miR-338-3p suppresses tumor growth of ovarian epithelial carcinoma by targeting Runx2

CHUNYAN WEN1, XIAOJUN LIU2, HONGXI MA3, WENJIE ZHANG1* and HAIFENG LI4*

Departments of 1Pathology and 2Obstetrics and Gynecology, China-Japan Union Hospital of Jilin University, Nanguan District, Changchun 13033; Departments of 3Pathology and 4Emergency Medicine, The First Hospital of Jilin University, Changchun 130021, P.R. China

Received January 12, 2015; Accepted March 2, 2015

DOI: 10.3892/ijo.2015.2929

Abstract. miR-338-3p, a recently discovered miRNA, has been shown to play important roles in tumorigenesis and metastasis in various cancers. However, the exact roles and mechanisms of miR-338-3p remain unknown in human ovarian epithelial carcinoma (EOC). The relationship between miR-338-3p expression pattern and clinicopathological features of patients with EOC were determined by real-time quantitative RT-PCR. Furthermore, the role of miR-338-3p and possible molecular mechanisms in EOC was investigated by several in vitro approaches and in a nude mouse model. We first showed that the expression of miR-338-3p was significantly downregulated in EOC tissues compared to those in adjacent normal tissues, and the value was negatively related to advanced FIGO stage, high histological grading and lymph node metastasis (P<0.01). An in vitro analysis revealed that the overexpression of miR‑338‑3p in EOC cells significantly inhibited cell proliferation, colony formation, migration and invasion, inducing cell apoptosis and enhancing caspase-3, -8, and -9 activities. Bioinformatic analysis and dual luciferase assays identified Runx2 as a direct target of miR-338-3p. We also found that enforced expression of miR -338-3p markedly inhibited the in vivo tumorigenicity in a nude mouse xenograft model system. Furthermore, overexpression of miR-338-3p inhibited phosphorylation of PI3K and AKT, which contributed to suppression of ovarian cancer cell growth. These findings revealed that miR-338-3p may act as a tumor suppressor that blocks the growth of human ovarian epithelial carcinoma through PI3K/AKT signaling pathways by targeting Runx2.

Introduction

Ovarian cancer (OC) is the fifth leading cause of cancer related deaths among women in the world and the most lethal gynecologic malignancy worldwide (1,2). Epithelial ovarian cancer (EOC) is a common entity accounting for 80-90% of OC cases, causing >140,000 deaths every year (1). Despite the great progress in surgical techniques, diagnostic methods, and new chemotherapy regimens, the 5-year survival rate of advanced-stage epithelial ovarian cancer remains <30% due to late diagnosis and chemoresistance (3). Therefore, the identification of new molecular biomarkers and the development of individualized treatment regimens remains a major challenge for EOC therapeutic care to improve the 5-year survival rate.

MicroRNAs (miRNAs) are a family of small non-coding, single-stranded ribonucleic acid (RNA) sequences (19-25 nucleotides in length) that regulate gene expression at the post-transcriptional level via partial base pairing to the 3' untranslated region (UTR) of their targets, thus leading to their translational repression or degradation, according to the degree of complementarity with them (4-6). It has been demonstrated that miRNAs are involved in the regulation of various cellular processes, such as the cell cycle, apoptosis, metabolism, differentiation, proliferation, oncogenesis, angiogenesis, cell migration and invasion (5-8). A growing body of evidence suggests that altered microRNA (miRNA) levels are related to the oncogenesis of many human cancers (11,12), including ovarian carcinoma (13,14), therefore, miRNAs are presently considered as potential novel targets for various cancers therapy (15).

The miR-338-3p, a recently discovered miRNA, was downregulated in several cancers including hepatocellular carcinoma (16,17), neuroblastoma (18), malignant melanoma (19), gastric cancer (20,21) and colorectal cancer (22,23). miR-338-3p acts as a tumor suppressor that inhibits cancer cell proliferation, invasion and migration, both in vitro and in vivo (16-18,20-23). However, our knowledge on clinicopathological impact and the exact roles of the miR-338-3p in EOC and the
underlying molecular mechanisms have not been reported previously.

