Int J Clin Exp Pathol 2017;10(2):1331-1339
www.ijcep.com /ISSN:1936-2625/IJCEP0042893

Original Article

MicroRNA-380-5p inhibits migration and invasion of ovarian cancer SKOV3 cells and regulates epithelial-mesenchymal transition factors

Haiying Su[1,2], Dongguang Liu[2], Chunyan Yan[3], Guoyun Wang[1]

[1]Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, China; [2]Department of Obstetrics and Gynecology, Jining No. 1 People's Hospital, Jining, China; [3]Department of Pathology, Jining Medical University, Jining, China

Received October 26, 2016; Accepted December 14, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: Ovarian cancer is a severe gynecological malignancy affecting the health of numerous female patients. microRNA (miRNA) plays key roles in regulating ovarian cancer metastasis, while the role of miR-380-5p in ovarian cancer is unclear. This study aims to uncover the function of miR-380-5p in ovarian cancer cell migration, invasion and epithelial-mesenchymal transition (EMT). miR-380-5p level was quantified in ovarian cancer OVCAR3 and SKOV3 cells and normal ovarian surface epithelial IOSE80 cells. Cell transfection with miRNA mimic was performed to elevate miR-380-5p in SKOV3, followed by MTT and Transwell assays on cell viability, migration and invasion changes. Transforming growth factor-β1 (TGF-β1) treatment was performed to induce EMT in SKOV3 cells, and then EMT factors were detected by Western blot. Results showed that miR-380-5p was significantly down-regulated in ovarian cancer cells ($P < 0.001$). miR-380-5p up-regulation in SKOV3 cells suppressed cell viability, migration and invasion ($P < 0.05$) and attenuated the expression changes of E-cadherin, N-cadherin and vimentin (VIM) that were induced by TGF-β1 ($P < 0.05$). miR-380-5p also inhibited ras-related C3 botulinum toxin substrate 1 (RAC1) protein expression ($P < 0.01$), while RAC1 overexpression promoted SKOV3 cell invasion ($P < 0.01$). Besides, RAC1 was up-regulated by TGF-β1 treatment, implying its involvement in ovarian cancer cell invasion and EMT. This study suggests the suppressive role of miR-380-5p in ovarian cancer cell viability, migration, invasion and EMT, which may be associated with its regulation on RAC1. Thus miR-380-5p may provide a potential option for the molecular therapy of ovarian cancer.

Keywords: Ovarian cancer, microRNA-380-5p, migration, invasion, epithelial-mesenchymal transition, ras-related C3 botulinum toxin substrate 1

Introduction

Ovarian cancer is a familiar tumor with a high morbidity and mortality in the reproductive organ of females, occupying the leading position among gynecological tumors. It jeopardizes the health of female patients and is therefore considered as a focus of cancer treatment. Unfortunately, the early symptom of ovarian cancer is relatively mild or inconspicuous, adding difficulties to timely diagnosis and treatment [1]. In the recent years, effective markers and potent regulators for ovarian cancer are being explored, which provide abundant supports for theoretical and clinical research on this disease [2, 3].

Ovarian cancer has a great potential of metastasis. Advanced ovarian cancer may lead to distant metastases to various tissue and organs like lung, bone and lymph node [4, 5]. Epithelial-mesenchymal transition (EMT) plays vital roles in enhancing the migration and invasion ability of ovarian cancer cells, thus facilitating ovarian cancer metastasis [6, 7]. Along with the progression of EMT during which the phenotype of epithelial cells alters to that of mesenchymal cells, various factors are regulated and modified, such as E-cadherin and N-cadherin [8]. Besides, transforming growth factor-β (TGF-β) pathway is a key mechanism inducing EMT. Both TGF-β1 and TGF-β2 have been detected at higher levels in malignant ovarian cancer tis-

sue compared to normal ovarian tissue [9]. Furthermore, obvious modification in cytoskeletal organization has been observed in ovarian cancer SKOV3 cells treated with TGF-β1 [10]. Hence, EMT is becoming a focus in studies on ovarian cancer cell migration and invasion.

microRNA (miRNA) is a kind of short non-coding RNA that exists extensively in eukaryotes. It regulates gene expression post-transcriptionally and influences a variety of biological processes. Among the miRNAs close related to cancer pathogenesis and progression, some have been reported in ovarian cancer [11]. For example, miR-200c regulates zinc finger E-box binding homeobox 2 (ZEB2) expression to suppress invasion of ovarian cancer ES-2 cells [12]. miR-22 suppresses migration and invasion abilities of SKOV3 cells and is predicted to regulate factors of cancer metastasis [13]. miR-125a inhibits EMT of ovarian cancer cells [14]. These findings on miRNAs provide theoretical proofs for the effective diagnosis and treatment of ovarian cancer.

miR-380-5p has been revealed as a suppressor of neuroblastoma apoptosis [15], which connected it with the modulation of cancer cells for the first time. A recent study predicted that the up-regulation of miR-380-5p might participate in cell apoptosis and adhesion, as well as the regulation of EMT components [16], which inspired us to speculate the possible functions of miR-380-5p in ovarian cancer cell migration, invasion and EMT. However, little evidence could be found in existed studies. Hence, the aim of this study was to investigate the role of miR-380-5p in ovarian cancer cell migration and invasion, and to elucidate its mechanism in regulating EMT. miR-380-5p level was regulated in SKOV3 cells by transfection with its specific mimic, and then cell viability, migration, invasion and expression of EMT factors were analyzed. These results were supposed to provide new evidence for the pivotal function of miR-380-5p in ovarian cancer cells, which might support molecular-targeted therapy for ovarian cancer.

