Original Article

MicroRNA-215 targets NOB1 and inhibits growth and invasion of epithelial ovarian cancer

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Abstract: MicroRNA-215 (miR-215) has been showed to play crucial roles in tumorigenesis and tumor progression in many types of cancer. However, its biological function and underlying mechanism in epithelial ovarian cancer (EOC) remains greatly unknown. The aims of this study were to investigate biological role and underlying mechanism of miR-215 in EOC. Here, we found that miR-215 expression was significantly decreased in EOC tissues or cell lines compared with adjacent normal tissues or normal ovarian cell line. Decreased miR-215 expression was significantly associated with International Federation of Gynaecology and Obstetrics (FIGO) stage and lymph node metastasis. Function analysis revealed that overexpression of miR-215 using miR-215 mimic significantly inhibit EOC cell proliferation, colony formation, migration and invasion in vitro. as well as suppress tumor growth in vivo. Moreover, we identified ribosome assembly factor NIN/RPN12 binding protein (NOB1) as a direct targets for miR-215 binding, resulting in suppression it expression, which in turn activated the MAPK signaling pathway. In clinical EOC specimens, NOB1 expression was upregulated, and inversely correlated with miR-215 expression (r = -0.675, P<0.001). Overexpression of NOB1 effectively rescued inhibition effect on EOC cells by induced miR-215 overexpression. Taken together, our findings suggested that miR-215 suppressed EOC growth and invasion by targeting NOB1.

Keywords: microRNAs, miR-215, epithelial ovarian cancer, NOB1

Introduction

Epithelial ovarian cancer (EOC), accounting for 90% of all ovarian cancers cases, is the most lethal of all gynaecological malignancies [1, 2]. Although current treatment strategies significantly improved the quality of life in patients with EOC, the estimated 5-year survival rate for all stages is 35-38% [3, 4], mainly due to advanced stage at diagnosis and the recurrence and metastasis [5]. Therefore, exploring the key molecular mechanisms of EOC involved in growth and metastasis would have great impact on the development of targeted therapy that can contribute to improve survival outcomes patients with EOC.

MicroRNAs (miRNAs) are single-stranded, small endogenous non-coding RNAs that negatively regulate gene expression at the post-transcriptional level in a sequence-specific manner, primarily via base pairing to the 3’-untranslated region (3’UTR) of the target messenger RNA transcripts [6]. It was well known that miRNAs were involved in various biological processes, such as cell growth, cycle, apoptosis, development, differentiation and endocrine homeostasis [7, 8]. Accumulating evidence suggests that miRNAs play essential roles in tumor initiation, development, and progression, which may provide a new and promising treatment target for cancer [9, 10]. A series of miRNAs has been found to be involved in EOC tumorigenesis, including oncogenesis, angiogenesis, progression, invasion, and metastasis [11, 12], suggesting that miRNAs can act as diagnosis marker or therapy agent.

MicroRNA-215 (miR-215), a transcript of chromosome 11q13.1, has been showed to play critical roles in tumorigenesis and tumor progression in multiple human cancers, such as glioma [13], myeloid leukemia [14], gastric cancer [15], non-small cell lung cancer [16], pancreatic carcinomas [17], breast cancer [18] and colon cancer [19]. Although a study showed
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Table 1. Correlation between clinicopathological features and miR-215 expression in EOC tissues

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. of cases</th>
<th>miR-215 expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>22</td>
<td>11 (50.0)</td>
<td>11 (50.0)</td>
</tr>
<tr>
<td>≥55</td>
<td>26</td>
<td>15 (57.7)</td>
<td>11 (42.3)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5</td>
<td>20</td>
<td>11 (55.0)</td>
<td>9 (45.0)</td>
</tr>
<tr>
<td>&lt;5</td>
<td>28</td>
<td>15 (53.6)</td>
<td>13 (46.7)</td>
</tr>
<tr>
<td>FIGO stage</td>
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<td></td>
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<tr>
<td>I-II</td>
<td>32</td>
<td>12 (37.5)</td>
<td>20 (62.5)</td>
</tr>
<tr>
<td>III-IV</td>
<td>16</td>
<td>14 (87.5)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Histological grading</td>
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<tr>
<td>1/2</td>
<td>29</td>
<td>15 (51.7)</td>
<td>14 (48.3)</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>11 (57.9)</td>
<td>8 (42.1)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>31</td>
<td>12 (38.7)</td>
<td>19 (61.3)</td>
</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>14 (82.4)</td>
<td>3 (17.6)</td>
</tr>
</tbody>
</table>

that miR-215 inhibited cell proliferation, promoted apoptosis and increased sensitivity to chemotherapy drugs in EOC cells by targeting the X-chromosome-linked inhibitor of apoptosis (XIAP) [20], the correlation between miR-215 dysregulation and clinicopathological characteristics of EOC has not yet been evaluated, and the biological roles of miR-215 in EOC, especially with regard to migration and invasion, remains poorly understood.