In the present study, we investigated miR-338-3p clinicopathological impact on patients with EOC, and found that the expression of miR-338-3p was significantly downregulated in EOC tissues compared to those in adjacent normal tissues, and the value was negatively related to advanced FIGO stage, high histological grading and lymph node metastasis (P<0.01). We also investigated the functional role of miR-338-3p in EOC, both in vitro and in vivo. We found that enforced expression of miR-338-3p in ovarian cancer cells significantly suppressed proliferation, migration and invasion in vitro and inhibited tumor growth in vivo. Furthermore, Runx2 was identified as a direct target of miR-338-3p.

Materials and methods

Patients and tissue samples. Fresh EOC tissue samples and the corresponding adjacent ovarian tissue were obtained from the 54 patients with primary EOC who underwent surgery at China-Japan Union Hospital of Jilin University (Changchun, China) from July 2012 to August 2014. Normal ovarian tissues adjacent to the tumor were taken from 5 cm away from the tumor cells, and then the absence of tumor cell infiltration was verified by pathological examination. Samples were immediately frozen in liquid nitrogen, and stored at -80°C until RNA extraction. None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. Informed consent was obtained from each patient prior to surgery and the study protocol and consent procedures were approved by the ethics committee of China-Japan Union Hospital of Jilin University (Changchun, China).

Cell culture. A human ovarian surface epithelial cell line (HOSEpiC) and three human ovarian cancer cell lines (SKOV3, OVCAR3 and A2780) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). A2780 and OVCAR3 cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (FBS, Gibco BRL, Gaithersburg, MD, USA), 100 U/ml penicillin and 100 mg/ml streptomycin. SKOV3 cells were cultured in DMEM medium (Gibco, Grand Island, NY, USA) supplemented with 10% FBS. The cells were harvested 24, 48 and 72 h after transfection and prepared for the subsequent study. Transfection efficiencies were evaluated in every experiment by qRT-PCR 48-h post-transfection.

Detection of cell viability and colony formation. To determine the cell proliferation capacity, cells were examined with cell viability assay and colony formation assay. Cell viability was determined by MTT assay. Briefly, cells (5x10^5 cells/well) were seeded into a 96-well plate with 100 µl of RPMI-1640 medium and incubated for 24 h. Thereafter, cell was transfected with miR-338-3p or miR-NC respectively, and were cultivated for additional 1-3 days. Cell viability was assessed using the MTT assay at a wavelength of 570 nm by an enzyme-linked immunosorbent assay reader (Thermo Labsystems, Finland).

For colony formation assay, Cells were transfected with miR-338-3p mimics or miR-NC for 48 h. Thereafter, cell were seeded into a 6-well plate at a low density (1,000 cells/well), and further cultured for 14 days. Then cells were fixed with 4% paraformaldehyde for 10 min and counted after staining with 1% crystal violet. The percentage colony formation was calculated by adjusting control (untreated cells) to 100%.

TUNEL assay. To determine whether overexpression of miR-338-3p promotes tumor cell death, TUNEL assays were performed. In brief, cells were transfected with miR-338-3p mimic or miR-NC for 48 h, respectively. After transfection, apoptotic cells were determined by using the In Situ Cell Death Detection kit (Roche, Mannheim, Germany) according to the manufacturer's instructions. The samples were analyzed by fluorescence-activated cell sorting (Becton-Dickinson, Franklin Lakes, NJ, USA).

In addition, the activity of caspase-3, -8 and -9 were detected as an additional indicator of apoptosis using caspase colorimetric protease assay kits (Millipore Corp., Billerica, MA, USA) according to the manufacturer's instructions.

