LncRNA-MSC-AS1 inhibits the ovarian cancer progression by targeting miR-425-5p

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Research

Keywords: LncRNA MSC-AS1, MicroRNA-425-5p, Proliferation, Apoptosis, Ovarian cancer

DOI: https://doi.org/10.21203/rs.3.rs-316559/v1

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Abstract

Background

Long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) were reported to be aberrantly expressed and related to the pathogenesis of ovarian cancer. However, the role and regulatory mechanism of MSC-AS1 in ovarian cancer has yet to be fully elucidated.

Methods

Expression of lncRNA MSC-AS1 (MSC-AS1) and microRNA-425-5p (miR-425-5p) in the ovarian cancer tissue samples and cell lines was examined by quantitative real-time polymerase chain reaction (qRT-PCR). The functions of MSC-AS1 on ovarian cancer cell proliferation, cell cycle and apoptosis were determined using MTT, colony formation and flow cytometry analyses. The protein expression levels were evaluated using western blot assay. The targeting relationship MSC-AS1 and miR-425-5p was verified via dual-luciferase reporter assay.

Results

MSC-AS1 expression level was lowly expressed, while miR-425-5p level was highly in ovarian cancer tissues and cells. Elevation of MSC-AS1 has the ability to significantly inhibit cell proliferation and facilitate cell apoptosis in SKOV3 cells. Moreover, MSC-AS1 targeted and negatively modulated miR-425-5p. MiR-425-5p up-regulation has been proved to partially reverse the tumor suppressive function of MSC-AS1 overexpression.

Conclusion

MSC-AS1 sponged miR-425-5p to inhibit the ovarian cancer progression. These findings may provide a promising therapeutic target for the treatment of ovarian cancer.

Introduction

Ovarian cancer is one kind of the most common gynecologic malignancy worldwide, which has high mortality [1, 2]. The patients was diagnose at advanced stage and the low diagnostic accuracy in the early stage [3]. Although the rise of great advancements in therapeutic strategies for ovarian cancer patients, including surgery, radiotherapy and chemotherapy, the five-year survival rate of ovarian cancer is still unsatisfied [4-6]. Nonetheless, molecules that are suitable for targeted therapies are still limited. Consequently, to explore the molecular mechanisms and to find potential targets were urgently needed for ovarian cancer diagnosis and therapy to improve the survival quality of ovarian cancer patients.
Long non-coding RNAs (lncRNAs) are a category of non-coding transcripts with longer than 200 nucleotides, which have attached more and more attention of researchers. A growing number of studies have disclosed that lncRNAs may play a tumorigenic or tumor-suppressive role in tumor carcinogenesis, ovarian cancer contained. For instance, lncRNA GAS5 regulates liver cancer proliferation and drug resistance by decreasing PTEN expression [7]. Wu et al. pointed out that knockdown of IncRNA PVT1 inhibits prostate cancer progression by regulating miR-15a-5p /KIF23 axis [8]. Besides, IncRNA H19 promotes cell proliferation and metastasis by inhibiting miR-200a expression in lung cancer [9]. It was also reported that downregulation of LncRNA GAS5 could promote liver cancer proliferation and drug resistance by decreasing PTEN expression [9]. However, no study was performed on the role of MSC-AS1 in ovarian cancer. Thus, its function in ovarian cancer requires further investigation.

MicroRNAs (miRNAs) are a family of small non-coding RNAs with the length of 18-22 nucleotides. These short miRNAs bind to the 3’ untranslated region (3’UTR) of their target genes. Similar to lncRNA, numerous studies have reported the abnormal expression of miRNAs participate in the development of tumors, such as proliferation, invasion, differentiation and apoptosis. For instance, previous studies depict that miR-23a promotes colorectal cancer cell migration and proliferation by targeting at MARK1 [10]. Furthermore, microRNA-21 could promotes cell proliferation and metastasis by targeting LZTFL1 in breast cancer [11]. Mai et al displayed that miR-18a promotes cancer progression through SMG1 suppression in nasopharyngeal carcinoma [12]. More importantly, a previous study indicated that miR-425-5p is associated with renal cell carcinoma cell migration, proliferation and apoptosis [13]. Therefore, elucidating the function and potential mechanisms of lncRNAs and miRNAs in ovarian cancer may provide markers for ovarian cancer diagnosis and therapy.