In this study, we examine miR-215 expression in human EOC tissues and cell lines and investigate the clinical significance of miR-215. Furthermore, we investigated the effect of miR-215 on the biological effect on EOC growth and metastasis. Here, we showed that miR-215 exerted suppressive roles in tumor growth and metastasis in EOC cells by targeting NOB1 and regulating MAPK signal pathway. Our data demonstrated the critical role of miR-215 in EOC growth and metastasis, and uncover a potential prognostic biomarker and molecular target for the treatment of EOC.

Materials and methods

Patients and clinical specimens

Paired EOC tumors and adjacent normal ovarian tissues (ANT) were obtained postoperatively from 48 patients at the Department of Obstetrics and Gynecology, the Second Hospital, Jilin University (Changchun, China) between September 2013 and October 2015. All samples were flash-frozen in liquid nitrogen immediately after resection and stored at -80°C until RNA extraction. None of the patients received neoadjuvant chemoradiotherapy prior to surgery. The clinical characteristics of all of EOC patients were recorded and described in Table 1. This study was approved by the Research Ethics Committee of the Second Hospital, Jilin University (Changchun, China), and written informed consent was obtained from all patient or family.

Cell culture and transfection

Human ovarian carcinoma cell lines (SKOV3, OVCAR3, A2780, and HEY), and a human ovarian surface epithelial cell line (HOSEpiC) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), and were maintained in RPMI-1640 medium (Gibco-BRL, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (HyClone, Logan, Utah, USA) and 1% penicillin/streptomycin and incubated in a humidified incubator (37°C, 5% CO₂).

The miR-215 mimic and negative control mimics (miR-NC) were brought from GenePharma (Shanghai, China). The coding sequences NOB1 were amplified by PCR and inserted into pcDNA3.1 vector (Invitrogen, Life Technologies, Carlsbad, USA) to generate NOB1 expression vectors pcDNA3.1-NOB1. Transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer protocol. NOB1 shRNA lentivirus (Lv/sh-NOB1) and scramble shRNA lentivirus (Lv/sh-Scramble) were constructed and infected into EOC cells according to our previous study [21].

RNA extraction and real-time PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. For detection of NOB1 mRNA analysis, complementary DNA was randomly primed from 1 µg of total RNA using the Prime Script RT reagent Kit (Takara, Dalian, China).
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Real-time PCR was subsequently performed in triplicate with 1:15 dilution of cDNA using Real-time PCR Mixture Reagent (Takara) on the 7900HT Fast real-time PCR machine (Applied Biosciences, Foster City, CA, USA). The primes of NOB1 and GAPDH were used in this study as described previously [21]. NOB1 mRNA quantification data were normalized to GAPDH using $2^{-\Delta\Delta C_t}$ method.

For detection of miR-215 level, real-time PCR was performed as above using TaqMan microRNA Assay (Applied Biosystems) according to the manufacturer's instructions using primes for miR-215 and U6 (Applied Biosystems). miR-215 data was normalized to U6 using $2^{-\Delta\Delta C_t}$ method.

Cell proliferation and colony formation assay

After transfection, SKOV3 cells were harvested, seeded into 96-well culture plates at a density of 5000 cells in 200 μl/well, and incubated at 37°C. At different time points (24, 48 or 72 h), 100 μl of MTT solution (0.5 mg/ml; Sigma, USA) was added to each well, and were cultured for another 4 h. Afterward, the MTT solution was removed and 150 μl dimethyl sulfoxide (DMSO, Sigma Aldrich, St. Louis, MO, USA) was added to each well to stop the reaction. The plates were gently shaken on a swing bed for 10 min and the absorbance at 490 nm in each well was measured by an enzyme-linked immunosorbent assay reader (Thermo Labsystems, Finland, USA).

