Iran J Public Health, Vol. 52, No. 11, Nov 2023, pp.2412-2416

Original Article

Lncrna FGFR3-AS1 Is a Prognostic Indicator for Ovarian Cancer and Induces Cell Proliferation and Hinders Apoptosis

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(Received 12 Mar 2023; accepted 10 May 2023)

Abstract

Background: Ovarian cancer is one of the most common malignant tumors in Gynecology, whose treatment was seriously limited by the unclear understanding of molecular mechanism in disease development. LncRNA FGFR3-AS1 is involved in human cancers. In this study, we aimed to clarify its regulatory effect on ovarian cancer.

Methods: Ovarian cell model was used in 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT) assay and flow cytometry for investigating the role of LncRNA FGFR3-AS1 in ovarian cancer. Western blot detection (CCND1、CDK6、MAPK9 and PIK3CA) tumor regulatory proteins. The study was conducted between March 2020 and October 2022 at General Hospital of Fushun Mining Bureau of Liaoning Health Industry Group, China.

Results: The results demonstrated the upregulation of LncRNA FGFR3-AS1 in ovarian cancer, whose expression was positively related to tumor grade and AJCC stage, and negatively correlated with patients' prognosis. LncRNA FGFR3-AS1 promoted ovarian cancer development through promoting cell growth and inhibiting cell apoptosis. PI3K signaling pathway-related proteins may be implicated in the LncRNA FGFR3-AS1 induced regulation of ovarian cancer.

Conclusion: LncRNA FGFR3-AS1 was involved in the development of ovarian cancer, which has the potential to become therapeutic target and prognostic indicator in ovarian cancer treatment.

Keywords: Ovarian cancer; Prognosis; Cell proliferation; Cell apoptosis

Introduction

Ovarian cancer is one of the most common malignant tumors in Gynecology. The incidence of ovarian cancer ranks third, while the mortality rate is the highest among female malignant tumors of the reproductive system (1). Ovarian cancer has the characteristics of concealed onset, high malignancy, and easy metastasis, which causes its poor prognosis (2). Although early-stage ovarian cancer is relatively easy to cure, patients are often in the middle and late stages of

the disease at the time of diagnosis because the ovary is located in the deep pelvic cavity and has no specific clinical symptoms in the early stage of the disease (3).

Although the treatment of ovarian cancer such as surgery, radiotherapy and chemotherapy has been greatly developed and advanced in recent years, the prognosis of advanced ovarian cancer has not been significantly improved. In recent years, the emergence of molecular-targeted therapy has brought a glimmer of light to the treatment of ovarian cancer, especially advanced ovarian cancer (4). Although researchers have developed a variety of targeted drugs for ovarian cancer and applied them in clinical treatment, the improvement of prognosis is still far from satisfactory (5, 6). Therefore, in-depth analysis of the molecular mechanism of ovarian cancer development and the search for regulatory factors that play an important role as drug targets are of great significance for improving the accuracy and efficiency of ovarian cancer targeted therapy (5).

The dysregulation of lncRNAs is related to many types of human cancer, including FGFR3 antisense transcript 1 (FGFR3-AS1) (7). FGFR3-AS1 (previous name: lncRNA-BX537709) oriented in an- tisense direction with respect to FGFR3, and formed a 'tail-to-tail' pairing pattern with 1053 nucleotides full complementarity. Although the function of LncRNA FGFR3-AS1 has not been clearly illustrated so far, it plays a role as a cancer-promoting factor in various human tumors such as osteosarcoma (7), and bladder cancer (8). FGFR3-AS1 increases the expression of its target gene FGFR3, which was previously identified as a potential biomarker for detection of bladder cancer (9, 10). FGFR3 decreases cell-cell and cell-matrix adhesion, and induces pro-proliferative and anti-apoptotic events in bladder cancer (11). However, the relationship be- tween FGFR3-AS1 and ovarian cancer is still unclear. Therefore, this work aims to clarify its regulatory effect on ovarian cancer.

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Materials and Methods

Cell Culture and Cell Transfection

The human ovarian epithelial cell line IOSE08 and human ovarian cancer cell lines 3AO, HO-8910, OVCAR-3 were obtained from ATCC. 3AO and HO-8910 cells were cultured in 90% RPMI-1640 containing 10% FBS in 6-well plates and OVCAR-3 were cultured in 90% DMEM containing 10% FBS. The culture environment was moist air containing 5% CO_2 at 37 °C. Prepared lentivirus was transfected to the HO-8910 cells using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, USA). After 72 h, the expression of green fluorescent protein was observed under a fluorescence microscope and the transfection efficiency was evaluated.

Western Blot and Antibody Array Assay

Western blot assay was applied to determine the LncRNA FGFR3-AS1 protein expression levels. Cells were collected and lysed with 1 × cold lysis buffer. Protein was extracted with BCA protein detection kit (Cat. #23225, HyClone-Pierce). 10% SDS-PAGE was used for Western analysis. Blots were transferred to a PVDF membrane and incubated with blocking liquid for 1 h. CCND1 antibody (1:3000, Cat. #ab134175, Abcam), CDK6 antibody (1:1000, Cat. #ab151247, Abcam), MAPK9 antibody (1:3000, Cat. #ab76125, Abcam), PIK3CA antibody (1:1000, Cat. #ab40776, Abcam), and internal standard GAPDH antibody (1:3000, Cat. #AP0063, Bioworld) were added overnight at 4 °C. After incubated with secondary antibody HRP goat anti-rabbit IgG polyclonal (1:3000, Cat. # A0208, Beyotime) for 1 h at room temperature, the membrane was color developed using ECL-PLUS/Kit (Cat. #RPN2232, Amersham).