The present study intended to probe the expression pattern and potential roles of MSC-AS1 in ovarian cancer. Our findings may provide strong evidence in the molecular mechanism through which MSC-AS1 regulates the ovarian cancer progression.

Materials And Methods

Cell culture

Ovarian cancer cell lines (OVCAR3, HOSE, SKOV3) and human ovarian epithelial cell line (IOSE80) were purchased from the Shanghai Bank of Cells, Chinese Academy of Science (Shanghai, China). The cells were maintained in RPMI-1640 (Hyclone, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) in a 37°C incubator with 5% of CO₂.

Cell transfection

MSC-AS1 overexpression vector (pcDNA 3.1 MSC-AS1) and empty vector (pcDNA3.1) were synthesized by GenePharma (Shanghai, China) which were employed to induce MSC-AS1 overexpression. Cell transfection was conducted utilizing Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.)
according with the manufacturer’s instruction. The samples were collected after 48 h transfection for further analysis.

**Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)**

Total RNA was extracted from cells utilizing TRizol reagent (Invitrogen). RNA was reverse transcribed to cDNA was acquired through Prime Script TM RT reagent kit (Takara, Dalian, China) and RNA quantification was assessed by Power SYBR Green (Takara, Dalian China) on ABI PRISM 7900 Real-time PCR system (Applied Biosystems). GAPDH and U6 were validated as the normalization. Gene relative expression level was calculated by the 2−ΔΔCt method. The reaction conditions were as follows: 94°C 3min, 94°C 45s, 57°C 45s, 72°C 45s, a total of 30 cycle, and finally extended at 72°C for 10min.

**Western blot analysis**

Total protein in cells was extracted by RIPA solution (GenePharma) and the protein concentration was estimated through BCA Protein Assay Kit (Beyotime). Equal amounts protein samples were loaded on each lane and separated by 10% SDS-PAGE and then transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). Next, the membrane were cultivated with primary antibodies against PCNA (ab92552, 1:1,000 dilution, abcam), Ki-67 (ab245113, 1:10,000 dilution, abcam), Bax (ab81083, 1:1,000 dilution, abcam), Caspase-3 (218161, 1:1000 dilution, abcam) and GAPDH (ab181603, 1:1,000 dilution, abcam) at 4°C overnight. The membrane was washed with PBST for three times. Then the membrane was incubated with the horseradish peroxidase (HRP)-conjugated secondary antibodies (ab6721, 1:1,000 dilution, abcam). The bands were visualized using a chemiluminescence detection kit (Beyotime).

**MTT assay**

Cell (1× 10^5) viability was determined by MTT Kit (Beyotime, Shanghai, China). The cells were seeded in 96-well plates and incubated for 24, 48, or 72 h. 10 μL MTT was added with for 4 h. Then, 100 μL DMSO was added to each well and incubated with for 2 h. The optical density (OD) value was measured at 490nm wavelength and each experiment was repeated for three times.

**Colony formation assay**

Cells (1× 10^5) were plated onto 6-well plates and maintained in complete culture medium for another 14 days. Then the cells were washed with PBS and fixed in 4% paraformaldehyde for 1 h, following stained with 0.1% crystal violet solution for 30 min at room temperature. Images of cells were captured under a light microscope and the number of clones was manually calculated in three randomly selected fields.

**5-ethynyl-20-deoxyuridine (EdU) analysis**

Cell proliferation was also determined using a EdU assay kit (Thermo Fisher Scientific) according to the manufacturer’s instructions. Cells (1 × 10^5) were maintained in 6-well plates. After 48 h, 100 μL EdU was added for 2 h. Cells were incubated with 4% formaldehyde, followed by 0.3% Triton X-100 for 10 min. EdU-
positive cells were analysed using fluorescence microscopy (Olympus, Tokyo, Japan) at the 20× objective.