For colony formation assay, 1,000 transfected cells were plated into a 6-well plate and cultured for 10 days. Then cells were fixed in a 10% acetic acid/10% methanol (in diH$_2$O) solution, and stained with 1% crystal violet (in methanol). Colonies were imaged and counted under light microscope (Olympus, Tokyo, Japan).

Cell migration and invasion assay

To determine the cell migration, wound healing assay was performed. Briefly, transfected cells (1 × 10$^5$/well) were seeded into 6-well plates and were serum-starved for 24 h at full confluence. An artificial homogenous wound was scratched into the monolayer using a sterile plastic micropipette tip. The cells were washed with PBS and then cultured for 24 h at full media. Cells were imaged at 0 and 24 h after the wounding using inverted Nikon Eclipse TS-100 phase-contrast microscope (Tokyo, Japan).

Wound closure was measured using Nikon NIS-Element Basic Research v3.2 software.

Cell invasion potential was evaluated using transwell chambers (8 μm pore; BD Biosciences, USA). Briefly, 2 × 10$^5$ cells (in serum-free media) were plated into the upper chamber with Matrigel (BD Biosciences), while media containing 10% FBS was added to the bottom chambers to attract cells. After 48 h incubation, noninvasive cells were removed from upper chamber with a cotton swab, while invaded cells were fixed with 95% ethanol, stained with 0.1% crystal violet, and counted at five randomly selected fields under a light microscope (Olympus, Tokyo, Japan).

Luciferase reporter assay

A fragment of the nob1 3'- UTR containing either the predicted binding site for miR-215 or a mutated 3'- UTR was inserted into the psiCHECK2 vector (Promega, Madison, WI, USA), and named as Wt-NOB1 and Mut-NOB1, respectively. After verification by DNA sequencing, the psiCHECK2 vector containing either the Wt-NOB1, or Mut-NOB1 3'- UTR and miR-215 mimic or miR-NC was cotransfected into SKOV3 cells using a Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. At 48 h after transfection, luciferase activity was measured using a dual-luciferase reporter assay system (Promega) and normalized to Renilla activity.

Western blot

Protein was extracted from cells using RIPA lysis buffer (Invitrogen) containing the protease inhibitors cocktail and PMSF according to the manufacturer’s protocol. The total concentration of protein was determined using BCA assay kit (Sigma). Equal amounts of protein lysates (30 μg each lane) were separated on a sodium dodecyl sulfate-polyacrylamide electrophoretic gel (SDS-PAGE) and transferred to nitrocellulose membranes (Bio-Rad, Munich, Germany). The membranes were blocked with 5% non-fat dry milk for 2 h and incubated with antibody against NOB1 p38MAPK, phosphorylated(p-) p38MAPK, p-ERK, ERK, JNK, p-JNK (all antibody from Santa Cruz Biotechnology, CA, USA) at 4°C overnight. The next day, the membrane was incubated with corresponding secondary antibodies HRP-conjugated for 2 h at room temperature. Protein bands were visualized with enhanced chemiluminescence reagent (ECL,
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Amersham, GE Healthcare, Velizy-Villacoublay, France) and exposed on an X-ray film. Blots were reprobed with GAPDH to control for loading variations.

In vivo tumor growth model

Twenty of five-week-old BALB/C nude male mice (20-25 g) were brought from the Experimental Animal Center of Changchun Institute for Biological Sciences (Changchun, China) and were maintained in the pathogen-free (SPF) conditions. All animal experiments were approved by National Institutes of Health Animal Care and the Use Committee guidelines of the Jilin University (Changchun, China).

For the in vivo tumor assay, SKVO3 cells (2 × 10⁶) transfected with miR-215 mimic or miR-NC were harvested and were washed and re-suspended in serum-free RPMI-1640 medium, and were injected into left side of the posterior flank of nude mouse( ten in each group). Tumor growth were measured with Vernier calipers every 5 days until 30 days, and tumor volume (mm³) was calculated using the formula: volume = (length × width²)/2. All mice were killed after 30 days inoculation, tumor tissues were stripped and weight. Parts of the tumor tissues were snap frozen in liquid nitrogen and stored at -80°C for detection of miR-215 and NOB1 expression.

Statistical analysis

The data shown are presented as the mean ± SD (standard deviation) of three independent experiments. Differences between groups were analyzed using the Student’s t-test, ANOVA or the chi-square test. Relationships between miR-215 expression and NOB1 mRNA levels were analyzed by Pearson correlation analysis. P values less than 0.05 (P<0.05) were considered as statistically significant.