Materials and methods

Cell culture

Human ovarian cancer cells OVCAR3 and SKOV3 (ATCC, Manassas, VA, USA) and normal ovarian surface epithelial cells IOSE80 (Bioleaf, Shanghai, China) were used in this study to compare the miR-380-5p level. The cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco) and incubated in humid atmosphere with 5% CO_2. The growth status of cells was observed regularly with an inverted microscope IX73 (Olympus, Tokyo, Japan). Cells were passaged at a confluency of about 80% after digested with trypsin-ethylene diamine tetraacetic acid (Trypsin-EDTA, Yeasen, Shanghai, China). Cells of the logarithmic phase were used in the following experiments.

Cell transfection

SKOV3 cells were seeded in 24-well plates (1×10^5 cells/well). When the confluency reached about 90%, the medium was changed into serum-free RPMI-1640. Cell transfection was performed by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. miR-380-5p mimic or mimic control (50 nM) designed and synthesized by RiboBio (Guangzhou, China) was added to up-regulate the level of miR-380-5p. The specific small interfering RNA (siRNA) for ras-related C3 botulinum toxin substrate 1 (si-RAC1, 20 pmol) or the control (si-control) synthesized by GenePharma (Shanghai, China) was added to knockdown *RAC1* gene. The RAC1 overexpression vector prepared by ligating the complete coding sequence of *RAC1* (GenBank Accession NM_006908) to pcDNA3.1 vector (0.8 μg/well, Thermo Scientific, Carlsbad, CA, USA) or the blank vector was added to overexpress RAC1. The cells were incubated at 37°C for 48 h and then analyzed by 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT), Transwell, qRT-PCR and Western blot.

TGF-β1 treatment

TGF-β1 treatment was performed before Western blot to analyze its influence on factor expression. For untransfected SKOV3 cells, Recombinant Human TGF-β1 (10 ng/mL, Peprotech, Rochy Hill, NJ, USA) was added to the medium [10], and the cells were treated for different time periods. For SKOV3 cells transfected with miR-380-5p mimic or mimic control, TGF-β1 (10 ng/mL) treatment for 24 h was performed before transfection.

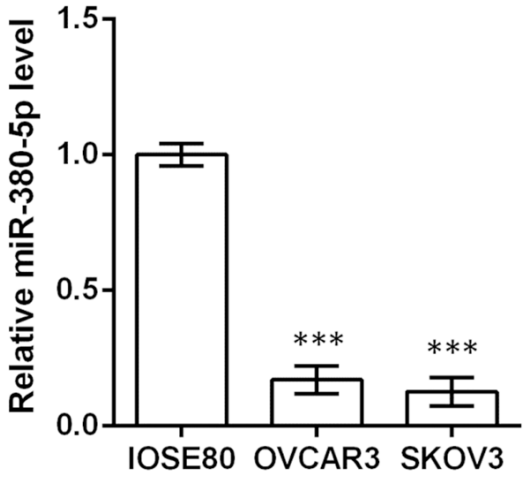

Figure 1. Relative miR-380-5p level in normal ovarian surface epithelial cells IOSE80 and ovarian cancer cells OVCAR3 and SKOV3. qRT-PCR indicates the significant down-regulated miR-380 level in OVCAR3 and SKOV3 (n = 5). ***P < 0.001 compared to IOSE80.

Cell viability

During the transfection with miR-380-5p mimic or mimic control, SKOV3 cells were detected by MTT assay at different time points (before transfection and transfected for 1, 2, 3 and 4 d). MTT assay was performed with MTT Cell Proliferation and Cytotoxicity Kit (Beyotime, Shanghai, China) according to the manufacturer's instruction. In brief, 10 μL of MTT solution was added to each well and the cells were incubated for 4 h, after which 100 μL of Formazan solution was added. The plates were incubated with gentle shaking until all purple crystals were dissolved. The absorbance at 570 nm was detected with a microplate reader Multiskan Go (Thermo Scientific, Carlsbad, CA, USA).