**Flow cytometry analysis**

Cells were harvested and fixed for about 8 h fixation. Then, the cells were incubated with RNase (50 μg/ml) and propidium iodide (PI) (50 μg/ml, Thermo Fisher, Waltham, MA, USA) for 30 min. Then, the cell cycle was measured by Flow Cytometry System (BD Accuri C6) and the relative ratios of G0/G1, S and G2/M phases were analyzed by FlowJo VX software (BD Biosciences, Franklin Lakes, NJ, USA).

**Dual luciferase reporter assay**

To validate the binding between MSC-AS1 and miR-425-5p, the wild type and mutant type of MSC-AS1 3′UTR (MSC-AS1-WT/MUT) were cloned into pmirGLO vector (Promega Corporation), and then co-transfected into SKOV3 cells with miR-425-5p mimic or mimic NC using Lipofectamine 2000 (Invitrogen). After transfection for 48 h, the luciferase activity was determined using dual-luciferase reporter assay system (Promega, Madison, WI, USA).

**Statistical analysis**

All experiments were performed in triplicate and data were presented as mean ± SD. SPSS 21.0 (IBM Corp., NY, USA) is applied for statistical analysis. Student’s t-test or one-way ANOVA were used for comparison differences between groups. P < .05 was considered statistically significant.

**Results**

**MSC-AS1 expression was reduced in ovarian cancer tissues and cell lines**

After browsing the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/help.html) website[14], we discovered that MSC-AS1 expression in ovarian cancer tissues was remarkably lower than that in normal tissues, as exhibited in Fig. 1A B. Consistently, qRT-PCR assay showed that the expression level of MSC-AS1 was markedly lower in ovarian cancer tissues comparison with that in adjacent non-tumor tissues (Fig. 1C). Subsequently, the level of MSC-AS1 in ovarian cancer cell lines was examined. Similarly, MSC-AS1 was lowly expressed in three ovarian cancer cell lines (OVCAR3, HOSE, SKOV3) compared with that in human IOSE80 cells (Fig. 1D).

**Ectopic of MSC-AS1 inhibits proliferation of ovarian cancer cells**

To probe the function of MSC-AS1 in ovarian cancer, overexpression plasmids were transfected into SKOV3 cells to upregulate MSC-AS1 expression. The efficiency of transfection was confirmed by qRT-PCR (Fig. 2A). The MTT assay unveiled that MSC-AS1 overexpression markedly suppressed the viability of SKOV3 cells (Fig. 2B). Consistently, the colony formation analysis revealed that pcDNA 3.1 MSC-AS1 significantly inhibited the proliferation of SKOV3 cells compared with NC group (Fig. 2C). In addition, flow
cytometry and western blot analysis was performed to explore the effects of MSC-AS1 on the cell cycle. As shown in Fig. 2D, a significantly greater proportion of S phase cells were observed in SKOV3 cells when MSC-AS1 was overexpression compared with NC group. Western blot analysis indicated that ectopic of MSC-AS1 decreased the expression levels of PCNA and Ki-67 compared to NC group (Fig. 2E).

**Overexpression of MSC-AS1 induces apoptosis of ovarian cancer cells**

Flow cytometry was used detect the effect of ectopic of MSC-AS1 on apoptosis of ovarian cancer cells. It was discovered from the results that pcDNA 3.1 MSC-AS1 induced of apoptosis rate in SKOV3 cells compared with NC group (Fig. 3A). Furthermore, apoptosis related proteins were detected by western blot assay, and the results showed that ectopic of MSC-AS1 resulted in an obvious raised in the expression levels of Bax and caspase-3 when transfected with pcDNA 3.1 MSC-AS1 in SKOV3 cells (Fig. 3B).

