MiR-381 regulates cell motility, growth and colony formation through PIK3CA in endometriosis-associated clear cell and endometrioid ovarian cancer

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Abstract. Ovarian cancer is the one of the most lethal gynecological cancer types. MicroRNAs (miRs) are noncoding RNAs that modulate the translation of their target mRNAs via binding to a complementary sequence in the target 3' untranslated region, and the dysregulation of certain miRs has been demonstrated to contribute to cancer progression. In this regard, the current study extended our previous work and used next-generation sequencing data to search for upstream regulators of genetic alterations that are common in ovarian cancer, as well as the miRs that are involved in controlling the expression of these regulators. An miR prediction program was used to identify miR-381 as an upstream regulator of phosphatidylinositol 3-kinase catalytic subunit α (PIK3CA) in the context of ovarian cancer. Levels of miR-381 were decreased in clear cell and endometrioid carcinoma ovarian cancer. Experimentally induced upregulation of miR-381 led to a decrease in the level of PIK3CA in ovarian cancer cells. Furthermore, experimentally induced upregulation of miR-381 inhibited the proliferation of ovarian cancer cells in vitro and their ability to form colonies and migrate. The observed decrease in miR-381 in ovarian cancer could be reversed upon overexpression of the gene encoding the tumor suppressor homeobox D10. The current results highlight the role of miR-381-mediated regulation of PIK3CA in the development and progression of ovarian cancer and suggest that restoration of miR-381 to normal levels in ovarian cancer cells may constitute a therapeutic strategy for patients.

Introduction

Ovarian cancer is the one of the most lethal gynecological cancer types. Approximately 85-90% of ovarian malignancies manifest as epithelial ovarian cancers (1). Ovarian cancer is a heterogeneous disease, which has been divided into two major subtypes based on histopathological, molecular and genetic criteria (2). Type I tumors comprise low-grade serous, clear cell, endometrioid and mucinous carcinomas, whereas Type II tumors comprise high-grade serous carcinomas, undifferentiated carcinomas and carcinosarcomas. Ovarian cancer remains a highly lethal malignancy, primarily as a result of inability to detect the cancer at an early, and thus more treatable, stage (3). Studies have demonstrated that ovarian cancer development is associated with the accumulation of certain gene mutations, as well as alterations in epigenetic modifications, which are associated with downstream activation of signaling pathways (4,5). Low-grade serous, clear cell, endometrioid and mucinous carcinomas are often associated with mutations in KRAS, BRAF, AT-rich interaction domain 1A (ARID1A), phosphatidylinositol 3-kinase catalytic subunit α (PIK3CA), CTNNB1 and ERBB2 but only very rarely in the tumor-suppressor gene tumor protein 53 (TP53) (6-9). Patients with high-grade serous ovarian cancer present with highly unstable genomes and harbor TP53 mutations in ~95% of cases (10). Although numerous studies have attempted to elucidate the general mechanisms of carcinogenesis in ovarian cancer, the molecular mechanisms, both genetic and epigenetic, that underlie ovarian tumorigenesis are not clearly understood.

MicroRNAs (miRNAs or miRs) are non-coding RNAs, 19-22 nucleotides in length, which are usually involved in the post-transcriptional downregulation of gene expression via base pairing with the 3'-untranslated region (3'-UTR) of target mRNAs. MiRNAs have been demonstrated to regulate both the translatability and turnover of numerous mRNAs involved in various biological processes, and hence miRNAs often serve as oncogenes or tumor suppressors (11). Mounting evidence indicates that miRNAs help define a particular
cancer phenotype via modulating the expression of genes and signaling pathways that are often involved in tumorigenesis (12). The signaling pathways involved in tumorigenesis that are frequently activated in human cancer thus represent attractive targets for therapies based on small molecules. Therefore, defining the networks of genetic and epigenetic interactions that govern tumorigenesis in any particular cancer type could inform molecular strategies for effective treatments across cancer subtypes.