Wound healing assay. To assess the effect of miR-338-3p on cell migration, wound-healing assay was performed. Briefly, transfected cells were seeded into 24-well tissue culture plates for 48 h. Thereafter, an artificial homogeneous wound was scratched into the monolayer using a sterile plastic micropette tip. After wounding, the debris was removed by washing the cells with serum-free medium. Migration of cells into the wound was observed at 0 and 24 h using an inverted phase-contrast microscope (Leica DMR, Germany). Individual cells were quantified as an average of at least five fields for each experiment.
Cell invasion assays. Cell invasion was assessed using Transwell Chambers (Corning, Tewksbury, MA, USA) in which two chambers were separated by a Matrigel-coated polycarbonate membrane (8-µm pore size). Briefly, 1x10⁵ transfected cells were placed into upper chambers precoated with Matrigel (BD, USA) in serum-free medium. Medium with 20% FBS were added to the lower chamber to serve as chemotactractant. After cells had been cultured at 37°C for 48 h, invaded cells were fixed with 70% ethanol for 30 min and stained with 0.2% crystal violet for 10 min. Images of five randomly selected fields of the fixed cells were taken and counted.

miRNA target prediction. Prediction of miR-338-3p targets was performed using three publicly available algorithms: TargetScan (http://www.targetscan.org/), miRanda (http://www.microrna.org/) and PicTar (http://pictar.mdc-berlin.de/).

Vector construction and luciferase reporter assay. The human Runx2 3’UTR oligonucleotides containing the wild-type (WT) or mutant (MT) miR-338-3p binding site were cloned into the pGL3-control vector (Ambion, Austin, TX, USA) at the NheI and XhoI sites. For luciferase assays, cells were transfected with miR-338-3p or miR-NC and then co-transfected with wild-type or mutant vectors using Lipofectamine 2000 reagent. After 48 h of transfection, luciferase activity was detected using the dual-luciferase assay system (Promega, Madison, WI, USA). Renilla-luciferase was used for normalization.

Western blotting. Protein was extracted from tissues and cells using RIPA lysis buffer containing protease inhibitor (Sigma, USA). Concentrations of total cellular protein were determined using a BCA assay kit (Pierce, Rockford, IL, USA) according to the manufacturer’s instructions. Twenty micrograms of protein mixed with 2X SDS loading buffer was loaded per lane, separated by 8-12% sodium dodecylsulfate-polyacrylamide gels (SDS-PAGE) and then transferred onto the nitrocellulose membrane (Bio-Rad, Munich, Germany). The membranes were blocked with 5% non-fat dry milk for 2 h and incubated with primary antibody overnight at -4°C as follows: anti-MMP-2 (1:1,000; Abcam, Cambridge, UK), anti-MMP-9 (1:2,500; Abcam), anti-GAPDH (1:2,000, Cell Signaling Technology, New England Biolabs); anti-Runx2 (1:1,000, Cell Signaling Technology); anti-pI3K (1:2,000, Cell Signaling Technology); anti-phosphorylated (p)-pI3K (Tyr458, 1:1,500, Cell Signaling Technology); anti-AKT (1:1,000, Cell Signaling Technology); anti-p-AKT (Ser473; 1:500, Cell Signaling Technology); anti-PI3K (1:2,000, Cell Signaling Technology) and anti-p-AKT (Thr308; 1:1,000, Cell Signaling Technology). The membranes were washed and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5,000; Santa Cruz Biotechnology, CA, USA). The protein bands were visualized on X-ray film with a chemiluminescent detection system (Beyotime, Shanghai, China). Blots were stripped and reprobed with anti-GAPDH to control for loading variations.

In vivo nude mouse tumorigenesis assay. Female BALB/c nude mice (5-6-week-old) were obtained from Experiments Animal Center of Changchun Biological Institute (Changchun, China), and maintained under specific pathogen-free conditions. This study was approved by the Animal Ethics Committee of Jilin University (Changchun, China).

