MEDICAL SCIENCE MONITOR

LAB/IN VITRO RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2019; 25: 4699-4674
DOI: 10.12659/MSM.914766

Received: 2018.12.23
Accepted: 2019.02.13
Published: 2019.06.24

The Long Non-Coding RNA (lncRNA) AGAP2-AS1 is Upregulated in Ovarian Carcinoma and Negatively Regulates lncRNA MEG3

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Source of support: Departmental sources

Background: lncRNA AGAP2-AS1 has been reported to promote several types of cancers, but its involvement in ovarian carcinoma (OC) is unknown. Our study was carried out to investigate the potential involvement of AGAP2-AS1 in OC.

Material/Methods: We enrolled 82 patients with ovarian carcinoma. Quantitative real-time PCR (qRT-PCR) was performed to analyze lncRNA AGAP2-AS1 expression in ovarian carcinoma and proliferation assays were performed.

Results: We found AGAP2-AS1 was upregulated in OC tissues compared to adjacent healthy tissues, and expression levels of AGAP2-AS1 increased with increased clinical stages. lncRNA MEG3 was downregulated in OC tissues and was inversely correlated with AGAP2-AS1. AGAP2-AS1 overexpression led to downregulation of MEG3, while MEG3 overexpression failed to affect AGAP2-AS1. AGAP2-AS1 overexpression promoted proliferation of OC cells, while MEG3 overexpression inhibited proliferation of OC cells. In addition, MEG3 overexpression attenuated the effects of AGAP2-AS1 overexpression on cancer cell proliferation, but AGAP2-AS1 overexpression did not obviously affect cancer cell invasion and migration.

Conclusions: lncRNA AGAP2-AS1 is upregulated in ovarian carcinoma and negatively regulates lncRNA MEG3 to participate in the regulation of cancer cell proliferation.

MeSH Keywords: Cell Proliferation • Ovarian Neoplasms • RNA, Long Noncoding

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/914766

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Background

The human genome transcribes both messenger RNAs (mRNAs) and non-protein-coding RNAs (ncRNAs) [1]. Although ncRNAs have no protein coding capacity, ncRNAs participate in cellular processes by gene expression at multiple levels, such as methylation, post-transcription, and translation [2,3]. Long ncRNAs (lncRNAs) are emerging classes of ncRNAs longer that 200 nt with critical functions in human diseases [4,5]. Regulation of lncRNA expression shows promise in disease control and treatment [6]. However, only a small portion of lncRNAs have been identified, and the functions of most lncRNAs are still unknown, which limits clinical applications.

Ovarian carcinoma (OC) is a common clinical malignancy and a leading cause of cancer-related deaths in females [7]. The development of OC is inevitably accompanied by tumor metastasis to the abdominal and pelvic cavities as well as many other major organs [8], and radical treatment approaches for metastatic OC remain lacking. Therefore, novel therapeutic targets are needed to improve the survival of OC patients. lncRNA AGAP2-AS1 promotes the development of several types of cancer, including lung cancer and gastric cancer [9,10], but its involvement in OC is unknown. Our preliminary RNA-seq data revealed the upregulated expression of more than 100 lncRNAs in tumor tissues of OC patients. Among those differentially expressed lncRNAs, AGAP2-AS1 is inversely correlated with lncRNA MEG3 (data not shown), which inhibits OC [11]. The present study investigated the function of AGAP2-AS1 in OC and explored its interaction with MEG3.

Material and Methods

Research subjects

Our study included 82 patients with OC at our hospital from May 2015 to May 2018. Pathological biopsy was performed by 3 experienced pathologists to confirm the disease. Inclusion criteria were: 1) first-time diagnosis and treatment, and 2) patients understood the experiment and provided signed informed consent. Exclusion criteria were: 1) patients who were transferred from other hospitals, and 2) treatment had been performed before admission. According to AJCC staging, there were 18 cases of stage I, 22 cases of stage II, 28 cases of stage III, and 14 cases of stage IV. Ages of patients ranged from 32 to 65 years, and the mean age was 48.6±5.9 years. This study was approved by the Ethics Committee of our hospital before the inclusion of patients.

Specimen and cell lines

Specimens of tumors and adjacent healthy tissues were collected from each patient through biopsy.