Cell migration and invasion

Cell migration was assessed with Transwell and 24-well plates (8.0 μm pore, BD Biosciences, San Jose, CA, USA) according to the manufacturer's instruction. SKOV3 cells were digested with Trypsin-EDTA and washed twice in phosphate-buffered saline (PBS), and then seeded in the plates containing serum-free medium (5×10^4 cells/well). The lower chamber was filled with RPMI-1640 medium supplemented with 20% FBS. Plates were incubated at 37°C for 24 h, after which the membrane was washed twice in PBS and fixed in methanol for 30 min at 4°C followed by staining in Crystal Violet Solution (Beyotime) for 20 min at room temperature. Then cells in the upper side of the membrane were wiped with cotton swabs, and those passed through the membrane were counted under a microscope (Olympus). The same procedures were also performed to detect cell invasion, but the membrane was pre-coated with Matrigel (BD Biosciences), incubated at 37°C for 30 min and infiltrated with culture medium for 2 h before cell seeding.

qRT-PCR

Total RNA was extracted from the 3 cell lines and transfected SKOV3 cells using TRIzol (Invitrogen) according the manufacturer's instruction. DNA contamination was removed by DNase I (Invitrogen) and then RNA samples were quantified with NanoDrop 2000 (Thermo Scientific). In reverse transcription, 1 μg of RNA from each cell sample was used in the reaction catalyzed by SuperScript III Reverse Transcriptase (Invitrogen). The specific primer (5'-CTCAA CTGGT GTCGT GGAGT CGGCA ATTCA GTTGA GGCGC ATGT-3') was used in hsa-miR-380-5p reverse transcription. qRT-PCR was conducted with LightCycler 480 (Roche, Basel, Switzerland). Each reaction system contained 20 ng of the complementary DNA, SYBR Green I Master (Roche) and the specific primer for miR-380-5p (forward: 5'-ACACT CCAGC TGGGT GGTTG GACCA TAGAA C-3' and reverse: 5'-TGGTG TCGTG GAGTC G-3') or *RAC1* (forward: 5'-CACGA TCGAG AAACT GAAGG A-3' and reverse: 5'-AGCAG GCATT TTCTC TTCCT C-3'). The relative level of miR-380-5p and *RAC1* was calculated by the $2^{-\Delta\Delta Ct}$ method normalized by *U6* (forward: 5'-CTCGC TTCGG CAGCA CATAT ACT-3' and reverse: 5'-ACGCT TCACG AATTT GCGTG TC-3') or *GAPDH* (forward: 5'-GAAGG TGAAG GTCGG AGTC-3' and reverse: 5'-GAAGA TGGTG ATGGG ATTTG-3'), respectively.

Western blot

Cell protein was extracted using ProteoPrep Total Extraction Sample Kit (Sigma-Aldrich, Saint Louis, MO, USA) according to the manufacturer's instruction. The protein (20 μg for each sample) was analyzed with sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred to a polyvinylidene fluoride membrane (Roche). The membrane was blocked in 5% milk in PBS for 4 h at room tem-

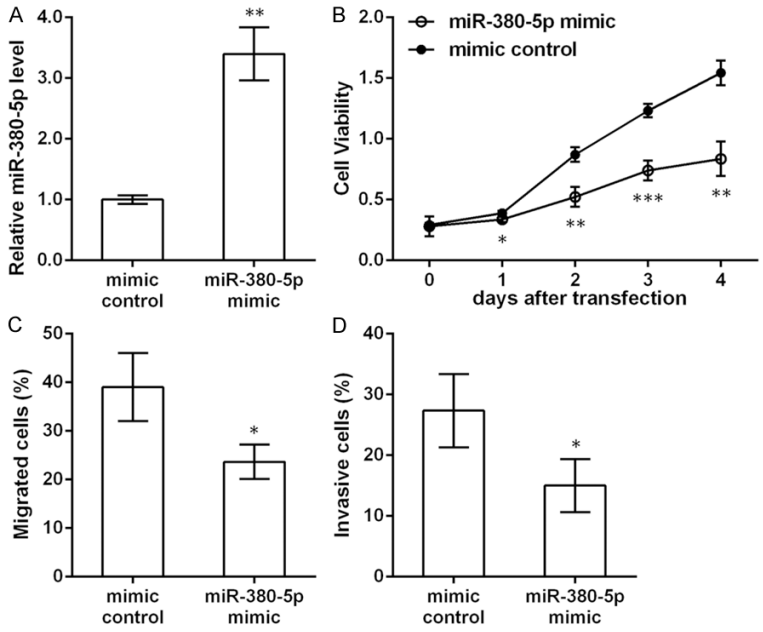

Figure 2. miR-380-5p inhibits viability, migration and invasion of ovarian cancer cells SKOV3. SKOV3 cells were transfected with miR-380-5p mimic or mimic control. qRT-PCR and Transwell assay were performed at 48 h post transfection. MTT assay was performed at 0, 1, 2, 3 and 4 d post transfection (n = 5). A: miR-380-5p level is significantly up-regulated by transfection of the mimic as shown by qRT-PCR. B: Cell viability is suppressed by miR-380-5p mimic when detected by MTT at 1, 2, 3 and 4 d post transfection. C: Percent of migrated cells is reduced by miR-380-5p mimic as revealed by Transwell. D: Percent of invasive cells is reduced by miR-380-5p mimic as revealed by Transwell. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$ compared to mimic control.