The aim of the current study was to investigate whether epigenetic drivers could cause genetic alterations that typically lead to ovarian cancer. Next-generation sequencing data from our previous study (13) was analyzed to identify potential miRNAs that, upon dysregulation, could significantly impact both growth factor signaling pathways and the rate of genetic mutations that often lead to ovarian cancer.

Materials and methods

Bioinformatics analysis. Target prediction tools TargetScan (version 7.1; http://www.targetscan.org/vert_71/) and miRanda (http://www.mirbase.org/) were used to search potential target miRNAs for PIK3CA, ARID1A, ETS1 and DNA-dependent protein kinase catalytic subunit (PRKDC).

Cell lines and culture. The ovarian cancer cell lines TOV21G and TOV112D were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were grown in a 1:1 mixture of complete Medium 199 (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Gibco; Thermo Scientific, Inc.). The levels of miRNAs were measured using specific TaqMan probes (Thermo Fisher Scientific, Inc.) and 18S mRNA served as the internal control. The reaction mixture included 2 µl cDNA, 5 µl TaqMan Universal PCR Master Mix II (2X; cat. no. KMUH-IRB-20140031), and informed written consent was obtained from each patient.

Serum collection. Serum samples were obtained from patients who underwent surgery with indications of gynecological disease at the Department of Obstetrics and Gynecology of Kaohsiung Medical University Hospital. A total of 9 subjects aged 21 to 55 years participated in the present study, including 3 healthy controls, 3 patients with endometriosis, and 3 patients with ovarian cancer (1 endometrioid and 2 clear cell carcinoma). In each case of endometriosis-associated ovarian cancer (EOC), a final diagnosis of endometriosis-associated clear cell cancer, endometrioid cancer or ovarian cancer was made according to FIGO and WHO criteria in patients who were surgically resected between June 2014 and April 2016 (17). Each diagnosis of endometriosis, either by laparoscopy or laparotomy, followed the classification of the revised American Society of Reproductive Medicine, 1997 (16). The patients with endometriosis and EOC were histologically confirmed by examination of a laparoscopy. All patients had regular menstrual cycles and had not received any hormone treatment in the 6 months prior to gynecological surgery. Healthy controls were diagnosed as having other benign diseases, including urinary incontinence, pelvic organ prolapse or ovarian hemorrhagic cyst. Exclusion criteria included post-menopausal status, endometrial cancer, hyperplasia, endometrial polyps, infectious diseases, adenomyosis, inflammatory diseases, malignancy, autoimmune disease or cardiovascular disease.

The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (approval no. KMUHIRB-G(I)-20150046), and written informed consent was obtained from each participant. Whole blood was drawn in EDTA-coated tubes (BD Biosciences, Franklin Lakes, NJ, USA) and centrifuged at 2,200 x g for 10 min at room temperature. The resultant sera were aliquoted into Eppendorf tubes and stored at -80˚C for use in reverse transcription (RT) and quantitative polymerase chain reaction (qPCR) studies.

RNA extraction, cDNA synthesis and qPCR. RNA was extracted from patient sera using bulk reagents from the MasterPure Complete DNA and RNA Purification kit (Epicentre; Illumina, Inc., San Diego, CA, USA) or purified from cultured cells using TRIzol reagent (Thermo Fisher Scientific, Inc.). RT was performed using the Deoxy + HiSpec RT kit (cat. FYT501-100R; Yeastern Biotech Co., Ltd., Taipei, Taiwan) and the TaqMan MicroRNA Reverse Transcription kit (cat. 4366596; Applied Biosystems; Thermo Fisher Scientific, Inc.). Then, qPCR was performed using the ABI 7900 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). mRNA was amplified using the Etimez Fast Real-Time PCR Premix (2X, SYBR-Green, ROX; Yeastern Biotech Co., Ltd.), and 18S mRNA served as the internal control. The levels of miRNAs were measured using specific primers to miR-381 (001093; Applied Biosystems; Thermo Fisher Scientific, Inc.). RNU6 served as the internal control. The reaction mixture included 2 µl cDNA, 5 µl TaqMan Universal PCR Master Mix II (2X; cat. no. 4440042; Applied Biosystems; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Gibco; Thermo Scientific, Inc.).
Fisher Scientific, Inc.), 1 µl specific primers and 2.5 µl RNase-free water for a final reaction volume of 10 µl. The thermocycling conditions were initiated by uracil-N glycosylase activation at 50˚C for 2 min and initial denaturation at 95˚C for 10 min, followed by 40 cycles at 95˚C for 15 sec, and annealing at 60˚C for 60 sec. Expression levels were normalized to that of an endogenous control. Relative miRNA and mRNA levels were determined using the 2^(-∆∆Cq) method (18).