OVCAR3 and A2780 human OC cell lines were used to perform all *in vitro* cell experiments. Cells of these 2 cell lines were bought from the Chinese Academy of Medical Science Tumor Cell Bank (Peking, China).

RPMI 1640 (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (FBS) was used to cultivate cells of the OVCAR3 cell line, and DMEM (HyClone, Logan, UT) containing 10% FBS was used to cultivate cells of the A2780 cell line, both at 37°C in a 5% CO_2 incubator.

Total RNA extraction and real-time quantitative PCR (RT-qPCR)

To detect the expression of AGAP2-AS1 and MEG3, total RNAs were extracted from tissues and *in vitro* cultivated cells using RNAzol reagent (Sigma-Aldrich, St. Louis, MO). the Applied Biosystems High-Capacity cDNA Reverse Transcription Kit was used to performed reverse transcription to obtain cDNA, and the Applied Biosystems™ Power™ SYBR™ Green Master Mix was used to make PCR reaction systems. All PCR reactions were performed on a CFX384 Touch™ Real-Time PCR Detection Systems instrument with 18S RNA as endogenous control. Primers of AGAP2-AS1, MEG3, and endogenous control 18S RNA were designed and synthesized by Sangon (Shanghai, China). According to 2-ΔΔCT method, expression of AGAP2-AS1and MEG3 was normalized to 18S RNA.

Cell transfection

Vector construction service was provided by Sangon. AGAP2-AS1 and MEG3 genomic DNAs were inserted into pcDNA 3.1 vector to construct AGAP2-AS1- or MEG3-expressing vectors. Lipofectamine 2000 reagent (Thermo Fisher Scientific) was used to perform cell transfection with vectors at a dose of 10 nM. Control cells were un-transfected cells, and negative control cells were cells transfected with empty vectors. Cells were collected at 36 h after transfection to perform subsequent experiments.

In vitro cell proliferation assay

Using the cell culture medium mentioned above, single-cell suspensions were prepared and cell density was adjusted to 3×10^4 cells/ml. Cells were transferred to a 96-well plate with 100 µl cell suspension in each well. The plate was incubated in an incubator (37°C, 5% CO_2), and 10 µl CCK-8 solution was added into each well every 24 h until 96 h. After that, cells were cultivated for an additional 4 h. Finally, 10 µl DMSO was added and OD values at 450 nm were measured and normalized to the OD value of the control group at 96 h, which was set to 100%.

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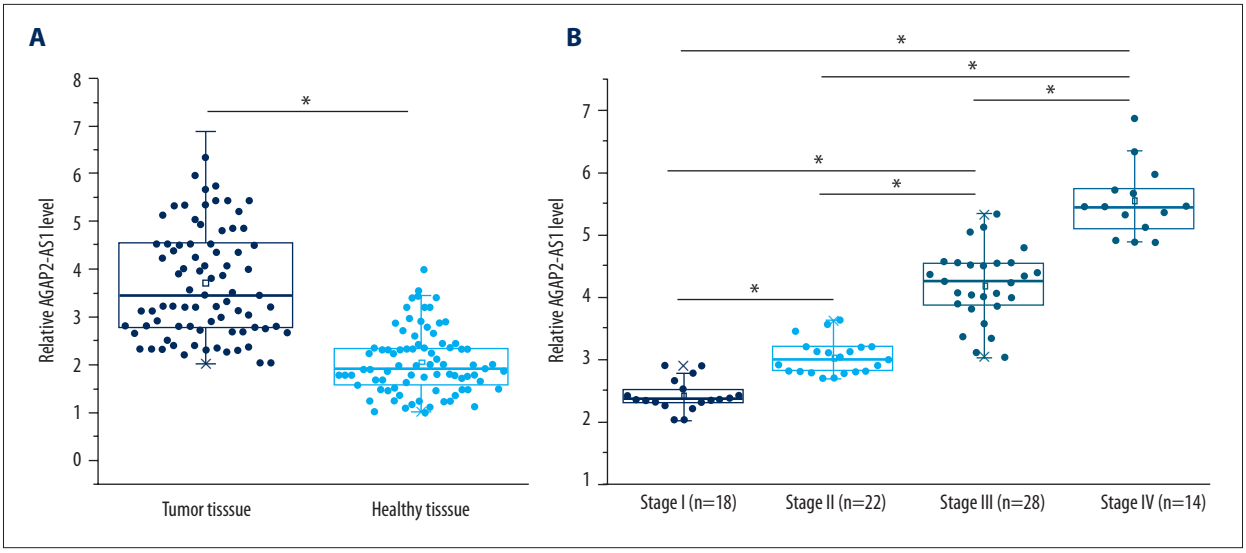