perature and then incubated in primary antibodies (1:1000) against RAC1 (ab33186, Abcam, Cambridge, UK), E-cadherin (ab1416), N-cadherin (ab18203), vimentin (VIM, ab8978), snail family zinc finger 1 (SNAI1, Saier, Tianjin, China), SNAI2 (Saier) and ZEB1 (Boster, Wuhan, China) overnight at 4°C. After washed in PBS for 3 times, the membrane was incubated in horseradish peroxidase-conjugated secondary antibodies (1:2000, ab6702 or ab6708, Abcam) for 2 h at room temperature followed by wash in PBS. Signals were developed using ECL Plus Western Blotting Substrate (Pierce, Carlsbad, CA, USA). Signal intensity was analyzed by ImageJ 1.49 software (National Institutes of Health, Bethesda, MD, USA) normalized with that of GAPDH (ab9485, Abcam).

Statistical analysis

All the experiments were repeated for 5 times and results were presented as the mean ± standard error of mean. Data were analyzed with one-way analysis of variance followed by Student's *t* test in SPSS 19 (IBM, New York, NY, USA). Differences between groups were considered statistically significant if $P < 0.05$.

Results

miR-380-5p is down-regulated in ovarian cancer cells

Before the functional analysis, miR-380-5p level was quantified by qRT-PCR in difference cell lines. It was indicated that miR-380-5p was significantly down-regulated in ovarian cancer OVCAR3 and SKOV3 cells compared to normal ovarian surface epithelial cells IOSE80 ($P < 0.001$, **Figure 1**). This result suggested the potential involvement of miR-380-5p in ovarian cancer pathogenesis, thus its effects on ovarian cancer cells were investigated in the following study.

miR-380-5p inhibits SKOV3 cell viability, migration, invasion and EMT

In ovarian cancer SKOV3 cells, miR-380-5p level was up-regulated by transfection with its mimic in order to investigate its role in ovarian cancer cell viability, migration and invasion. Before the investigation, the transfection effectiveness was verified: miR-380-5p level was significantly up-regulated by transfection with its mimic compared to the mimic control ($P < 0.01$, **Figure 2A**), suggesting the successful cell transfection. Thus the cells were used in the following experiments.

MTT assay suggested that miR-380-5p markedly reduced SKOV3 cell viability at 1, 2, 3 and 4 d post transfection compared to mimic control ($P < 0.05$, **Figure 2B**). Transwell assay showed that the percent of migrated or invasive SKOV3 cells was significantly suppressed by miR-380-5p ($P < 0.05$, **Figure 2C** and **2D**). Taken together, these results suggested the suppressive function of miR-380-5p in SKOV3 cell viability, migration and invasion.

Since EMT was closely related to the pathogenesis and progression of ovarian cancer, we next

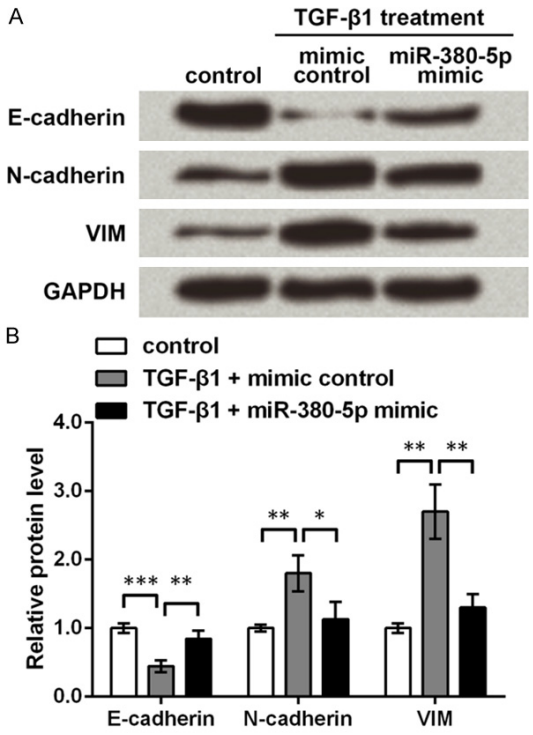

Figure 3. Transforming growth factor β1 (TGF-β1) treatment and miR-380-5p regulate E-cadherin, N-cadherin and vimentin (VIM) in ovarian cancer cells SKOV3. SKOV3 cells were transfected with miR-380-5p and treated with TGF-β1 for 48 h, after which Western blot was performed to detect the protein level of the three factors (n = 5). A: TGF-β1 inhibits E-cadherin and promotes N-cadherin and VIM, but its effects are attenuated by miR-380-5p as revealed by Western blot. GAPDH was used as an internal control. B: Quantitated results of Western blot indicate significant differences between groups. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

detected the expression of EMT-related factors after SKOV3 cells were treated with TGF-β1. Western blot showed that TGF-β1 suppressed E-cadherin and induced N-cadherin and VIM protein expression ($P < 0.01$, **Figure 3A** and **3B**), which indicated the promoted EMT process. However, miR-380-5p could abrogate the effects of TGF-β1, which was significantly different from the mimic control groups ($P < 0.05$). These results implied the possibility that miR-380-5p might suppress EMT in SKOV3 cells.