Approximately 2x10⁶ logarithmically growing untreated A2780 cells, stably expressing miR-338-3p A2780 cells or miR-NC A2780 cells suspended in 100 µl of PBS (containing 10% Matrigel) were injected into the flanks of mice (n=10), respectively. Mice were monitored weekly for tumor growth. Tumor volume was measured every week using digital Vernier calipers, and was calculated according to the formula: \[ \frac{1}{6} x \text{length} \times \text{width} \times \text{height} \]. Five weeks after inoculation, mice were sacrificed, and tumors were stripped and weighed. Cell apoptosis of tumor tissues were determined by TUNEL by using the In Situ Cell Death Detection kit (Roche) according to the manufacturer’s instructions.

Statistical analysis. The data are shown as mean ± SD (standard deviation), and the experiments of in vitro were repeated at least three times. Comparisons between the groups were analyzed with one-way ANOVA or two-tailed Student’s t-test. SPSS version 16.0 software (SPSS, Chicago, IL, USA) and the GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA) for Windows® were used for statistical analyses. A P-value of <0.05 was considered statistically significant.

Results

Downregulation of miR-338-3p is associated with clinico-pathological features of EOC patients. The expression of miR-338-3p in EOC patients was examined in tumor tissues and paired adjacent normal ovarian tissues from 54 EOC patients. Results of real-time quantitative RT-PCR (qRT-PCR) showed that expression of miR-338-3p in EOC patients was significantly downregulated compared to corresponding normal ovarian tissues (P<0.01) (Fig. 1A). In addition, the levels of miR-338-3p expression in the SKOV3, OVCAR3 and A2780 human ovarian cancer cell lines were examined by qRT-PCR (Fig. 1B). In all three ovarian cancer cell lines, the miR-338-3p expression level was less than that in a control human ovarian surface epithelial cell line (HOSEpIC). The A2780, which possessed the lowest levels of miR-338-3p expression among the three cell lines, was selected for further studies.

The association between miR-338-3p expression and the clinico pathological parameters of the patients, including age, CA125 level, FIGO stage, histological grading and lymph node metastasis was assessed (Table I). We found that the level of miR-338-3p expression in tissues was significantly decreased in the patients with high histological grading, advanced FIGO stage and lymph node metastasis (P<0.01), which are all indicators of poor prognosis. There was no correlation between miR-338-3p expression and other tumor characteristics including age and CA125 level. These data suggested that miR-338-3p might play a key role in EOC development.

miR-338-3p inhibited the proliferation and colony formation of ovarian cancer cells. The effect of miR-338-3p on cell proliferation was further investigated in the human EOC cell line A2780. The overexpression of miR-338-3p in the cell line transfected with miR-338-3p was confirmed by qRT-PCR
miR-338-3p SUPPRESSES TUMOR GROWTH OF EOC BY TARGETING Runx2

Figure 1. Downregulation of miR‑338‑3p in ovarian cancer tissues and cell lines. (A) Relative miR‑338‑3p expression of EOC tissue (n=54) and corresponding adjacent normal tissue were determined by real-time quantitative RT‑PCR (qRT‑PCR). (B) Relative miR‑338‑3p expression levels of three ovarian cancer cell lines (SKOV3, OVCAR3 and A2780) and a normal ovarian surface epithelial cell line (HOSEpiC; OE) were examined with qPCR assay.

Figure 2. Stable miR‑338‑3p overexpression inhibited the proliferation and clonogenic ability formation of A2780 cells. (A) The expression level of miR‑338‑3p was significantly upregulated in A2780 cells after transfected with miR‑338‑3p mimic, assessed by qRT‑PCR. (B) Effects of overexpression of miR‑338‑3p on the proliferation of A2780 cells were examined with MTT assay and shown as growth curve. (C) Effects of ectopic expression of miR‑338‑3p on the clonogenic ability of A2780 cells. The left panel represents dishes by colony formation assay, and the right panel illustrates the number of colonies formed. *P<0.05; **P<0.01, versus miR‑NC group.

### Analysis

The effect of miR‑338‑3p on cell proliferation was assessed with the MTT assay. As shown in Fig. 2B, overexpression of miR‑338‑3p significantly decreased cell growth of ovarian cancer cells compared with their corresponding control (miR‑NC).

