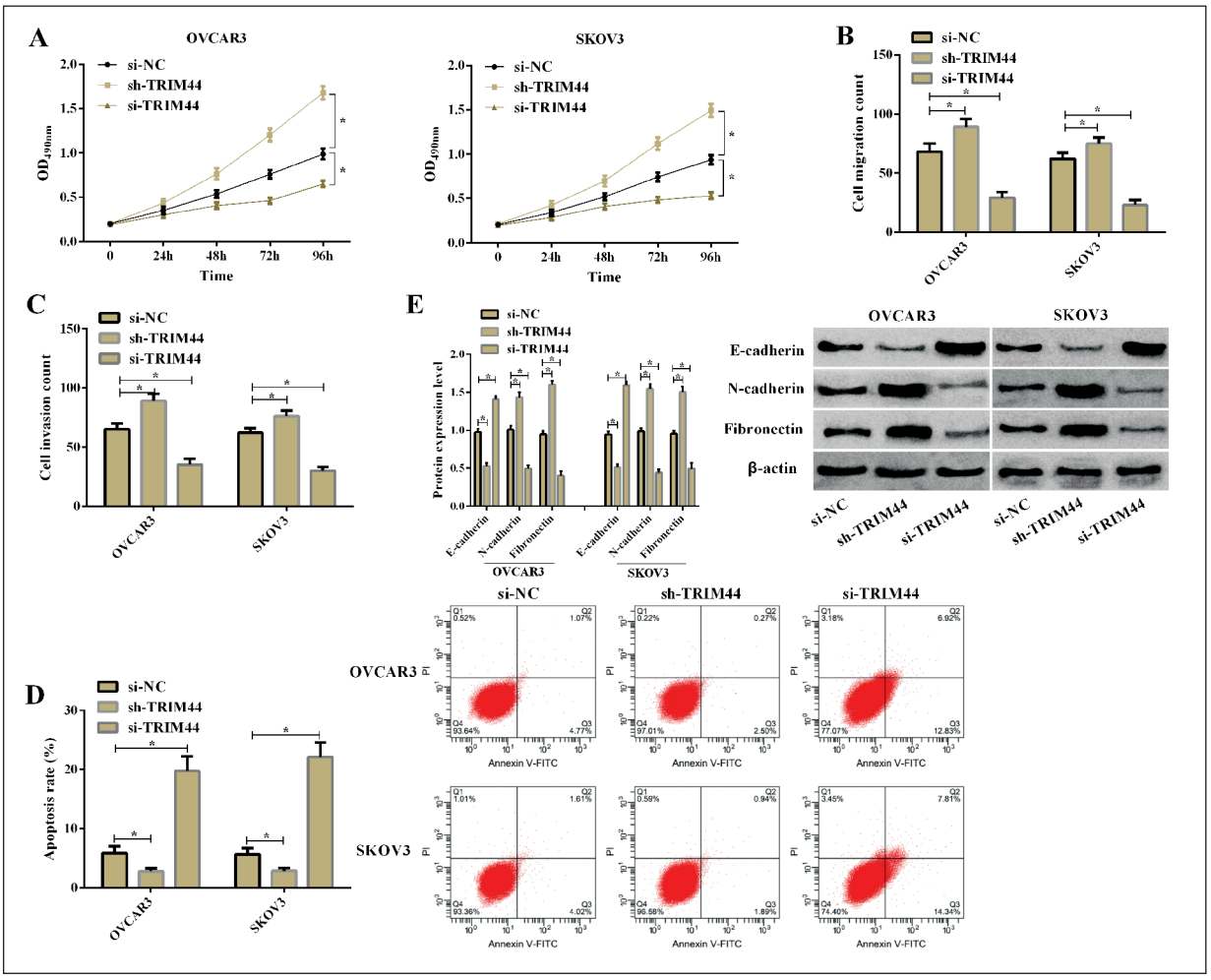

Figure 4. TRIM44 plays a role in the biological behavior of OC cells. **A,** MTT analysis of transfected cells of OVCAR3 and SKOV3 after si-NC, sh-TRIM44 and si-TRIM44; **B,** Migration assay of OVCAR3 and SKOV3 cells transfected with si-NC, sh-TRIM44 and si-TRIM44; **C,** Invasion of OVCAR3 and SKOV3 cells transfected with si-NC, sh-TRIM44 and si-TRIM44; **D,** Determination of apoptosis rate and apoptosis map of OVCAR3 and SKOV3 cells transfected with si-NC, sh-TRIM44 and si-TRIM44; **E,** Expression of E-cadherin, N-cadherin, Fibronectin protein and its protein bands in OVCAR3, SKOV3 cells after transfection of si-NC, sh-TRIM44, si-TRIM44. Note: *indicates that $p<0.05$.

TRIM44. We further investigated the role of miR-34a-5p and TRIM44 co-transfection on the biological function of OC cells. The OVCAR3 and SKOV3 cells were moved into miR-34a-5pmiccs+miR-nc, sh-TRIM44+miR-NC, miR-34a-5pmiccs+sh-trim44, miR-NC+si-NC. Compared with transfected with sh-TRIM44+miR-NC, transfected with miR-34a-5pmics+miR-NC, miR-34a-5pmics+SH-Trim44, miR-NC+si-NC could evidently hinder the proliferation, migration and invasion, and could induce the apoptosis. E-cadherin in cells was enhanced, while N-cadherin and Fibronectin were reduced. These biological behaviors of miR-34a-5pmics+miR-nc were most obviously inhibited, while those of cells transfected with miR-34a-5pmics+sh-trim44 and miR-NC+si-NC were not evidently changed (Figure 5).

Discussion

OC is the malignant tumor with the highest cancer mortality rate among women[20]. In this research, miR-34a-5p was low expressed in OC serum, tissues and cells, while TRIM44 was highly expressed and could act as a diagnostic marker for OC. In addition, increase or decrease of miR-34a-5p in OC cells could lead to down-regulation and up-regulation of TRIM44. Our study re-