miR-380-5p regulates RAC1 and EMT-related factors

Given that RAC1 is involved in the malignancy of ovarian cancer [17], the expression of RAC1 was detected in the transfected cells. Western blot indicated that RAC1 protein level was significantly suppressed by miR-380-5p ($P < 0.01$, **Figure 4A**), which implied that miR-380-5p might regulate the expression of RAC1. So next we explored whether RAC1 was involved in the mechanism of miR-380-5p. RAC1 expression level was successfully altered by its overexpression vector and siRNA compared to the corresponding control groups ($P < 0.01$ and $P < 0.001$, **Figure 4B**). Besides, RAC1 protein level showed consistent changing patterns (**Figure 4C**). Transwell assay indicated that RAC1 overexpression obviously increased the percent of invasive cells ($P < 0.01$, **Figure 4D**) and that knockdown of RAC1 suppressed cell invasion ($P < 0.05$), which suggested that the role of RAC1 in SKOV3 cells was opposite to miR-380-5p. Collectively, RAC1 might be involved in the regulatory function of miR-380-5p in SKOV3 cell invasion.

Western blot was further conducted to investigate whether RAC1 was related to EMT. Results showed that with the prolonged TGF-β1 treatment duration, E-cadherin was gradually decreased, and N-cadherin and VIM were elevated (**Figure 5**). Moreover, RAC1 protein level also increased with TGF-β1 treatment, suggesting that RAC1 was induced by EMT. Besides, the expression of EMT factors including SNAI1, SNAI2 and ZEB1 was also analyzed. It was found that SNAI1 and SNAI2 were hardly detected when SKOV3 was treated by TGF-β1 for less than 4 h, while they were gradually promoted by TGF-β1 of longer treatment duration. Similarly, ZEB1 was also increased along with TGF-β1 treatment, further supporting the induced EMT in TGF-β1-treated SKOV3 cells. These results implied that RAC1 was involved in the EMT process, which might help to elucidate the functional mechanism of miR-380-5p in EMT of SKOV3 cells.

Discussion

In light of the potential function of miR-380-5p in ovarian cancer that has been implied in existed research [16], this study performed cell transfection, MTT, Transwell and factor expression assays to investigate the effect of miR-380-5p on SKOV3 cell migration, invasion and EMT. miR-380-5p was detected in a significant lower level in ovarian cancer OVCAR3 and SKOV3 cells. miR-380-5p suppressed SKOV3 cell viability, migration and invasion and inhib-

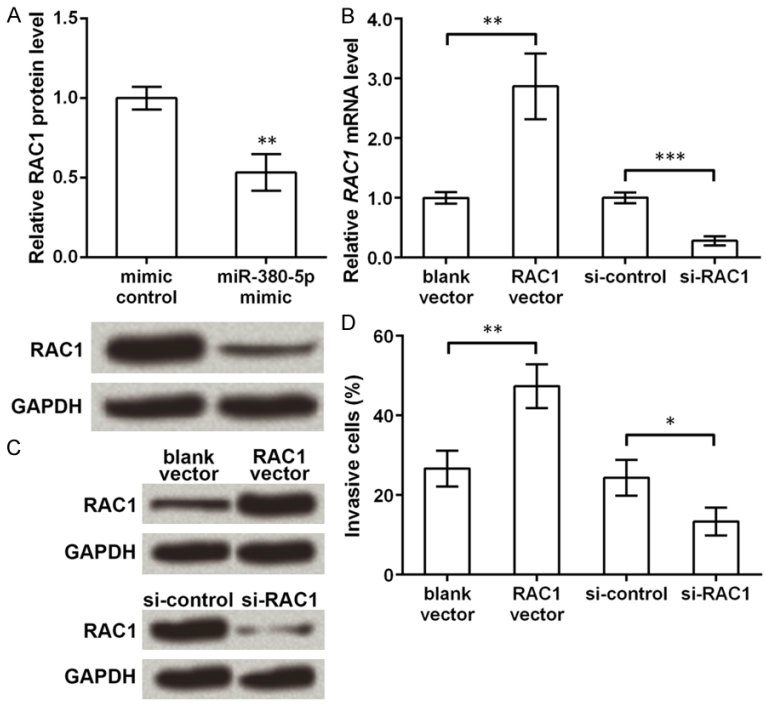

Figure 4. Ras-related C3 botulinum toxin substrate 1 (RAC1) is suppressed by miR-380-5p and can promote ovarian cancer cells SKOV3. SKOV3 cells were transfected with the overexpression vector of RAC1 (RAC1 vector) or the specific small interfering RNA of RAC1 (si-RAC1). qRT-PCR, Western blot and Transwell assay were performed at 48 h post transfection (n = 5). A: miR-380-5p inhibits RAC1 protein expression as shown by Western blot. GAPDH was used as an internal control. B: Transfection with RAC1 vector or si-RAC1 successfully induces changes in *RAC1* mRNA level compared to the corresponding control (blank vector or si-control) as revealed by qRT-PCR. C: Transfection with RAC1 vector or si-RAC1 successfully induces changes in RAC1 protein level compared to blank vector or si-control as revealed by Western blot. D: RAC1 overexpression increases and si-RAC1 decreases percent of invasive cells as revealed by Transwell. $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