The qPCR primers for HOXD10, 18S, PIK3CA, PRKDC, ETS1 and ARID1A were synthesized by Genemessenger Scientific Co., Ltd. (Kaohsiung, Taiwan), and their sequences are listed in Table I.

Transient transfection of cells. TOV21G and TOV112D cells were transiently transfected with 45 nM of miRNA mimics, inhibitors, and 1 µg of short hairpin RNAs (shRNAs) or other plasmids was performed using Turbofect Transfection Reagent (Thermo Fisher Scientific, Inc.). The miRIDIAN miRNA mimic (miR-381 mimics, cat. no. C-300690-03-0010) and mimic negative control (cat. no. CN-001000-01-05) were purchased from GE Healthcare Dharmacon, Inc. (Lafayette, CO, USA). The mirVana miRNA inhibitor (miR-381 mimics, cat. no. C-300690-03-0010) and mimic negative control were used as controls. The following day, 90 µl culture medium and 10 µl CCK-8 reagent were added to each well. Cell viability was assayed after 2 h using an enzyme-linked immunosorbent assay reader (Multiskan EX; Thermo Fisher Scientific, Inc.) at 450 nm (reference, 650 nm). In each case, three independent experiments were performed.

Cell viability assay. Cell viability was measured using a Cell Counting Kit-8 (CCK-8) assay (Honeywell Fluka; Thermo Fisher Scientific, Inc.). Briefly, ovarian cancer cell lines were seeded into 96-well plates at a density of 5x10^3 cells/well in a total volume of 100 µl medium with 15% (v/v) FBS, transfected with miRNA mimics, inhibitors or scrambled-sequence controls, and incubated at 37˚C in an atmosphere of 5% CO_2. The following day, 90 µl culture medium and 10 µl CCK-8 reagent were added to each well. Cell viability was assayed after 2 h using an enzyme-linked immunosorbent assay reader (Multiskan EX; Thermo Fisher Scientific, Inc.) at 450 nm (reference, 650 nm). In each case, three independent experiments were performed.

 Colony formation assay. Ovarian cancer cells transfected with the miR-381 mimic, miR-381 inhibitor, or scrambled-sequence controls were plated in triplicate into 6-well plates (500 cells/well), cultured in a humidified incubator at 37˚C with 5% CO_2 for 2 weeks, then fixed with 4% paraformaldehyde and stained with 0.1% crystal violet at room temperature for 1 h. Colonies were counted by visual inspection. Digital images of the colonies were obtained using a camera. Colonies were counted using ImageJ software (version 1.47; National Institutes of Health, Bethesda, MD, USA). Triplicate wells were measured for each treatment group.