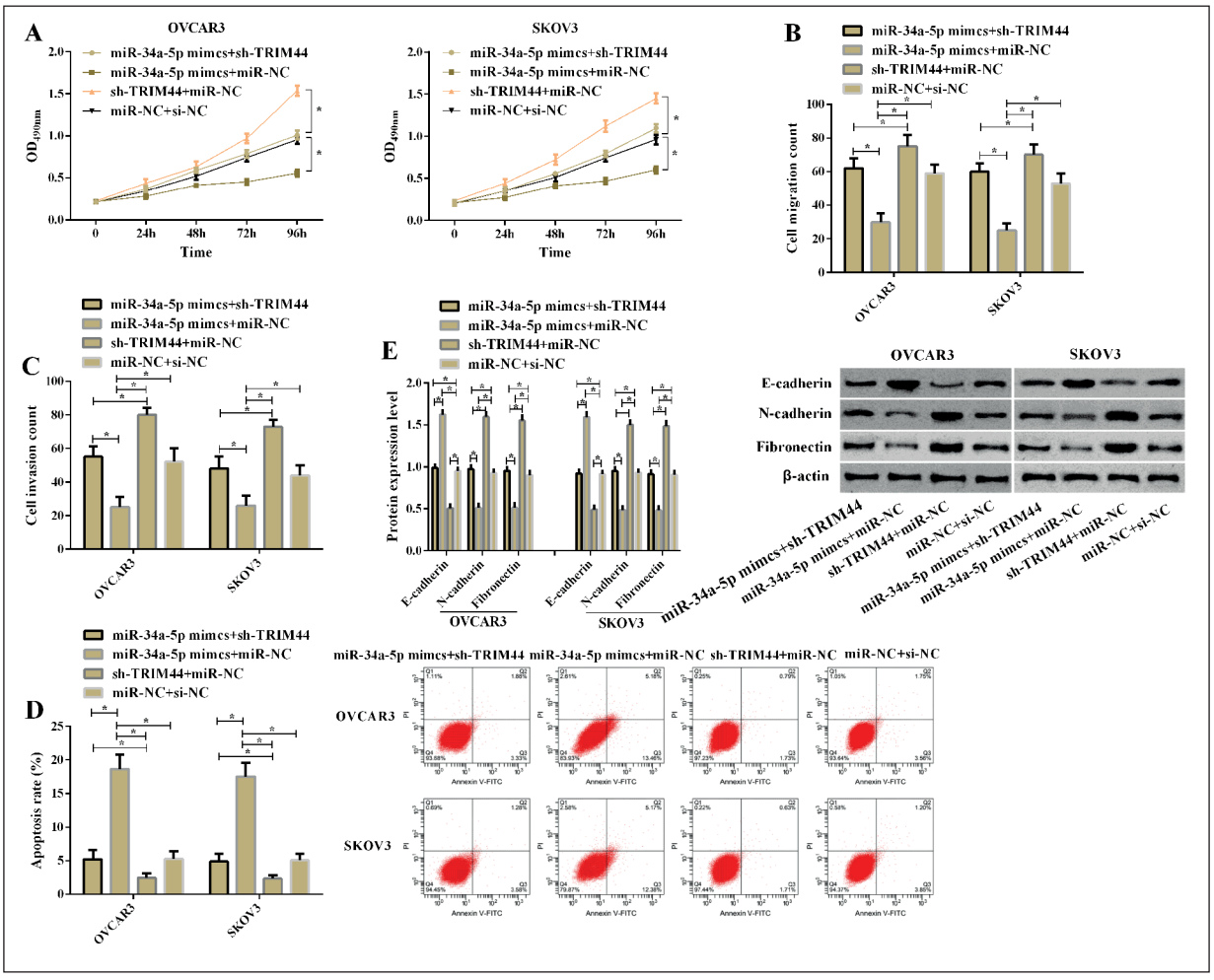

Figure 5. Co-transfection of miR-34a-5p and TRIM44 affects the biological behavior of OC cells. **A,** MTT analysis of transfected OVCAR3 and SKOV3 cells; **B,** Migration assay of OVCAR3 and SKOV3 cells after transfection; **C,** Determination of invasiveness of OVCAR3 and SKOV3 cells after transfection; **D,** Determination of apoptosis rate of OVCAR3 and SKOV3 cells after transfection and the apoptosis figure; **E,** Expression of E-cadherin, N-cadherin, Fibronectin protein and its protein band in transfected OVCAR3 and SKOV3 cells. Note: *indicates that $p<0.05$.

vealed for the first time that miR-34a-5p/TRIM44 can be used as a diagnostic marker of ovarian cancer and found their roles in the occurrence and progression of ovarian cancer.

Previously, the main diagnostic methods for OC were transvaginal ultrasound, pelvic examination or marker carbohydrate antigen 125, but these methods had limited effect in early OC[21]. miRNA acted in the biological process of tumors, and they are related to the development of tumors[22]. It was shown that miR-34a-5p had anti-cancer function in various cancers[23]. Previous researchers[24,25] have concluded that the decrease of miR-34a-5p indicates a poor prognosis for nasopharyngeal carcinoma and colorectal cancer. However, the function of miR-34a-5p in OC has never been reported. For this reason, we detected miR-34a-5p in serum and tissues of OC patients by qRT-PCR and conclude that miR-34a-5p was decreased in OC, and lower expression predicted higher FIGO stage and lymph node metastasis. This indicated that miR-34a-5p acted in the occurrence and progression of OC. Further visualizing the ROC curve found that the AUC value of miR-34a-5p in the diagnosis of OC was 0.885, which had good clinical diagnostic value in OC. Although our research has confirmed the effect of miR-34a-5p in OC, the adjusting mechanism is still unclear. Therefore, we predicted that TRIM44 was a latent target of miR-34a-5p through TargetScan and miRBD databases. TRIM44 was a member of TRIM family, and

participated in various cell developments, such as invasion, differentiation, and proliferation[26]. Liu et al[27] reported that TRIM44 was enhanced in epithelial OC (EOC), and overexpression of TRIM44 in tissues had a correlation with the poor prognosis of patients. Similarly, TRIM44 was enhanced in OC serum and tissue expression in our study, which was related to FIGO stage and lymph node metastasis and could be used in diagnosis for OC.