Next, colony forming was performed to assess the role of miR‑338‑3p in cancer cell growth. Compared with miR‑NC group and control group, the numbers of A2780 colonies were reduced significantly by overexpression of miR‑338‑3p (P<0.05, Fig. 2C).

miR‑338‑3p induces the apoptosis and increases the activity of caspase‑3, ‑8 and ‑9 of ovarian cancer cells. To determine the effect of miR‑338‑3p on apoptosis, A2780 cells were transfected with miR‑338‑3p mimic and then analyzed using the TUNEL assay. Compared with miR‑NC group and control...
group, the percent of apoptosis of A2780 cells was increased significantly (P<0.05) following overexpression of miR‑338‑3p (Fig. 3A).

To determine the potential mechanism of cell apoptosis in vitro, the activity of caspase-3, -8 and -9 were detected in A2780 cells after transfected with miR-338-3p mimic. It was found that the activity of caspase-3, -8 and -9 was significantly increased in miR-338-3p treatment groups compared to the control group and miR-NC groups (P<0.05, Fig. 3B-D).

miR‑338‑3p inhibited the migration and invasion of ovarian cancer cells. To test whether miR-338-3p overexpression suppresses tumor cell migration and invasion, the migration and invasion of A2780 cells were measured by wound healing assay and transwell assay, respectively. We found that overexpression of miR-338-3p significantly suppressed migration (Fig. 4A) and invasion (Fig. 4B) in ovarian cancer cells. To further investigate whether the inhibitory effect of miR-338-3p on migration and invasion was mediated by matrix metalloproteinases (MMPs), we examined the expression of MMP-2 and MMP-9 since there are a major group of enzymes that regulate basement membrane (BM) and extracellular matrix (ECM) composition during normal development and pathological responses. As expected, miR-338-3p overexpression decreased the expression level of MMP-2 and MMP-9 in ovarian cancer cells (Fig. 4C). Taken together, these findings suggest that miR-338-3p could impede invasion mediated by regulating MMP-2 and MMP-9 expression.

miR‑338‑3p targeting the Runx2 gene. We next determined the potential targets of miR-338-3p by bioinformatic databases (TargetScan, PicTar, and miRanda), and found that miR-338-3p may bind to Runx2 3'-UTR mRNA sequences (Fig. 5A). We

Table I. Correlation between miR-338-3p status and clinical characteristics in patients with EOC.

<table>
<thead>
<tr>
<th>Feature</th>
<th>N</th>
<th>miR-338-3p expression</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60</td>
<td>26</td>
<td>0.44±0.12</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>≥60</td>
<td>28</td>
<td>0.42±0.13</td>
<td></td>
</tr>
<tr>
<td>CA125 (U/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>24</td>
<td>0.42±0.11</td>
<td>P&gt;0.05</td>
</tr>
<tr>
<td>≥500</td>
<td>30</td>
<td>0.44±0.12</td>
<td></td>
</tr>
<tr>
<td>Histological grading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/2</td>
<td>40</td>
<td>0.47±0.13</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>0.32±0.13</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>0.35±0.11</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Negative</td>
<td>32</td>
<td>0.49±0.14</td>
<td></td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>35</td>
<td>0.50±0.15</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>III-IV</td>
<td>19</td>
<td>0.31±0.10</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Stable miR-338-3p overexpression induced apoptosis and increased caspase-3, -8 and -9 activities of A2780 cells. (A) Effects of overexpression of miR-338-3p on the apoptosis of A2780 cells were examined with TUNEL assay. (B-D) Effects of ectopic expression of miR-338-3p on the activity of caspase-3, -8 and -9 in A2780 cells were detected by using caspase colorimetric protease assay kits. *P<0.05; **P<0.01, versus miR-NC group.
constructed luciferase reporter vectors to determine whether miR-338-3p was binding to target the Runx2 gene. After co-transfection of miR-338-3p mimic with Runx2-wild-type or mutated 3’-UTR luciferase reporter vector into A2780 cells, miR-338-3p reduced wild-type Runx2 3’-UTR luciferase activity relative to miR-NC group. Conversely, no reduction or increase in luciferase activity was detected by miR-338-3p with the mutated Runx2 3’-UTR luciferase reporter (Fig. 5B), suggesting that Runx2 expression may be negatively regulated via miR-338-3p 3’-UTR miRNA binding sites.