Results

miR-215 is downregulated in EOC tissues and cell lines

To investigate the role of miR-215 in EOC occurrence and development, we first detected its expression in 48 EOC specimens and corresponding adjacent normal tissue (ANT) specimens by real-time quantitative reverse transcription-PCR (qRT-PCR). Data showed that miR-215 was significantly downregulated in EOC tissues compared to matched adjacent normal tissues (P<0.05; Figure 1A). To further assess the clinical relevance of miR-215 in EOC, all patients were divided into 2 groups: high miR-215 expression and low miR-215 expression using the median (0.417) of miR-215 expression in all EOC patients as a cutoff. Our results showed that decreased miR-215 expression was significantly associated with FIGO stage and lymph node metastasis (Table 1), but not with patients’ age, histological grading, or tumor size. In addition, we also measured miR-215 expression in four ovarian cancer cell lines (SKOV3, OVCAR3, A2780, and HEY) as well as normal ovarian surface epithelial cell line (HOSEpiC). Compared with ovarian surface epithelial cell line (HOSEpiC), miR-215 expression was significantly downregulated in four ovarian cancer lines (Figure 1B). SKOV3 cells exhibited...
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Figure 2. miR-215 inhibits EOC cell proliferation, colony formation, migration and invasion. A. qRT-PCR analysis confirmed increased miR-215 expression in SKOV3 cells transfected with miR-215 mimic or miR-NC. B-E. Cell proliferation, colony formation, migration and invasion were determined in SKOV3 cells transfected with miR-215 mimic or miR-NC. *P<0.05, **P<0.01 compared to miR-NC.
miR-215 inhibits EOC cell proliferation, colony formation, migration and invasion

Given that miR-215 was aberrantly expressed in EOC specimens, we hypothesized that miR-215 may regulate EOC growth and metastasis. To test our hypothesis, we overexpressed miR-215 in SKOV3 cells by transfected miR-205 mimic. Successful increase of miR-215 expression in SKOV3 cells was confirmed by qRT-PCR (Figure 2A). Then we performed MTT assay at 24 h, 48 h, and 72 h posttransfection. Our results demonstrated that overexpression of miR-215 significantly inhibited the proliferation activity in SKOV3 cells (Figure 2B). Consistent with this result, we also found that miR-215 overexpression significantly inhibited colony formation in SKOV3 cells compared to miR-NC group. Additionally, to investigate whether miR-215 effect on EOC migration and invasion, wound healing and transwell invasion assays were performed in SKOV3 cells transfected with miR-215 mimic or miR-NC. Our results demonstrated that overexpression of miR-215 significantly inhibited cell migration (Figure 2C) and invasion (Figure 2D) in SKOV3 cells.

miR-215 activates MAPK signal pathway

It has been showed that NOB1 could regulate MAPK signal pathway in EOC cells [22]. Given NOB1 is a direct target of miR-215, we wonder if miR-215 could regulate MAPK signal pathway. The p38MAPK, phosphorylated-p38MAPK (p-p38MAPK), ERK, p-ERK, JNK and p-JNK protein expression were measured in SKOV3 cells transfected with miR-215 mimic or miR-NC by Western blot. Our results showed that miR-215 overexpression resulted in a remarked addition of p-p38MAPK, p-ERK and p-JNK in ovarian cancer cells, without modifying that of the total protein levels of p38MAPK, ERK and JNK in each group (Figure 4). Consistent with this result, we also found that downregulation of NOB1 by infection of LV/sh-NOB1 also increased p-p38MAPK, p-ERK and p-JNK protein in ovarian cancer cells (Figure 4). These results suggested that miR-215 could regulate MAPK signal pathway in EOC.

NOB1 reversed the impaired EOC cell growth and metastasis mediated by miR-215

Since above results demonstrated that miR-215 targeted and regulated the expression of NOB1, thus, we investigated the ability of NOB1 to alter the effect of miR-215 on EOC growth and invasion. SKOV3 cells were cotransfected with miR-215 mimic or miR-NC and overexpression of NOB1 plasmids (pCDNA3.1-NOB1) or blank vector (pCDNA3.1). As shown in Figure 5A and 5B, the expression levels of NOB1 on mRNA level and protein level were significantly increased in SKVO3 cells cotransfection of NOB1 overexpression plasmid and miR-215 compared with cells cotransfected with miR-215 and blank vector. In addition, we found that overexpression of NOB1 could rescued inhibition effect on cell proliferation (Figure 5C), colony formation (Figure 5D), migration (Figure 5E) and invasion (Figure 5F) of SKVO3 cells induced by miR-215 overexpression. These findings suggested that miR-215 function as tumor suppressor by targeting NOB1.