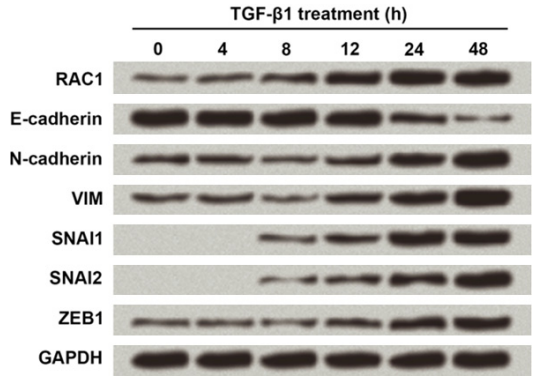

Figure 5. Transforming growth factor β1 (TGF-β1) treatment regulates the protein expression of ras-related C3 botulinum toxin substrate 1 (RAC1), E-cadherin, N-cadherin, vimentin (VIM), snail family zinc finger 1 (SNAI1), SNAI2 and zinc finger E-box binding homeobox 1 (ZEB1) in a time-dependent manner. Western blot was performed at 0, 4, 8, 12, 24 and 48 h post TGF-β1 treatment (n = 5). GAPDH was used as an internal control.

ited RAC1, while RAC1 overexpression promoted SKOV3 cell invasion. Besides, miR-380-5p could regulate E-cadherin, N-cadherin and VIM expression that was altered by TGF-β1 treatment. Meanwhile, RAC1 expression was also induced by TGF-β1 treatment.

The aberrant level of miR-380-5p has been reported in several diseases. Its elevated level is detected in embryonic stem cells and neuroblastomas, which may be correlated with the poor outcome of neuroblastomas [15]. However, miR-380-5p is suppressed in other cancers, for example, non-small cell lung cancer [18] and oral squamous cell carcinoma [19]. In this study, miR-380-5p was in a significantly lower level in ovarian cancer OVCAR3 and SKOV3 cells compared to normal cell line IOSE80. This result is in line with the research in non-small cell lung cancer and oral squamous cell carcinoma, which implies the potential role of miR-380-5p in ovarian cancer cells.

TGF-β1 is crucial for EMT progression and has been used to induce EMT in various cells [20-22]. Based on these reports, this study treated SKOV3 cells with TGF-β1 to induce EMT, and the effects were examined by the expression of E-cadherin, N-cadherin and VIM. EMT is usually accompanied by the switch from E-cadherin to N-cadherin and the up-regulation of VIM [23, 24]. This study also detected suppressed E-cadherin and promoted N-cadherin and VIM expression by TGF-β1 treatment, which indicated the induced EMT. Furthermore, miR-380-5p alleviated the effect of TGF-β1 on these factors, which may imply its repressive role in EMT of SKOV3 cells.

Up-regulation of miR-380-5p in ovarian cancer SKOV3 cells led to obvious suppression in cell viability, migration and invasion, suggesting the regulatory function of miR-380-5p in ovarian cancer cells. Limited evidence has been report-

ed before. However, we tried to verify the role of miR-380-5p by testing the function of RAC1, whose protein expression was remarkably inhibited by miR-380-5p. It has been reported that RAC1 is capable of inducing cancer cell migration and invasion [25-27] and is considered to be a promising target for controlling cancer metastasis [28]. Consistently, RAC1 overexpression in this study markedly increase SKOV3 cell invasion, while its knockdown showed opposite effects. Together with the suppressed RAC1 expression by miR-380-5p, these results further support that miR-380-5p has inhibitory roles in ovarian cancer cell viability, migration and invasion, which may involve the mechanism of RAC1 regulation.

As aforementioned that RAC1 could be inhibited by miR-380-5p and involved in SKOV3 invasion, RAC1 expression was then assessed, together with several factors related to EMT to reveal its function in EMT. RAC1 expression was gradually increasing with the prolonged TGF-β1 treatment, implying its association with EMT, which is well founded because numerous studies have suggested RAC1 is correlated with EMT [29, 30]. Especially, both *in vitro* and *in vivo* studies support that RAC1 overexpression is associated with increased EMT in epithelial ovarian cancer [31]. Hence the up-regulated RAC1 level by TGF-β1 indicated that RAC1 is also involved in EMT of SKOV3 cells. Similarly, E-cadherin, N-cadherin and VIM possessed decreasing or increasing expression changes with the prolonged TGF-β1 treatment. Moreover, SNAI1, SNAI2 and ZEB1, three factors triggering EMT [32-34], were also detected to be promoted by TGF-β1 treatment, further indicating the elevated EMT in SKOV3 cells. Taken together, RAC1 is important to the regulation of invasion and EMT in SKOV3 cells, and its suppression by miR-380-5p therefore suggests that RAC1 may help to elucidate the mechanism of miR-380-5p in regulating SKOV3 cell invasion and EMT.