Table I. Primer sequences.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>18S</td>
<td>F: 5'-CATGGCCGTTCTTAGTGTG-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CGCTGACCAGTCGATG-3'</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>F: 5'-TTCTTACATTTTCGAA GTTACTCA-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CGAAGGTATTTGTTAAGCAGAA-3'</td>
</tr>
<tr>
<td>PRKDC</td>
<td>F: 5'-TGCACTGACATGAGCTATA-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-TGGCAACTTACGTGTTGTGA-3'</td>
</tr>
<tr>
<td>ETS1</td>
<td>F: 5'-GAACGAATTTTGGAACATGC-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CTTCCTTCATCACC TCCT-3'</td>
</tr>
<tr>
<td>ARID1A</td>
<td>F: 5'-CCAGCAGAACTCTACGACC-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-CTGAGGCAGAGCAAGAAAGG-3'</td>
</tr>
<tr>
<td>RNU6B</td>
<td>CGCAAGGATGACACGCAAATTCGTAAGCGTTTCATATTTT</td>
</tr>
<tr>
<td>miR-381</td>
<td>UAUACAAGGCCAACUCUCUGU</td>
</tr>
<tr>
<td>mi-203</td>
<td>GUGAAAGUUGUAGGACACAUAG</td>
</tr>
<tr>
<td>MiR-381 ChIP (-565 to -574)</td>
<td>F: 5'-GTGTTGTCTCATAGCCGTCTTT-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-ACAGCTCCCACGGCTCAT-3'</td>
</tr>
<tr>
<td>MiR-381 ChIP (-682 to -691)</td>
<td>F: 5'-CGGAGACATGGCTCTGCTTA-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-GACTGCAGCTGAGCTCAT-3'</td>
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PIK3CA, phosphatidylinositol 3-kinase catalytic subunit α, PRKDC, DNA-dependent protein kinase catalytic subunit; ARID1A, AT-rich interaction domain 1A; miR, microRNA; ChIP, chromatin immunoprecipitation.
In vitro migration assay. A total of 5x10^4 cells were seeded into the upper chamber of each Transwell insert (8 μm pore size) in a 24-well plate in serum-free medium. In the lower chambers, medium containing 15% FBS was added as a chemoattractant. Following incubation for 18 h at 37˚C, non-migrated cells in the upper chamber were removed with cotton swabs. The migrated cells on the lower chamber surface were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet at room temperature for 15-20 min. The number of migrated cells was calculated by counting three different fields of view under a phase-contrast microscope. In each case, three independent experiments were performed.

Western blotting. Proteins were extracted using radioimmuno-precipitation lysis buffer (EMD Millipore, Billerica, MA, USA) containing several protease and phosphatase inhibitors (Roche Applied Science, Madison, WI, USA). Protein concentration was determined with the Bio-Rad DC Protein Assay kit from Bio-Rad Laboratories, Inc. (Hercules, CA, USA). Cell lysates were analyzed by western blotting. Proteins (30 μg) were resolved on a 10% BIS-TRIS SDS gel) at 100 V and transferred onto nitrocellulose membranes at 90 V for 1.5 h. Non-specific binding was blocked by incubating the membranes in 5% nonfat dry milk in PBS for 1 h. Then, the membranes were incubated with primary antibodies overnight at 4˚C. Primary antibodies were as follows: Mouse monoclonal anti-HOXD10 (cat. no. TA800777; 1:500; OriGene Technologies, Inc., Rockville, MD, USA) or rabbit monoclonal anti-PIK3CA (cat. no. TA302178; 1:1,000; OriGene Technologies, Inc.). Expression of β-actin was used as a loading control and was detected with monoclonal anti-actin (cat. no. A5316; 1:5,000, Sigma-Aldrich; Merck KGaA). The secondary antibodies used were horseradish peroxidase-conjugated goat anti-mouse IgG or anti-rabbit IgG (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Secondary antibodies were incubated at room temperature for 1 h. Enhanced chemiluminescence reagents (EMD Millipore) were used for immunodetection.