Figure 1. AGAP2-AS1 was upregulated in OC and was associated with clinical stage. AGAP2-AS1 was upregulated in OC tissues compared with healthy tissues around tumors (**A**), and expression levels of AGAP2-AS1 increased with increase of clinical stages (**B**); * p<0.05.

Statistical analysis

All experiments were repeated 3 times and results are expressed as mean ± standard deviation. The paired *t* test was used for comparisons of expression levels of AGAP2-AS1 and MEG3 between tumor and adjacent healthy tissues. Comparisons of expression levels of AGAP2-AS1 and MEG3, as well as cell proliferation data among cells with different treatments, were performed by ANOVA (one-way) and Tukey test. Correlations between expression levels of AGAP2-AS1 and MEG3 were performed by linear regression. Differences with p<0.05 were considered statistically significant.

Results

AGAP2-AS1 was upregulated in OC and affected by clinical stage

Expression of AGAP2-AS1 was detected by RT-qPCR. Compared with adjacent healthy tissues, AGAP2-AS1 was significantly upregulated in tumor tissues (Figure 1A, p<0.05). In addition, increased expression levels of AGAP2-AS1 in tumor tissues were observed with increases clinical stages (Figure 1B, p<0.05).

lncRNA MEG3 was downregulated in OC tissues and was inversely correlated with AGAP2-AS1

Expression of MEG3 was also detected by RT-qPCR. Compared with adjacent healthy tissues, expression levels of MEG3 were significantly decreased in tumor tissues (Figure 2A, p<0.05). Linear regression analysis showed that expression levels of AGAP2-AS1 and MEG3 were inversely and significantly correlated in tumor tissues (Figure 2B), but no significant correlation between expression levels of AGAP2-AS1 and MEG3 was observed in adjacent healthy tissues (Figure 2B).

AGAP2-AS1 is an upstream inhibitor of MEG3 in OC cells

The inverse correlation between AGAP2-AS1 and MEG3 in tumor tissues of OC patients indicated the possible interaction between AGAP2-AS1 and MEG3 in OC. To further investigate the interaction between AGAP2-AS1 and MEG3 in OC, AGAP2-AS1 and MEG3 expression vectors were transfected into cells of OVCAR3 and A2780 cell lines. As shown in Figure 3A, overexpression of AGAP2-AS1 and MEG3 was reached at 36 h after transfection (p<0.05). Compared with the control (C) and negative control (NC) groups, AGAP2-AS1 overexpression led to downregulation of MEG3 in cells of OVCAR3 and A2780 cell lines (Figure 3B, p<0.05), while MEG3 overexpression failed to significantly affect AGAP2-AS1 expression (Figure 3C, p<0.05).

AGAP2-AS1 overexpression promoted OC cell proliferation, but not migration and invasion, through MEG3

Results of *in vitro* cell proliferation assay showed that, compared with control (C) and negative control (NC) groups, AGAP2-AS1 overexpression promoted, but MEG3 inhibited, proliferation of cells of OVCAR3 (Figure 4A) and A2780 (Figure 4B) cell lines (p<0.05). In addition, MEG3 overexpression reduced the effects of AGAP2-AS1 overexpression on proliferation of cancer cell (p<0.05).