Next, we determined the expression of Runx2 protein in the ovarian cell lines SKOV3, OVCAR3 and A2780 and the human ovarian surface epithelial cell line HOSEpiC by western blot analysis. The results of western blot analysis showed that Runx2 protein expression was obviously upregulated in three ovarian cancer cell lines compared to ovarian surface epithelial cell line HOSEpiC (Fig. 5C). The result showed that Runx2 protein was overexpressed in EOC tissue samples compared with their corresponding normal adjacent tissues (Fig. 5D). These results indicate that miR-338-3p directly recognizes the 3’-UTR of Runx2 mRNA and inhibits it translation.

miR-338-3p regulates the PI3K/AKT signaling pathway. To further investigate the possible molecular mechanisms of miR-338-3p inhibited cell proliferation and migration and invasion, we detected the protein expression level of Runx2 and the related signal pathway regulators by western blotting after transfection with miR-338-3p mimic or miR-NC. Our results show that miR-338-3p reduced the expression of Runx2 protein and the phosphorylation of PI3K (Tyr458) phosphory-
translation of p-AKT (Serine473, Thr308), whereas the total PI3K and AKT remained unchanged (Fig. 6).

miR-338-3p inhibits tumor growth in a mouse xenograft model. We next examined whether miR-338-3p overexpression could suppress ovarian cancer tumor growth in vivo, in nude mice. Untreated A2780 ovarian cancer cells, A2780 cells with overexpressed miR-NC or miR-338-3p were subcutaneously inoculated in nude mice (n=10 for each group). It was found that the tumor sizes derived from A2780-miR-338-3p overexpressing group were smaller than those in control group (untreated group) and A2780-miR-NC group (Fig. 7A and B). Additionally, the tumors formed from the A2780-miR-338-3p overexpressing group weighed significantly less as compared to control group and A2780-miR-NC group (Fig. 7C). Furthermore, we also determined cell apoptosis of tumor tissue from each group by TUNEL. The data demonstrated that the percent of cell apoptosis from A2780-miR-338-3p overexpressing group obviously increased compared to control group and A2780-miR-NC group (Fig. 7D). These data indicated that overexpression of miR-338-3p was able to suppress tumor growth of ovarian cancer in vivo.

Discussion

Ovarian cancer remains as one of common cancer types and is still a leading cause of lethal gynecologic malignancy worldwide (1,2), with low 5-year survival and poor prognosis, partly due to late diagnosis and chemoresistance (3), thus, it is urgent to develop new diagnostic markers and therapeutic strategies. During the past years, dysregulation of miRNAs has been shown to play a role in control of cell proliferation, metastasis, and cell cycle in ovarian cancer, suggesting that miRNAs hold great promise for novel therapeutic approaches for treating human ovarian cancers (24,25). The miR-338, located on chromosome 17q25, is typically downregulated in several malignancies, such as hepatocellular carcinoma (16,17), neuroblastoma (18), gastric cancer (20,21), and colorectal cancer (22,23). However, investigations for its clinical impact or functional assessments have not been reported. We investigated the miR-338-3p expression in EOC tissues samples and three ovarian cancer lines by qRT-PCR, and found that miR-338-3p was frequently downregulated in both EOC cell lines and human ovarian surface epithelial cell line HOSEpiC, respectively. In addition, low miR-338-3p expression was significantly associated with
Negative prognostic clinicopathological parameters, such as high histological grading, advanced FIGO stage (III/IV), and lymph node metastasis, suggesting that low miR-338-3p expression may present a useful biomarker of poor prognosis. To our knowledge, this is the first report that miR-338-3p expression is downregulation, and low miR-338-3p expression correlates with poor prognostic parameters of ovarian cancer patients.