It was well known that miRNA could play a biological function in tumors by binding mRNA 3'-UTR[28]. We preliminarily indicated the function of miR-34a-5p and TRIM44 in OC in the above research, but the relationship between the two regulatory mechanisms in OC has not been investigated. Therefore, we first observed the biological functions of miR-34a-5p and TRIM44 in OC and found that an evident increase of miR-34a-5p or decrease of TRIM44 could hinder the biologic behaviors of OC cells, while miR-34a-5p decrease or TRIM44 increase could accelerate the biologic behaviors of OC cells. In addition, the double luciferase reporter revealed that miR-34a-5p could combine with TRIM44, indicating that miR-34a-5p regulated the biological behavior of OC cells by targeting TRIM44. Previous studies have showed that the knockdown of TRIM44 can hinder the growth of non-small cell lung carcinoma and is carried out simultaneously with the increase of epithelial markers and the decrease of mesenchymal markers. Overexpression of TRIM44 could induce EMT and increase the metastatic ability of lung cancer cells[29]. EMT acts in the process of tumor invasion and metastasis, but the mechanism involved is still unclear[30]. In glioma, miR-101-3p could directly target TRIM44 to weaken EMT induced by TRIM44 and regulate proliferation and migration of glioma[31]. In nasopharyngeal carcinoma, NEAT1 knock-down could inhibit the biologic behaviors and EMT through miR-34a-5p[32]. These reports revealed that miR-34a-5p and TRIM44 may affect tumor EMT. Next, we observed miR-34a-5p and TRIM44 on EMT and found that an evident increase of miR-34a-5p or decrease of TRIM44 could elevate EMT-related protein E-cadherin and reduce N-cadherin and Fibronectin. Moreover, miR-34a-5p knockdown or TRIM44 overexpression can downregulate E-cadherin, while upregulate N-cadherin and Fibronectin. Further, through co-transfection, it was verified that overexpression of miR-34a-5p eliminated the biologic behaviors and EMT of TRIM44 on OC cells, and induced cell apoptosis. Therefore, miR-34a-5p may regulate EMT by directly targeting TRIM44. However, there are still some defects in our research. Firstly, there are no in *vivo* experiments to observe the role of miR-34a-5p and TRIM44 on tumors. Secondly, miR-34a-5p and TRIM44 need further practical verification in clinical diagnosis or treatment.

Conclusions

MiR-34a-5p and TRIM44 can be used as diagnostic markers for OC. MiR-34a-5p can act in cell proliferation, migration, invasion, apoptosis, and EMT by targeting TRIM44 in OC. This may provide new ideas for the treatment of OC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Lv XL, Zhu Y, Liu JW, Al H. The application value of the detection of the level of tissue polypeptide antigen, ovarian cancer antigen X1, cathepsin L and CA125 on the diagnosis of epithelial ovarian cancer. Eur Rev Med Pharmacol Sci 2016; 20: 5113-5116.
2) Meng X, Müller V, Milde-Langosch K, Trillsch F, Pantel K, Schwarzenbach H. Circulating cell-free miR-373, miR-200a, miR-200b and miR-200c in patients with epithelial ovarian cancer. Adv Exp Med Biol 2016; 924: 3-8.
3) Zuberi M, Mir R, Das J, Ahmad I, Javid J, Yadav P, Masroor M, Ahmad S, Ray PC, Saxena A. Expression of serum miR-200a, miR-200b, and miR-200c as candidate biomarkers in epithelial ovarian cancer and their association with clinicopathological features. Clin Transl Oncol 2015; 17: 779-787.
4) Tang X, Zeng X, Huang Y, Chen S, Lin F, Yang G, Yang N. MiR-423-5p serves as a diagnostic indicator and inhibits the proliferation and invasion of ovarian cancer. Exp Ther Med 2018; 15: 4723-4730.
5) 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.
6) Li Y, Gu Y, Tang N, Liu Y, Zhao Z. MiR-22-notch signaling pathway is involved in the regulation of the apoptosis and autophagy in human ovarian cancer cells. Biol Pharm Bull 2018; 41: 1237-1242.
7) Zhou J, Zhang C, Zhou B, Jiang D. MiR-183 modulated cell proliferation and apoptosis in ovarian