In summary, miR-380-5p has suppressive effects on viability, migration, invasion of ovarian cancer SKOV3 cells, which may be related to its regulation on RAC1 and the EMT process. This study enriches information on miR-380-5p studies and provides basic evidence for potential application of miR-380-5p in the molecular-targeted therapy for ovarian cancer.

Disclosure of conflict of interest

None.

Address correspondence to: Guoyun Wang, Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, 107 Wenhua West Road, Lixia District, Jinan 250012, China. E-mail: wangguoyun012@126.com

References

[1] Rossing MA, Wicklund KG, Cushing-Haugen KL and Weiss NS. Predictive value of symptoms for early detection of ovarian cancer. J Natl Cancer Inst 2010; 102: 222-229.

[2] Tian C, Sargent DJ, Krivak TC, Powell MA, Gabrin MJ, Brower SL and Coleman RL. Evaluation of a chemoresponse assay as a predictive marker in the treatment of recurrent ovarian cancer: further analysis of a prospective study. Br J Cancer 2014; 111: 843-850.

[3] Cruet-Hennequart S, Maubant S, Luis J, Gauduchon P, Staedel C and Dedhar S. alpha(v) integrins regulate cell proliferation through integrin-linked kinase (ILK) in ovarian cancer cells. Oncogene 2003; 22: 1688-1702.

[4] Dauplat J, Hacker NF, Nieberg RK, Berek JS, Rose TP and Sagae S. Distant metastases in epithelial ovarian carcinoma. Cancer 1987; 60: 1561-1566.

[5] Pradeep S, Kim SW, Wu SY, Nishimura M, Chaluvally-Raghavan P, Miyake T, Pecot CV, Kim SJ, Choi HJ, Bischoff FZ, Mayer JA, Huang L, Nick AM, Hall CS, Rodriguez-Aguayo C, Zand B, Dalton HJ, Arumugam T, Lee HJ, Han HD, Cho MS, Rupaimoole R, Mangala LS, Sehgal V, Oh SC, Liu J, Lee JS, Coleman RL, Ram P, Lopez-Berestein G, Fidler IJ and Sood AK. Hematogenous metastasis of ovarian cancer: rethinking mode of spread. Cancer Cell 2014; 26: 77-91.

[6] Ponnusamy MP, Lakshmanan I, Jain M, Das S, Chakraborty S, Dey P and Batra SK. MUC4 mucin-induced epithelial to mesenchymal transition: a novel mechanism for metastasis of human ovarian cancer cells. Oncogene 2010; 29: 5741-5754.

[7] Yagi H, Yotsumoto F and Miyamoto S. Heparin-binding epidermal growth factor-like growth factor promotes transcoelomic metastasis in ovarian cancer through epithelial-mesenchymal transition. Mol Cancer Ther 2008; 7: 3441-3451.

[8] Chen L, Munoz-Antonia T and Cress WD. Trim28 contributes to EMT via regulation of E-cadherin and N-cadherin in lung cancer cell lines. PLoS One 2014; 9: e101040.