Chromatin immunoprecipitation (ChIP) assay. ChIP assays were performed using the Chromatin Immunoprecipitation Assay kit (Upstate Biotechnology, Inc., Lake Placid, NY, USA). The ALGGEN-PROMO prediction software (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3) was used to identify potential transcription factor binding sites within the miR-381 promoter (19). Chromatin was incubated with anti-HOXD10 (cat. no. TA800777; 1:500; OriGene Technologies, Inc.) or goat anti-mouse IgG (control, cat. no. TA13004; 1:500; OriGene Technologies, Inc.). PCR was conducted with primers complementary to the miR-381 promoter region. The primer sequences are presented in Table I. RT semi-quantitative PCR was performed to amplify the transcription factor binding sequence of miR-381. RT-PCR analysis of relative expression was performed with the one-step Emerald Amp GT PCR Master mix (Takara Bio, Inc., Otsu, Japan). Thermocycling was performed under the following conditions: 94˚C for 5 min; 35 cycles of 94˚C for 30 sec, 58˚C for 30 sec and 72˚C for 30 sec. PCR products were analyzed by electrophoresis with 2% agarose gels and visualized by UV light following staining with EthB’Out’ Nucleic Acid Staining Solution (cat. no. FYD007-200P; Yeastern Biotech Co., Ltd).

Statistical analysis. Data are presented as the mean ± standard deviation from at least three independent experiments by GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA, USA). For the in vitro cell migration assays, groups were compared using the Mann-Whitney U test. For all other results, one-way analysis of variance followed by Tukey’s honest significant difference test was used to analyze differences among multiple groups. Student’s t-test was used to analyze differences between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Analysis of differentially expressed miRNAs and prediction of target genes. In our previous work, the genetic alterations that are commonly observed in endometriosis-associated ovarian cancers were characterized; the genes PIK3CA, ARID1A, ETS1 and PRKDC were identified to be the most frequently mutated genes in patient samples (13). Further examination revealed potential interactions between miRNAs and the mutated mRNAs in ovarian tumors. To characterize the mechanisms by which genetic alterations promote ovarian cancer, TargetScan (version 7.1) and miRanda were used to predict potential upstream regulators of the mutated gene (20). The two most commonly mutated genes encoded mRNAs are regulated by two miRNAs, namely miR-203 and miR-381 (Fig. 1A). Therefore, in the present study, the expression level of miR-203 and miR-381 was determined in tumor samples from patients with ovarian cancer and in ovarian cancer cell lines. The results revealed that miR-381 and miR-203 were expressed at a significantly lower level in the sera of patients with ovarian cancer compared with healthy patients and endometriosis patients, and in ovarian cancer cell lines compared with controls (Fig. 1B and C). Subsequent studies focused on miR-381 because it was the most consistently downregulated miRNA in patient sera (Fig. 1B) and ovarian cancer cell lines (Fig. 1C).

Identification of potential miRNA-mRNA interactions specific to ovarian cancer. To address whether miR-381 affects the cellular levels of mRNAs encoding PIK3CA, ARID1A, ETS1 and PRKDC, ovarian cancer cell lines were transfected with an miRNA mimic or miRNA inhibitor specific for these individual mRNAs and then mRNA expression was assessed. The PIK3CA mRNA level was significantly reduced in ovarian cancer cell lines transfected with miR-381 mimics compared with a non-specific control mimic; by contrast, PIK3CA mRNA level was significantly increased following transfection with an miR-381 inhibitor compared with a control non-specific inhibitor (Fig. 2A and B). However, the cellular levels of ARID1A, ETS1 and PRKDC mRNAs did not respond consistently to transfection with various miRNA mimics and inhibitors (Fig. 2A and B). Next, the effect of various concentrations of miR-381 mimics or inhibitors on the cellular level of PIK3CA mRNA was examined in the various transiently transfected ovarian cancer cell lines (Fig. 2C). The results indicated that 45 nM of miR-381 mimic or inhibitor affected the cellular level of PIK3CA mRNA and protein (Fig. 2C and D), suggesting that miR-381 effectively suppresses the cellular level of both PIK3CA mRNA and protein.
Experimental upregulation of miR-381 suppresses cell proliferation, cell migration and colony formation. To investigate how miR-381 suppression promotes ovarian cancer progression, cell proliferation and migration assessed following targeted knockdown or overexpression of miR-381. Cell proliferation was evaluated by CCK-8 and colony formation assays. The downregulation of miR-381 significantly increased cell proliferation, and inhibition of miR-381 activity significantly decreased proliferation (Fig. 3A). Similarly, in a colony formation assay, which is an in vitro cell survival assay based on the ability of a single cell to grow into a colony (21), clonogenic survival decreased following transfection of cells with miR-381 mimics, whereas survival was enhanced in miR-381 inhibitor-transfected cells (Fig. 3B). Furthermore, cell migration was evaluated by Transwell assays, in which overexpression of miR-381 significantly decreased migration, whereas an miR-381 inhibitor significantly increased migration (Fig. 3C). These results indicated that miR-381 suppresses the growth and migration of ovarian cancer cells. 