**MiR-425-5p is a direct target of MSC-AS1**

To explore the mechanism underlying MSC-AS1 in ovarian cancer, StarBase database was utilized. As shown in Fig. 4A, 3‘-UTR of miR-425-5p has been predicted as the candidate and contains the MSC-AS1 binding site. Next, the level of miR-425-5p in ovarian cancer tissues was examined and the result showed that the expression level of miR-425-5p was significantly up-regulated in ovarian cancer tissues comparing to normal tissues (Fig. 4B). Subsequently, the level of miR-425-5p was measured in OVCAR3, HOSE, SKOV3 cells, and normal epithelial cells line (IOSE80). As presented in Fig. 4C, miR-425-5p expression in ovarian cancer cell lines was significantly higher compared to that in IOSE80 cells. Next, the target relationship between MSC-AS1 and miR-425-5p was verified through luciferase reporter assay. As expected, a significantly lower activity of the luciferase was observed in the combination of miR-425-5p mimic and MSC-AS1-WT but not MSC-AS1-MUT was weakened obviously after overexpression of miR-425-5p (Fig. 4D). qRT-PCR analysis was used to measure the expression of miR-425-5p (Fig. 4E). We demonstrated that ectopic expression of MSC-AS1 decreased expression level of miR-425-5p in SKOV3 cells.

**MSC-AS1 inhibits the ovarian cancer progression by targeting miR-425-5p**

Following the above experiments, we attempted to elucidate the mechanism underlying MSC-AS1. To perform rescue experiments, the miR-425-5p mimic was transfected into SKOV3 cells with or without MSC-AS1 overexpression. Transfection efficiency is presented in Fig. 5A, B. The results presented that pcDNA 3.1-MSC-AS1 transfection decreased but miR-425-5p mimic transfection increased miR-425-5p expression, and that the level of miR-425-5p in cells co-transfected with pcDNA 3.1 MSC-AS1 and miR-425-5p mimic was almost similar to the original level of miR-425-5p. Then, the participation of miR-425-5p in MSC-AS1-mediated ovarian cancer cellular activity was explored. In MTT assay, it was observed that MSC-AS1 overexpression obviously boosted cell viability and ectopic miR-425-5p expression rescued the promotion impact of MSC-AS1-overexpression on cell viability (Fig. 5C). Same trends of cell proliferation were viewed in EdU experiments (Fig. 5D).
As shown in Fig. 6A, the flow cytometry experiment unveiled that MSC-AS1 promotion markedly increased the proportion of S phase cells were observed in SKOV3 cells, while up-regulated miR-425-5p expression antagonized the impact of the pcDNA 3.1-MSC-AS1. Besides, flow cytometry results also revealed that after up-regulated MSC-AS1 expression, the apoptosis rate of SKOV3 cells was significantly induced, while up-regulating miR-425-5p led to an opposite effect and up-regulating miR-425-5p was found to reverse the effects of MSC-AS1 overexpression on SKOV3 cell apoptosis (Fig. 6B).

**Discussion**

Although the rise of great advancements in therapeutic strategies, the survival rate of ovarian cancer patients remains very poor[15, 16]. Therefore, it is significant to further discover the molecular mechanisms related to ovarian cancer development and progression. The complex process of ovarian cancer includes gene expression, signal pathway and epigenetic changes. To study the abnormal expression of core genes[17], elucidate the molecular mechanism of ovarian cancer, and find potential biomarkers are of great significance for early diagnosis and prognosis, and help to find new treatment methods and improve the prognosis of patients with ovarian cancer.

In the past few decades, abnormal expression of lncRNAs has been widely found and plays a vital role in malignancies. Multiple studies have suggested that lncRNAs can participate in many pathological processes, including proliferation, apoptosis, invasion and differentiation [18-20]. With the development of high-throughput sequencing technology, TCGA and GEO have been established which as the major approach for selecting lncRNAs. We selected MSC-AS1 as a potential essential lncRNA for ovarian cancer progression and diagnosis and further studied the molecular mechanisms.

MSC-AS1 abnormal expressed in many tumors, such as pancreatic cancer, nasopharyngeal carcinoma, osteosarcoma and hepatocellular carcinoma [21-24]. In the present study, MSC-AS1 expression was discovered to be down-regulated in ovarian cancer tissues and cell lines. Considering that the role of MSC-AS1 in ovarian cancer development and progression remained elusive, we intended to explore the precise role of MSC-AS1 in ovarian cancer. Subsequently, gain-of-function experiments validated that up-regulation of MSC-AS1 could significantly inhibit cell proliferation, clone formation, and induce apoptosis capacity of ovarian cancer cells. These data hinted that MSC-AS1 also played an anti-oncogenic role in ovarian cancer.