[9] Inan S, Vatansever S, Celik-Ozenci C, Sanci M, Dicle N and Demir R. Immunolocalizations of VEGF, its receptors flt-1, KDR and TGF-beta's in epithelial ovarian tumors. Histol Histopathol 2006; 21: 1055-1064.
[10] Vergara D, Merlot B, Lucot JP, Collinet P, Vinatier D, Fournier I and Salzet M. Epithelial-mesenchymal transition in ovarian cancer. Cancer Lett 2010; 291: 59-66.
[11] Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Menard S and Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res 2007; 67: 8699-8707.
[12] Wu Q, Guo R, Lin M, Zhou B and Wang Y. MicroRNA-200a inhibits CD133/1+ ovarian cancer stem cells migration and invasion by targeting E-cadherin repressor ZEB2. Gynecol Oncol 2011; 122: 149-154.
[13] Li J, Liang S, Yu H, Zhang J, Ma D and Lu X. An inhibitory effect of miR-22 on cell migration and invasion in ovarian cancer. Gynecol Oncol 2010; 119: 543-548.
[14] Cowden Dahl KD, Dahl R, Kruichak JN and Hudson LG. The epidermal growth factor receptor responsive miR-125a represses mesenchymal morphology in ovarian cancer cells. Neoplasia 2009; 11: 1208-1215.
[15] Swarbrick A, Woods SL, Shaw A, Balakrishnan A, Phua Y, Nguyen A, Chanthery Y, Lim L, Ashton LJ, Judson RL, Huskey N, Blelloch R, Haber M, Norris MD, Lengyel P, Hackett CS, Preiss T, Chetcuti A, Sullivan CS, Marcusson EG, Weiss W, L'Etoile N and Goga A. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. Nat Med 2010; 16: 1134-1140.
[16] Maria RA and Alexandrina B. Changes in microRNA expression induced by three-dimensional aggregation of mesenchymal stem cells. Annals of R.S.C.B. 2015; 53-61.
[17] Wang H, Linghu H, Wang J, Che YL, Xiang TX, Tang WX and Yao ZW. The role of Crk/Dock180/Rac1 pathway in the malignant behavior of human ovarian cancer cell SKOV3. Tumour Biol 2010; 31: 59-67.
[18] Yu X, Wei F, Yan F, Yu W, Li H and Ren X. Low expression profiling of microRNA in non-small cell lung cancer tissues. Chinese Journal of Clinical Oncology 2014; 41: 291-295.
[19] Manikandan M, Rao AKDM, Arunkumar G, Rajkumar KS, Rajaraman R and Munirajan AK. Down regulation of miR-34a and miR-143 may indirectly inhibit p53 in oral squamous cell carcinoma: a pilot study. Asian Pac J Cancer Prev 2015; 16: 7619-7625.
[20] Doerner AM and Zuraw BL. TGF-beta1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1beta but not abrogated by corticosteroids. Respir Res 2009; 10: 100.
[21] Slabáková E, Pernicová Z, Slavíčková E, Staršíchová A, Kozubík A and Souček K. TGF-β1-induced EMT of non-transformed prostate hyperplasia cells is characterized by early induction of SNAI2/Slug. Prostate 2011; 71: 1332-1343.
[22] Serrano I, McDonald PC, Lock FE and Dedhar S. Role of the integrin-linked kinase (ILK)/Rictor complex in TGFbeta-1-induced epithelial-mesenchymal transition (EMT). Oncogene 2013; 32: 50-60.
[23] Rogers CD, Saxena A and Bronner ME. Sip1 mediates an E-cadherin-to-N-cadherin switch during cranial neural crest EMT. J Cell Biol 2013; 203: 835-847.
[24] Otsuki S, Inokuchi M, Enjoji M, Ishikawa T, Takagi Y, Kato K, Yamada H, Kojima K and Sugihara K. Vimentin expression is associated with decreased survival in gastric cancer. Oncol Rep 2011; 25: 1235-1242.
[25] Yang WH, Lan HY, Huang CH, Tai SK, Tzeng CH, Kao SY, Wu KJ, Hung MC and Yang MH. RAC1 activation mediates Twist1-induced cancer cell migration. Nat Cell Biol 2012; 14: 366-374.
[26] Johnson E, Seachrist DD, DelLeon-Rodriguez CM, Lozada KL, Miedler J, Abdul-Karim FW and Keri RA. HER2/ErbB2-induced breast cancer cell migration and invasion require p120 catenin activation of Rac1 and Cdc42. J Biol Chem 2010; 285: 29491-29501.
[27] Imam JS, Plyler JR, Bansal H, Prajapati S, Bansal S, Rebeles J, Chen HI, Chang YF, Panneerdoss S, Zoghi B, Buddavarapu KC, Broaddus R, Hornsby P, Tomlinson G, Dome J, Vadlamudi RK, Pertsemlidis A, Chen Y and Rao MK. Genomic loss of tumor suppressor miRNA-204 promotes cancer cell migration and invasion by activating AKT/mTOR/Rac1 signaling and actin reorganization. PLoS One 2012; 7: e52397.
[28] Bid HK, Roberts RD, Manchanda PK and Houghton PJ. RAC1: an emerging therapeutic option for targeting cancer angiogenesis and metastasis. Mol Cancer Ther 2013; 12: 1925-1934.
[29] Gulhati P, Bowen KA, Liu J, Stevens PD, Rychahou PG, Chen M, Lee EY, Weiss HL, O'Connor KL, Gao T and Evers BM. mTORC1 and mTORC2 regulate EMT, motility, and metastasis of colorectal cancer via RhoA and Rac1 signaling pathways. Cancer Res 2011; 71: 3246-3256.
[30] Chen X, Cheng H, Pan T, Liu Y, Su Y, Ren C, Huang D, Zha X and Liang C. mTOR regulate EMT through RhoA and Rac1 pathway in prostate cancer. Mol Carcinog 2015; 54: 1086-1095.

[31] Leng R, Liao G, Wang H, Kuang J and Tang L. Rac1 expression in epithelial ovarian cancer: effect on cell EMT and clinical outcome. Med Oncol 2015; 32: 329.
[32] Casas E, Kim J, Bendesky A, Ohno-Machado L, Wolfe CJ and Yang J. Snail 2 is an essential mediator of Twist1-induced epithelial mesenchymal transition and metastasis. Cancer Res 2011; 71: 245-254.
[33] Kaufhold S and Bonavida B. Central role of snail 1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res 2014; 33: 62-80.
[34] Argast GM, Krueger JS, Thomson S, Sujka-Kwok I, Carey K, Silva S, O'Connor M, Mercado P, Mulford IJ, Young GD, Sennello R, Wild R, Pachter JA, Kan JL, Haley J, Rosenfeld-Franklin M and Epstein DM. Inducible expression of TGFβ, snail and Zeb1 recapitulates EMT in vitro and in vivo in a NSCLC model. Clin Exp Metastasis 2011; 28: 593-614.