cancer through the TGF-β/Smad4 signaling pathway. Int J Mol Med 2019; 43: 1734-1746.

8) Wang XJ, Zhang DL, Fu C, Wei BZ, Li GJ. MiR-183 modulates multi-drug resistance in hepatocellular cancer (HCC) cells via miR-183-IDH2/SOCS6-HIF-1α feedback loop. Eur Rev Med Pharmacol Sci 2016; 20: 2020-2027.
9) Cha YH, Kim NH, Park C, Lee I, Kim HS, Yook JI. MiRNA-34 intrinsically links p53 tumor suppressor and Wnt signaling. Cell Cycle 2012; 11: 1273-1281.
10) Ren F, Zhang X, Liang H, Luo D, Rong M, Dang Y, Chen G. Prognostic significance of MiR-34a in solid tumors: a systemic review and meta-analysis with 4030 patients. Int J Clin Exp Med 2015; 8: 17377-17391.
11) Ayaz L, Çayan F, Balci Ş, Görür A, Akbayir S, Yıldırım Yaroğlu H, Doğruer Unal N, Tamer L. Circulating microRNA expression profiles in ovarian cancer. J Obstet Gynaecol 2014; 34: 620-624.
12) Pu Y, Zhao F, Wang H, Cai S. MiR-34a-5p promotes multi-chemoresistance of osteosarcoma through down-regulation of the DLL1 gene. Sci Rep 2017; 7: 44218.
13) Ding N, Wu H, Tao T, Peng E. NEAT1 regulates cell proliferation and apoptosis of ovarian cancer by miR-34a-5p/BCL2. Onco Targets Ther 2017; 10: 4905-4915.
14) Gao J, Li N, Dong Y, Li S, Xu L, Li X, Li Y, Li Z, Ng SS, Sung JJ, Shen L, Yu J. MiR-34a-5p suppresses colorectal cancer metastasis and predicts recurrence in patients with stage II/III colorectal cancer. Oncogene 2015; 34: 4142-4152.
15) Xiong D, Jin C, Ye X, Qiu B, Jianjun X, Zhu S, Xiang L, Wu H, Yongbing W. TRIM44 promotes human esophageal cancer progression via the AKT/mTOR pathway. Cancer Sci 2018; 109: 3080-3092.
16) Zhou X, Yang Y, Ma P, Wang N, Yang D, Tu Q, Sun B, Xiang T, Zhao X, Hou Z, Fang X. TRIM44 is indispensable for glioma cell proliferation and cell cycle progression through AKT/p21/p27 signaling pathway. J Neurooncol 2019; 145: 211-222.
17) Luo Q, Lin H, Ye X, Huang J, Lu S, Xu L. Trim44 facilitates the migration and invasion of human lung cancer cells via the NF-κB signaling pathway. Int J Clin Oncol 2015; 20: 508-517.
18) Ledermann JA, Raja FA, Fotopoulou C, Gonzalez-Martin A, Colombo N, Sessa C; ESMO Guidelines Working Group. Newly diagnosed and relapsed epithelial ovarian carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2013; 24: vi24-vi32.
19) He PY, Yip WK, Chai BL, Chai BY, Jabar MF, Dusa N, Mohtarrudin N, Seow HF. Inhibition of cell migration and invasion by miR-29a-3p in a colorectal cancer cell line through suppression of CDC42BPA mRNA expression. Oncol Rep 2017; 38: 3554-3566.