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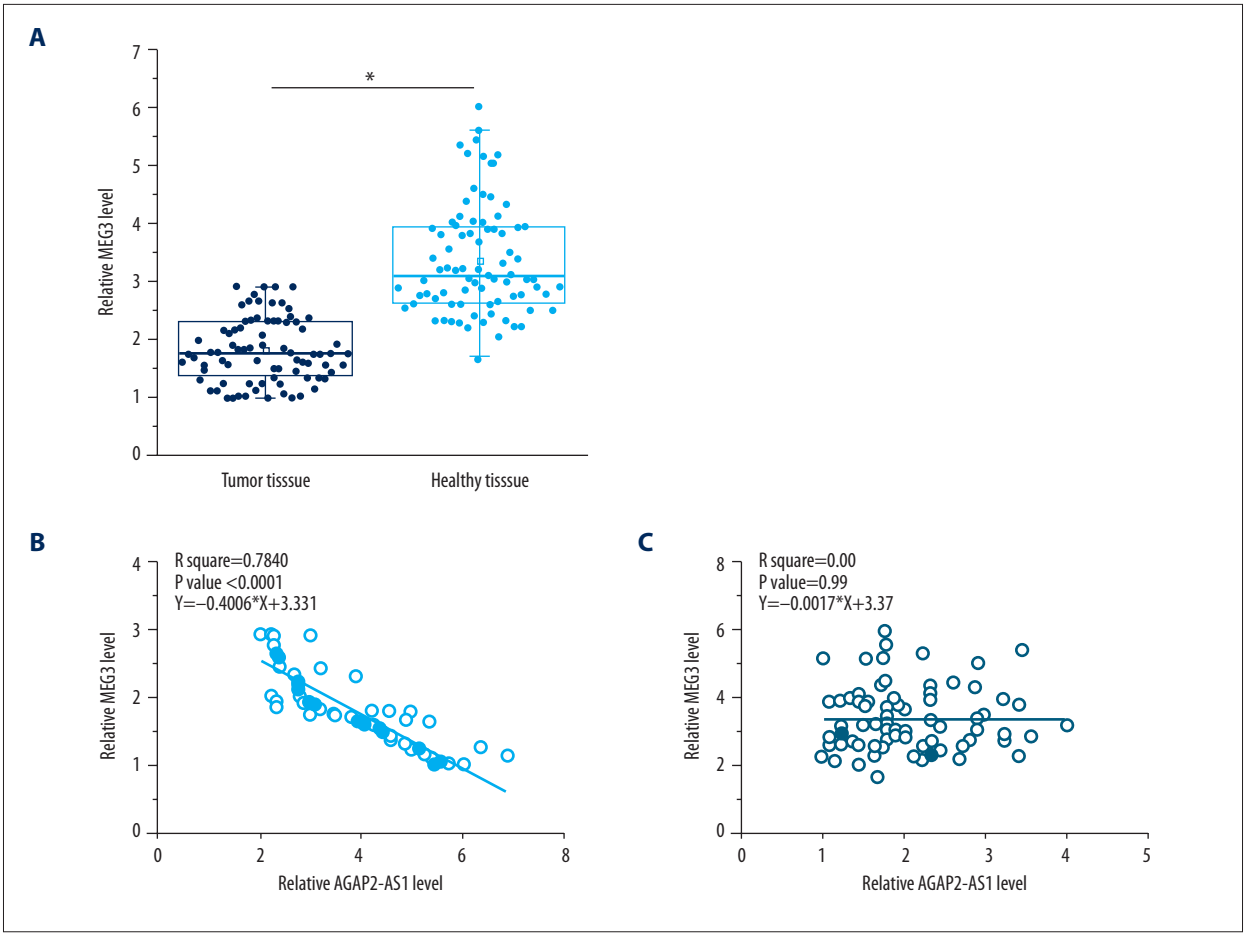

Figure 2. LncRNA MEG3 was downregulated in OC tissues and was inversely correlated with AGAP2-AS1. Expression levels of MEG3 were significantly decreased in tumor tissues (**A**); * p<0.05. Linear regression analysis revealed that expression levels of AGAP2-AS1 and MEG3 were inversely and significantly correlated in tumor tissues (**B**), but not in healthy tissues around tumors (**B**).

Discussion

The unknown function of most lncRNAs in cancer biology is a major challenge for application of lncRNAs in the diagnosis and treatment of cancer. The key finding of the present study is that AGAP2-AS1 is upregulated in OC and may act as an oncogene in OC by promoting cell proliferation through the inhibition of MEG3.