Growing evidence has demonstrated that the interaction between lncRNAs and miRNAs can form a complex regulatory network, which plays a vital role in the biological process of various malignancies [25, 26]. The possible mechanism of MSC-AS1 in ovarian cancer cells was also revealed. To validate the possible targets of MSC-AS1, miRDB (http://mirdb.org/) and StarBase 3.0 (http://starbase.sysu.edu.cn/) were utilized to predict the potentially target genes. 3'-UTR of miR-425-5p has been predicted as the candidate and contains the MSC-AS1 binding site. In addition, the dual luciferase reporter assay concluded that the interaction between MSC-AS1 and miR-425-5p is direct. Subsequently, Rescue assays were also conducted to confirm that ectopic the miR-425-5p expression could partially reverse the tumor
suppressive function of MSC-AS1 overexpression, suggesting that MSC-AS1 can inhibit the development of ovarian cancer cells by down-regulating miR-425-5p expression.

In summary, we demonstrated that a new mechanism by which MSC-AS1 elevated miR-425-5p expression, which retard the progression of ovarian cancer, manifesting that MSC-AS1 serves as a promising target for the treatment of ovarian cancer patients.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest for this work.

Availability of data and material
All data generated or analyzed using this study was included in this published article.

Founding
Not applicable.

Acknowledgements
Not applicable.

Authors' contributions
HQ, XB conceived of the study, and participated in its design and coordination. YZ, DY witted the manuscript. DZ, TX, AH carried out the experiments. LJ, CL performed the statistical analysis. All authors read and approved the final manuscript

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Figures
Figure 1

MSC-AS1 expression was reduced in ovarian cancer tissues and cell lines. A, B The expression of MSC-AS1 in ovarian cancer tissues and adjacent tissues was predicted using bioinformatics analysis based on GEPIA database. C, D qRT-PCR was used to detect the expression level of MSC-AS1 in ovarian cancer tissues and cell lines. *P<0.05, **P<0.01 vs. Normal tissues or IOSE80.
Figure 2

Ectopic of MSC-AS1 promotes proliferation of ovarian cancer cells. A, The upregulation efficacies of MSC-AS1 in SKOV3 cells were validated through qRT-PCR. B, C The impact of MSC-AS1 on cell proliferation was explored by MTT and colony formation analyses. D, Flow cytometry analysis was performed to explore the effects of MSC-AS1 on the cell cycle. E, The protein expression levels of PCNA and Ki-67 were analyzed using western blot assay. *P<0.05, **P<0.01 vs. pcDNA 3.1.
Figure 3

Impact of MSC-AS1 on ovarian cancer cell apoptosis. A. The apoptosis property of SKOV3 cells was investigated by tunel assay. B. The apoptosis related protein expressions were investigated via western blot assay. *P<0.05, **P<0.01 vs. pcDNA 3.1.
Figure 4

miR-425-5p is a direct target of MSC-AS1. A. The binding relationship between MSC-AS1 and miR-425-5p was assumed by StarBase database. B. Level of miR-425-5p in ovarian cancer tissues and normal tissues was evaluated by qRT-PCR assay. C. miR-425-5p expression was detected in ovarian cancer cell lines. D, Luciferase reporter assay assessed the target of MSC-AS1 to miR-425-5p. E, qRT-PCR analysis was used to measure the expression of miR-425-5p under transfection of pcDNA 3.1-MSC-AS1 or NC. **P<0.01 vs. Normal tissues, IOSE80, or pcDNA 3.1.
Figure 5

MSC-AS1 inhibits the ovarian cancer progression by targeting miR-425-5p. A, B. Transfection efficiency was analyzed. C, D. Cell proliferation was measured by using MTT and EdU assays. **P<0.01, ***P<0.001 vs. pcDNA 3.1. ##P<0.01 vs. pcDNA 3.1 MSC-AS1.
Figure 6

MSC-AS1 inhibits the ovarian cancer progression by targeting miR-425-5p. A, B. Determination of cell cycle and apoptosis by flow cytometry analysis. *P<0.05, **P<0.01 vs. pcDNA 3.1. #P<0.05, ##P<0.01 vs. pcDNA 3.1 MSC-AS1.

Supplementary Files

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