Original Article

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

Haiying Su1,2, Dongguang Liu2, Chunyan Yan3, Guoyun Wang1

1Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, China; 2Department of Obstetrics and Gynecology, Jining No. 1 People’s Hospital, Jining, China; 3Department 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-
Role of miR-380-5p in ovarian cancer

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 cancerogenesis 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% CO₂. 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 × 10⁵ 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-phenyltetrazoliumromide (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.
Role of miR-380-5p in ovarian cancer

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 GTGGA GTGGT GGCGA 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 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 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-
Role of miR-380-5p in ovarian cancer

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 \( (P < 0.01, \text{Figure 2A}) \), 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 \( (n = 5) \). 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.

**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 different 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 \( (P < 0.001, \text{Figure 1}) \). 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 \( (P < 0.01, \text{Figure 2A}) \), 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 \( (P < 0.05, \text{Figure 2B}) \). Transwell assay showed that the percent of migrated or invasive SKOV3 cells was significantly suppressed by miR-380-5p \( (P < 0.05, \text{Figure 2C} \text{ and 2D}) \). 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...
Role of miR-380-5p in ovarian cancer

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.

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 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.

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
Role of miR-380-5p in ovarian cancer

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. 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-
Role of miR-380-5p in ovarian cancer

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 Weihua West Road, Lixia District, Jinan 250012, China. E-mail: wangguoyun012@126.com

References


Role of miR-380-5p in ovarian cancer