Increased miR-215 expression suppresses tumor growth in vivo

To further evaluate the potential effect of miR-215 on EOC growth in vivo, SKOV3 cells transfected with miR-215 or miR-NC were injected into mouse, and tumor volume was measured.
miR-215 inhibits EOC growth and invasion by targeting NOB1

Figure 3. NOB1 is a target of miR-215 in EOC cells. A. Conservation of the miR-215-targeting sites in the NOB1 3’UTR and the miR-215 mutant sequence that abrogates miR-215 binding to target mRNA. B. Luciferase reporter assays in SKOV3 cells after co-transfected with wild-type or mutant 3’UTR of NOB1 and miR-215 mimic or miR-NC. Wt: wide-type, Mut: Mutant-type. *P<0.05, **P<0.01 compared to miR-NC. C, D. NOB1 expression on mRNA level and protein level in SKOV3 cells transfected with miR-215 mimic or miR-NC. GAPDH was used as internal control. *P<0.05, **P<0.01 compared to miR-NC. E. NOB1 mRNA expression in human EOC tissues and corresponding adjacent normal tissues (ANT) was determined by qRT-PCR. GAPDH was used as an internal control. * *P<0.01 compared to ANT. F. The reverse relationship between NOB1 mRNA expression and miR-215 expression was analyzed in EOC tissues by Pearson correlation analysis.

every five days until mice was killed. As shown in Figure 6A, the tumors growth of the miR-215 overexpressing group was lower than that of the miR-NC overexpressing group. At the end of
miR-215 inhibits EOC growth and invasion by targeting NOB1

Figure 4. miR-215 activate MAPK signal pathway. The p38MAPK, phosphorylated-p38MAPK (p-p38MAPK), ERK, p-ERK, JNK and p-JNK protein expression were measured in SKOV3 cells transfected with miR-215 and miR-NC or infected with Lv/sh-NOB1 or Lv/sh-Scramble by Western blot. GAPDH was used as internal control.

the experimental period, the tumor tissues were striped and weighted. It was found that the tumor size and weight of miR-215 overexpression group was significantly smaller than that of miR-NC overexpressing group (Figure 6B and 6C). In addition, we also detected miR-215 and NOB1 expression in tumor tissues. We found that miR-215 expression was upregulated (Figure 6D), while NOB1 expression both on mRNA level and protein level was decreased in miR-215 overexpressing group compared to miR-NC overexpressing group (Figure 6E and 6F). Collectively, these data suggest that miR-215 may inhibit tumor growth in vivo by targeting NOB1.

Discussion

Accumulating evidence supports miRNAs as effective diagnostic and prognostic molecular biomarkers as well as therapeutic targets in cancer [9, 10]. Accumulating studies have identified a lot of miRNAs with aberrant expression in EOC tissues or cells, and as tumor suppressor or oncogene in EOC [11, 12]. For instance, miR-338-3p was found to significantly inhibit EOC cell proliferation, colony formation, migration and invasion, as well as induce cell apoptosis and enhanced caspase-3, -8, and -9 activities through PI3K/AKT signaling pathways by targeting Runx2 [23]. miR-494 was showed to drastically inhibit cell proliferation, colony formation, migration and invasion, induce cell apoptosis and cell cycle arrest at G1 stage in vitro, as well as reduced tumor growth in vivo by regulating IGF1R [24]. miR-205, acting as an oncogenic miRNA, may promote the clinical progression of EOC patients and enhance the cellular migration and invasion in vitro by directly and negatively regulating ZEB1 [25]. Here, we demonstrated a critical role for miR-215 in EOC development and progression. We found that miR-215 expression was significantly decreased in EOC tissues and cell lines, and its expression was significantly associated with advanced FIGO stage and lymph node metastasis. We also showed that miR-215 inhibited EOC cell growth and invasion by suppressing the expression of NOB1. Importantly, NOB1 could partially reverse the effects of miR-215 on EOC suppression of growth and invasion. These results suggested that miR-215 could act as a novel potential therapeutic strategy for EOC.