20) Oza AM, Cook AD, Pfisterer J, Embleton A, Ledermann JA, Pujade-Lauraine E, Kristensen G, Carey MS, Beale P, Cervantes A, Park-Simon TW, Rustin G, Joly F, Mirza MR, Plante M, Quinn M, Poveda A, Jayson GC, Stark D, Swart AM, Farrelly L, Kaplan R, Parmar MK, Perren TJ; ICON7 trial investigators. Standard chemotherapy with or without bevacizumab for women with newly diagnosed ovarian cancer (ICON7): overall survival results of a phase 3 randomised trial. Lancet Oncol 2015; 16: 928-936.
21) Kossaï M, Leary A, Scoazec JY, Genestie C. Ovarian cancer: a heterogeneous disease. Pathobiology 2018; 85: 41-49.
22) Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK. MiRNA deregulation in cancer cells and the tumor microenvironment. Cancer Discov 2016; 6: 235-246.
23) Maroni P, Puglisi R, Mattia G, Carè A, Matteucci E, Bendinelli P, Desiderio MA. In bone metastasis miR-34a-5p absence inversely correlates with Met expression, while Met oncogene is unaffected by miR-34a-5p in non-metastatic and metastatic breast carcinomas. Carcinogenesis 2017; 38: 492-503.
24) Song P, Ye LF, Zhang C, Peng T, Zhou XH. Long non-coding RNA XIST exerts oncogenic functions in human nasopharyngeal carcinoma by targeting miR-34a-5p. Gene 2016; 592: 8-14.
25) Song H, He P, Shao T, Li Y, Li J, Zhang Y. Long non-coding RNA XIST functions as an oncogene in human colorectal cancer by targeting miR-132-3p. J BUON 2017; 22: 696-703.
26) Yamada Y, Takayama KI, Fujimura T, Ashikari D, Obinata D, Takahashi S, Ikeda K, Kakutani S, Urano T, Fukuhara H, Homma Y, Inoue S. A novel prognostic factor TRIM44 promotes cell proliferation and migration and inhibits apoptosis in testicular germ cell tumor. Cancer Sci 2017; 108: 32-41.
27) Liu S, Yin H, Ji H, Zhu J, Ma R. Overexpression of TRIM44 is an independent marker for predicting poor prognosis in epithelial ovarian cancer. Exp Ther Med 2018; 16: 3034-3040.
28) Ren K, Liu QQ, An ZF, Zhang DP, Chen XH. MiR-144 functions as tumor suppressor by targeting PIM1 in gastric cancer. Eur Rev Med Pharmacol Sci 2017; 21: 3028-3037.
29) Xing Y, Meng Q, Chen X, Zhao Y, Liu W, Hu J, Xue F, Wang X, Cai L. TRIM44 promotes proliferation and metastasis in non-small cell lung cancer via mTOR signaling pathway. Oncotarget 2016; 7: 30479-30491.
30) Ye Z, Zhou M, Tian B, Wu B, Li J. Expression of lncRNA-CCAT1, E-cadherin and N-cadherin in colorectal cancer and its clinical significance. Int J Clin Exp Med 2015; 8: 3707-3715.
31) Li L, Shao MY, Zou SC, Xiao ZF, Chen ZC. MiR-101-3p inhibits EMT to attenuate proliferation and metastasis in glioblastoma by targeting TRIM44. J Neurooncol 2019; 141: 19-30.
32) Ji Y, Wang M, Li X, Cui F. The long noncoding RNA NEAT1 targets miR-34a-5p and Drives Nasopharyngeal Carcinoma Progression via Wnt/β-catenin signaling. Yonsei Med J 2019; 60: 336-345.