MEG3 acts as a tumor suppressor, with downregulated expression during the development of different types of cancer, including OC [11–13]. Consistently, our study also observed downregulated expression of MEG3 in OC tissues. In addition, overexpression of MEG3 inhibited OC cell proliferation. Our data further confirmed the role of MEG3 in ovarian cancer. lncRNAs achieve their biological functions in cancer biology through interactions with tumor suppression or oncogenic pathways [14,15]. It is also observed that lncRNAs can also interact with other ncRNAs, such as miRNAs, to promote or inhibit cancer development and progression [16,17]. However, the interactions between lncRNAs still are not well studied. Our study showed that AGAP2-AS1 was overexpressed in OC and can promote OC by downregulating MEG3. In addition, the downregulation of MEG3 by AGAP2-AS1 is likely involved in the regulation of OC cell proliferation. It has been reported that MEG3 can regulate the PTEN signaling pathway to inhibit OC. Our study identified an upstream regulator of MEG3/PTEN signaling in OC.

However, the molecular mechanism of the interactions between AGAP2-AS1 and MEG3 is still unknown. We speculated that AGAP2-AS1 indirectly interacts with MEG3, and disease-related factors may be involved. This speculation is supported by the observation that expression levels of AGAP2-AS1 and MEG3 were closely correlated in OC tissues but not in healthy tissues.

Interestingly, MEG3 has inhibitory effects on OC cell migration, proliferation, and invasion abilities [11]. In our study,

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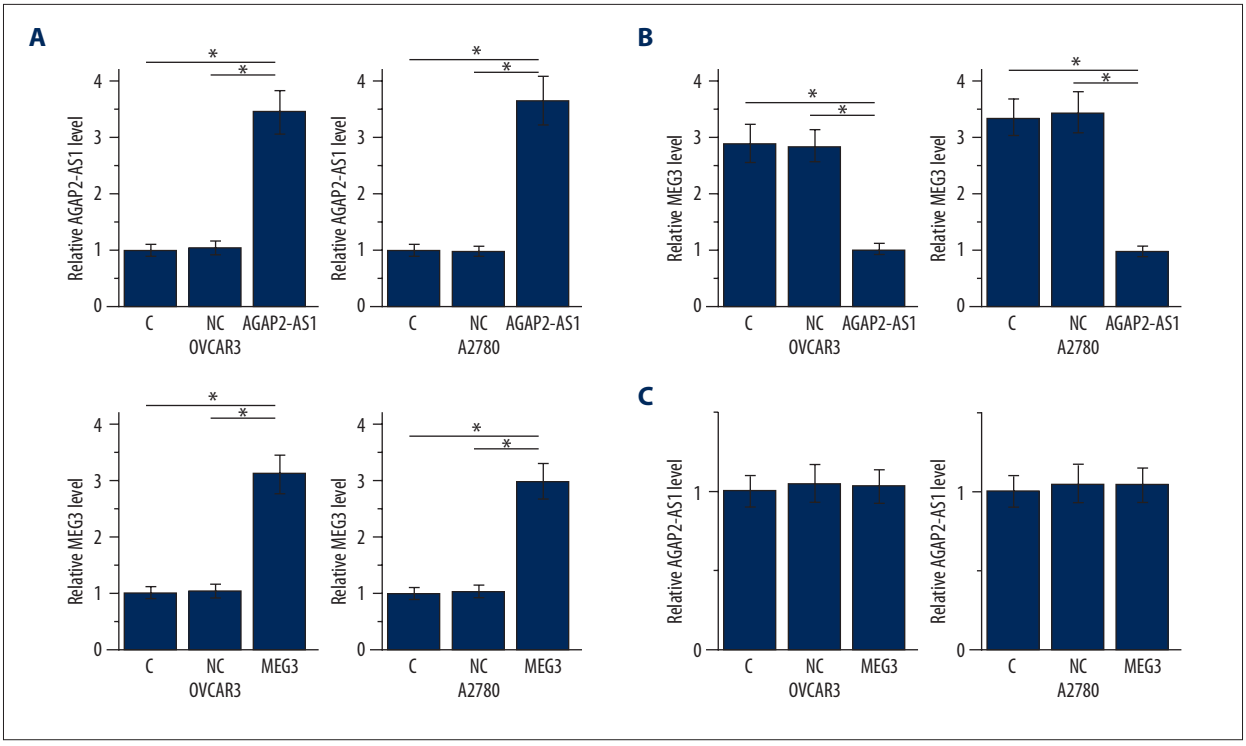