HOXD10 drives the expression of miR-381. The current results indicated that miR-381 is an upstream regulator of PIK3CA in ovarian cancer cells. Previous studies have identified that dysregulation of the phosphatidylinositol 3-kinase (PI3K) pathway, which is commonly observed in human tumors, drives tumorigenesis; therefore, the PI3K pathway is an appealing therapeutic target (22). Hence, the upstream regulators of miR-381 with respect to the progression of ovarian cancer were investigated. The ALGGEN-PROMO prediction program (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3) was used to identify potential transcription factor binding sites within the miR-381 promoter (19,21,23). HOXD10 is a tumor-suppressor gene that suppresses tumor invasion and hence could potentially modulate the cellular level of miR-381 (24). To determine whether miR-381 level is regulated by HOXD10, ovarian cancer cells were transfected with an overexpression plasmid encoding HOXD10. Subsequently, HOXD10 was efficiently overexpressed (Fig. 4A), resulting in a significantly increased cellular level of miR-381 expression (Fig. 4B). Conversely,
HOXD10 knockdown resulted in a decreased level of miR-381 in cells (Fig. 4B). Furthermore, it was investigated whether HOXD10 modulates the cellular level of miR-381. The ALGGEN-PROMO program was used to predict the promoter region for miR-381. ChIP assays revealed that HOXD10 positively regulated transcription of the miR-381 gene by binding to the region -565 to -574 bp upstream of the miR-381 coding sequence (Fig. 4C).
Figure 4. HOXD10 upregulates miR-381 expression. Ovarian cancer cells were transfected with an HOXD10 overexpression plasmid or HOXD10 shRNA. (A) Western blot analysis of HOXD10 in cells following transfection with the HOXD10 plasmid or HOXD10 shRNA. (B) Levels of miR‑381 in cells following transfection with the HOXD10 plasmid or HOXD10 shRNA. Expression levels were normalized to that of Run6. (C) Schematic diagram of the HOXD10‑binding element identified within the MIR381 promoter (left panel). ChIP assay of HOXD10 binding to the 5' flanking region (-565 to -574) of the MIR381 promoter (right panel). IgG was used as the control antibody. Data are presented as the mean ± standard deviation from three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. control group. HOXD10, homeobox D10; ChIP, chromatin immunoprecipitation; miR‑381, microRNA-381; shRNA, short hairpin RNA; IP, immunoprecipitating antibody; Input, internal control for the amount of genomic DNA used in the immunoprecipitation.