MicroRNA-215 (miR-215) have showed been to function as oncogene in glioma [13], myeloid leukemia [14], gastric cancer [15], hepatocellular carcinoma [26], and cervical cancer [27], as tumor suppressor in non-small cell lung cancer [16], pancreatic carcinomas [17], breast cancer [18], colon cancer [19] and renal cell carcinoma (RCC) [28]. These controversial findings suggest that miR-215 functions as an oncogene or a tumor suppressor depended on the specific cancer type and context. Although recently a report demonstrated that the miR-215 is downregulated in ovarian cancer tissues and cell lines, overexpression of miR-215 in EOC cells inhibited cell proliferation, increased of apoptosis and increased of sensitivity to chemotherapy drugs by targeting XIAP [20], the clinical importance of miR-215 in EOC progression, the biological function and underlying molecular mechanism involved in EOC progression of miR-215 remains largely unclear. In current study, we found that miR-215 expression was significantly decreased in EOC tissues or cell lines compared with adjacent normal tissues or normal ovarian cells, which was consistent with previous result [20]. Further statistical analysis first demonstrated that decreased miR-215 expression was significantly associated with advanced FIGO stage and lymph node metastasis. In addition, our results showed that miR-215 could remarkably inhibit proliferation, colony formation, migration and invasion of EOC cells in vitro, as well as suppressed
miR-215 inhibits EOC growth and invasion by targeting NOB1

Figure 5. NOB1 reversed the impaired EOC cell growth and metastasis mediated by miR-215. SKOV3 cells were cotransfected with miR-215 mimic or miR-NC and NOB1 overexpression plasmids (pCDNA3.1-NOB1) or blank vector (pCDNA3.1). The following experiments were performed with the above cells. A, B. Expression of NOB1 on mRNA and protein level was measured by qRT-PCR and western blotting, respectively. GAPDH was used as internal control. C-F. Cell proliferation, colony formation, migration and invasion were determined in above cells. *P<0.05, **P<0.01 compared to miR-215 + Vector.
miR-215 inhibits EOC growth and invasion by targeting NOB1

It is well known that miRNAs execute their biological functions by regulating the expression of target genes [8]. With regard to miR-215, several targets have been confirmed in previous studies, such as RUNX1 [15], ZEB2 [16], XIAP [20], RB1 [29] and ACVR2B [30]. Here, we confirmed that NOB1 is a direct target of miR-215 as evidenced by the fact that ectopic expression of miR-215 reduced luciferase activity of the NOB1 promoter and miR-215 downregulated NOB1 expression (Figure 3). NOB1, located on chromosome 16q22.1, has been reported to be upregulated in various malignancies, such as prostate carcinoma, thyroid cancer, breast infiltrating ductal carcinoma and nonsmall lung cancer [31-34]. For ovarian cancer, our recent studies showed that NOB1 expression was upregulated, and silencing NOB1 inhibited cell proliferation, colony formation, and induced cell arrest at G1 stage [21], and increased TRAIL sensitivity of EOC cells [22]. These results suggested that NOB1 act as an oncogene in EOC. Here, we identified NOB1 as a target of miR-215, and found that overexpression of NOB1 effectively rescued inhibition effects on proliferation, colony formation, migration and invasion of EOC cells induced by miR-215 overexpression. These data indicated that miR-215 exert inhibition role in EOC cells by targeting NOB1.

It has been showed that NOB1 could regulate the mitogen-activated protein kinase (MAPK) signal pathway [22], which is a critical pathway for human cancer cell survival, dissemination, and resistance to drug therapy [35, 36]. Therefore, in this study, we investigated the effect of miR-215 on MAPK signal pathway. We found that overexpression of miR-215 or downregulation of NOB1 could increased the phosphorylation of p38MAPK, ERK and JNK expression. These results suggested that miR-215 exert tumor suppressor role in EOC by repressing NOB1, and indirectly activation MAPK pathway.

In summary, we demonstrated that miR-215 expression was downregulated in EOC cell lines and tissues compared with normal ovarian cell line and adjacent normal ovarian tissues, and its expression was significantly associated with FIGO stage and lymph node metastasis. miR-215 impaired EOC cell proliferation, colony formation, migration and invasion in vitro, as well as suppressed tumor growth in vivo by inhibiting NOB1. The results of this study contribute to improve our understanding molecular mechanism of EOC development and...
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progression, and provide potential new therapeutic targets for the management of EOC.

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Disclosure of conflict of interest

None.

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References

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