Figure 3. AGAP2-AS1 is an upstream inhibitor of MEG3 in OC cells. Overexpression of AGAP2-AS1 and MEG3 was reached at 36 h after transfection (**A**). AGAP2-AS1 overexpression led to downregulation of MEG3 in cells of OVCAR3 and A2780 cell lines (**B**), while MEG3 overexpression did not obviously affect AGAP2-AS1 expression (**C**); * p<0.05.

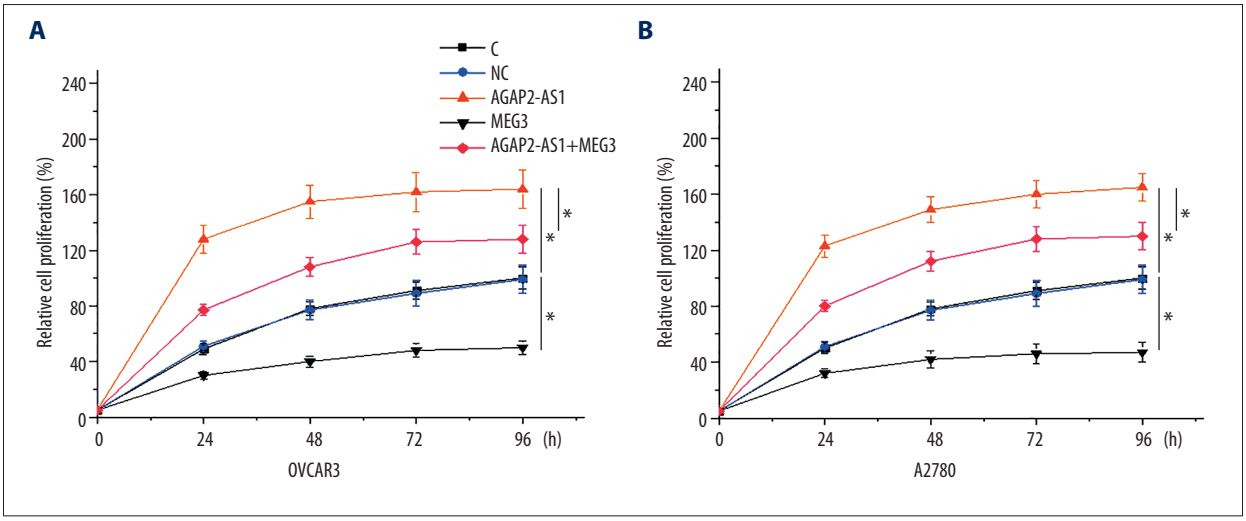

Figure 4. AGAP2-AS1 overexpression promoted OC cell proliferation, but not migration and invasion, through MEG3. AGAP2-AS1 overexpression promoted, but MEG3 overexpression inhibited, proliferation of cells of OVCAR3 (**A**) and A2780 (**B**) cell lines. In addition, MEG3 overexpression reduced the effects of AGAP2-AS1 overexpression on cancer cell proliferation; * p<0.05.

AGAP2-AS1 overexpression led to inhibited MEG3 expression but showed no significant effects on OC cell migration and invasion. This controversial observation suggests that AGAP2-AS1 can interact with multiple pathological factors to finely regulate different behaviors of OC cells.

Although our study failed to elucidate the molecular mechanism of the actions of AGAP2-AS1/MEG3 signaling in OC, previous studies have shown that AGAP2-AS1 can regulate downstream oncogenes of tumor suppressors, such as EZH2 and LSD1, to promote cancer development [9], while MEG3 also interacts with downstream proteins such as c-MET to inhibit

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cancer development [13]. Therefore, the interaction between EZH2 and LSD1 forms a complex gene expression network to affect OC progression.

Conclusions

AGAP2-AS1 is overexpressed in OC and can promote OC cell proliferation by downregulating MEG3.

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Conflict of Interest

None.

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