Figure 3. Effect of miR-381 on cell viability and migration in vitro. TOV21G and TOV112D cells were transfected with miR-381 mimics or mimic control, or with miR-381 inhibitors or inhibitor control. (A) A Cell Counting Kit-8 assay was performed to determine the viability of ovarian cancer cell lines. (B) Colony formation assays were performed to determine the relative rate of proliferation of ovarian cancer cells. Left panel: Representative images of the colony-forming assay. Right panel: Quantification of the colony formation rate for TOV21G and TOV112D cells. (C) Cell migration was assessed using a Transwell assay, and the cells on the bottom of each Transwell membrane were counted. Magnification, x100. Data are presented as the mean ± standard deviation from three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. respective control group. miR-381, microRNA-381.
Discussion

Emerging evidence has demonstrated that several miRNAs are mutated in human cancers, resulting in their dysregulation. These miRNAs may thus act as tumor suppressors or oncogenes. MiRNAs serve as important regulators of gene expression in various human cancer types and hold promise for the development of cancer diagnoses or therapies (25,26). Several studies have investigated the association between miRNAs and the various phenotypes observed in ovarian tumors (27-29). However, the associations between mutations in miRNAs and/or gene-coding regions and cancer risk have not been fully elucidated. The present study was an extension of our previous work, in which next-generation sequencing was used to identify the majority of endometriosis-associated somatic mutations associated with ovarian cancer, including mutations in ARID1A, PIK3CA, ETS1 and PRKDC, among others (33). Based on the results of that previous study, in the present study it was identified that miR-381 is an upstream regulator of PIK3CA using an miR prediction program and the regulation and role of miR-381 in ovarian cancer cells was explored. MiR-381 was observed to be downregulated in ovarian cancer samples and clear cell and endometrioid cancer cells. Exploration of the effect of miR-381 on ovarian cancer cells confirmed that miR-381 inhibits cell migration and proliferation.

MiR-381 has been identified to be involved in a variety of tumorigenic processes, including cell proliferation, apoptosis, migration and invasion, complicating the regulatory network in human cancer. For instance, miR-381 has been identified to be diversely deregulated in several cancer types, including colorectal and breast cancers, and this deregulation could affect epithelial-mesenchymal transition as well as proliferation, invasion, and migration of cancer cells via targeting TWIST and CXCR4 mRNAs (30,31). In addition, Xia et al (32) reported the downregulation of miR-381 in ovarian epithelial cancer tissue. The current findings are consistent with these previous studies in that miR-381 was identified to act as a tumor suppressor in various cancer types.

Chemotherapy is the principal treatment for ovarian cancer; however, the acquisition of chemoresistance is a major problem with respect to patient long-term survival. Previous studies have demonstrated that the level of miR-381 correlates with the development of drug resistance following chemotherapy (33,34). Consistent with this, the PI3K-AKT pathway and its components (principally PIK3CA), which target miR-381, are often mutated, and dysregulation of this pathway or its components increases the chemoresistance observed in ovarian cancer (35-37). Therefore, it is proposed that the deregulation of miR-381 may have consequential effects on the PI3K-AKT pathway and thus contribute to chemoresistance. Additionally, it was identified in the current study HOXD10 is a novel transcription factor for miR-381. HOXD10 may serve as a tumor suppressor (38,41). A previous study revealed that HOXD10 is a direct target of miR-10b, which promotes cell invasion through the RhoC/AKT signaling pathway (24).

In addition to the aforementioned results, a role for HOXD10 has been established in angiogenesis and in modulating cell motility in numerous cancer types (24,39,42,43). Similar to the results reported previously, the current results establish a functional connection between HOXD10, miR-381 and PIK3CA.

In summary, the current results demonstrate that miR-381 is significantly downregulated in ovarian cancer cells and that miR-381 overexpression may inhibit the migration and proliferation ability of ovarian cancer cells in vitro. Additionally, expression of the miR-381 gene is directly regulated by HOXD10, and HOXD10 may mediate the effect of miR-381 on ovarian cancer cell migration and proliferation. The current results suggest that miR-381 functions as a tumor suppressor through targeting PIK3CA in ovarian cancer.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors’ contributions

CYH and EMT designed the experiments. CYH and EMT conducted the experiments and wrote the manuscript. THH, TKE, CCT and HSC provided the research materials and conducted the experiments and wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (approval no. KMUHIRB-G(1)-20150046), and written informed consent was obtained from each participant.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
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