Next, we analyzed the function of miR-338-3p on ovarian cancer cells by several in vitro approaches and in a nude mouse model, we demonstrated that overexpression of miR-338-3p impaired proliferation, colony formation, invasion and migration, and induced apoptosis of various ovarian cancer cells, as well as suppressed tumor growth in a nude mouse model, which is in accordance with previous studies that demonstrated miR-338-3p-mediated suppression of cell growth in liver cancer, gastric cancer, and colorectal cancer cells (16,17,20-23). These findings suggest that miR-338-3p functions as a tumor suppressor and inhibits ovarian carcinoma cell growth in vitro and in vivo, suggesting that the miR-338-3p mimic is a promising therapeutic strategy for this malignancy.

Although the mechanism of the inhibitory effect of miR-338-3p on tumor cell growth has not been fully elucidated, a recent study demonstrated that miR-338-3p suppresses the expression of PREX2a by binding to its 3'-UTR, leading to inhibition of neuroblastoma cell growth (18). Other studies indicated that miR-338-3p targets PREX2a and SSX2IP in gastric cancer cells (20,21), and smoothened, cyclinD1 and hypoxia inducible factor-1 in liver cancer cells (16,17,26). In the present study, we predicted the Runx2 oncogene, which was reportedly overexpressed in ovarian cancer tissue (27), as a potential target of miR-338-3p by bioinformatics analyses, and further confirmed miR-145 directly targets Runx2 by a luciferase reporter assay and western blot analysis, which is in accordance with previous studies that demonstrated miR-338-3p targets the Runx2 in odontoblast (28) and bone marrow stromal cells (29).

Runx2, an important member of runt-related transcription factor (Runx) gene family, is a key regulator of normal bone development, homeostasis and remodeling (30). Runx2 is aberrantly expressed in several cancer types (31-34), and play a role in invasive breast (31), prostate (32), bone (34), thyroid (35) and pancreatic cancer (36). For ovarian cancer, Runx2 expression was upregulated and its expression also associated with EOC tumor progression and poor prognosis (27), which was in agreement with our results that Runx2 expression is decreased in EOC tissues and ovarian cancer lines. Knockdown of the RUNX2 expression in EOC cells resulted in a sharp decrease of cell proliferation and significantly inhibited EOC cell migration and invasion (37). In addition, recently a study demonstrated that Runx2 was critical for activating PI3K/Akt signaling (38), and down-regulation of expression of Runx2 inhibited the activation of PI3K/Akt signaling pathway (38,39). Our present results show that miR-338-3p functions as a tumor suppressor in ovarian cancer and directly targets Runx2, and that overexpression of miR-338-3p can reduce the expression of Runx2 protein and the phosphorylation of PI3K (Tyr458) phosphorylation of p-AKT (Serine473, Thr308), suggesting that miR-338-3p inhibits ovarian cancer cell proliferation, migration and...
invasion through PI3K/AKT signaling pathways by targeting Runx2.

In conclusion, the results presented herein demonstrate that miR-338-3p expression level was decreased in EOC tissue and its expression level was significantly associated with negative prognostic clinicopathological parameters, such as high histological grading, advanced FIGO stage (II/IV), and lymph node metastasis. Additionally, miR-338-3p functions as a tumor suppressor and suppresses tumor growth of EOC in vitro and in vivo. Moreover, we identified runx2 as a crucial target gene of miR-338-3p, and found that the expression of miR-338-3p was regulated by targeting PI3K/AKT signaling pathways, suggesting that miR-338-3p may be a novel tumor suppressor that blocks the growth of ovarian cancer cells through PI3K/AKT signaling pathways by targeting Runx2. Based on the multiple functions of miR-338-3p in tumor growth of ovarian cancer, miR-338-3p may present not only a useful biomarker of poor prognosis, but also a therapeutic target for patients with ovarian carcinoma.

References