Apoptotic Assay

The proportion of LncRNA FGFR3-AS1 HO-8910 cell apoptosis was detected by flow cytometry. HO-8910 cells were seeded and cultured for 5 days in a 6 cm-well dish. Afterwards, cells were digested with trypsin and re-suspended with 1 ×

binding buffer. Before detected by FACScan, cells were stained by Annexin V-APC for 15 min in the dark. Cell phase percentage was determined to assess the apoptotic rate

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) Assay

Proliferation of LncRNA FGFR3-AS1 HO-8910 cells was detected via MTT assay with five 96-well plates. 2500 cells/well were seeded to the 96-well plates for culturing. In the next 5 days (one time one day), 20 μL 5 mg/mL MTT solution was added into per well, and 4 h later, the culture medium was discarded and 100 μL DMSO was added for shaking 5 min. The OD490 was measured with a Microplate Reader and the cell viability was calculated.

Statistical Analysis

All our cell experiments were performed in triplicate and the data were expressed as mean ± SD ($n \geq 3$) and statistical analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism software 7.0. The χ 2 test, Mann-Whitney U-test, Spearman rank correlation analysis, Kaplan-Meier survival analysis were applied to estimate the association between the expression level of LncRNA FGFR3-AS1 and clinical characteristics. *t*-test were used to compare the difference. *P* values < 0.05 were defined as significant.

Results

LncRNA FGFR3-AS1 promoted Cell Proliferation

MTT assay was utilized to study the effects of LncRNA FGFR3-AS1 on cell proliferation. Through detecting cell viability of LncRNA FGFR3-AS1 and control cells in 5 days of culturing, it was demonstrated that the proliferation rate of cells with downregulated LncRNA FGFR3-AS1 was significantly restrained (Fig. 1; $P < 0.05$). We demonstrated the promotion of ovarian cancer cell growth by LncRNA FGFR3-AS1, which was in consistent with our deduction.

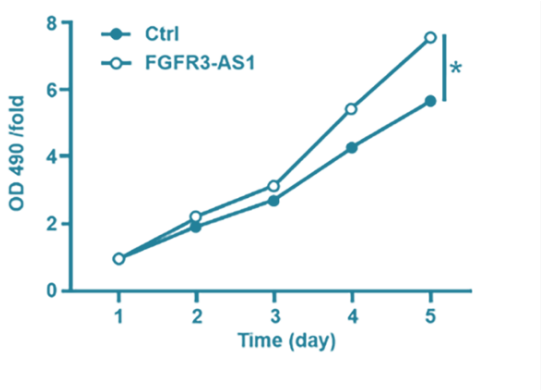

Fig. 1: MTT assay was performed to assess the effects of LncRNA FGFR3-AS1 on cell proliferation of HO-8910 cells. *$P < 0.05$

LncRNA FGFR3-AS1 inhibited apoptosis of ovarian cancer cells

Finally, we evaluated the role of LncRNA FGFR3-AS1 in ovarian cancer cell apoptosis through flow cytometry. The results indicated that LncRNA FGFR3-AS1 significantly inhibited cell apoptosis, which was also in consistent with the above results (Fig. 2; $P < 0.001$). Moreover, several well-known tumor regulator proteins such as CCND1, CDK6, MAPK9 and PIK3CA were also subjected to detection. CCND1, CDK6 and PIK3CA, which were generally PI3K signaling-related proteins, were upregulated in LncRNA FGFR3-AS1 cells, while MAPK9 was downregulated (Fig. 3).

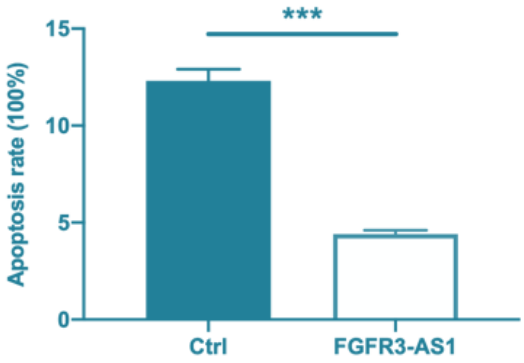

Fig. 2: LncRNA FGFR3-AS1 inhibited cell apoptosis of ovarian cancer. Data were shown as mean with SD. ***$P < 0.001$

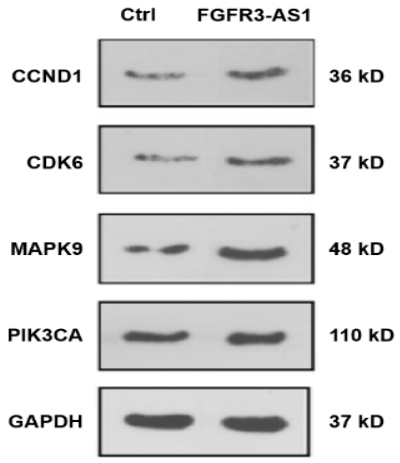

Fig. 3: LncRNA FGFR3-AS1 regulated ovarian cancer through regulating cancer-related proteins. The expression of CCND1, CDK6, MAPK9 and PIK3CA was detected in ovarian cancer cells with or without LncRNA FGFR3-AS1

LncRNA FGFR3-AS1 Was Upregulated in Ovarian Cancer and Predicted Poor Prognosis

We detected the endogenous expression of LncRNA FGFR3-AS1 in ovarian epithelial cell IOSE80 and ovarian cancer cell HO-8910, 3AO and OVCAR-3, which showed that LncRNA FGFR3-AS1 expression was higher in ovarian cancer cells than normal cells, and higher in HO-8910 cells than 3AO and OVCAR-3 cells (Fig. 4; $*P < 0.05$, $**P < 0.01$).

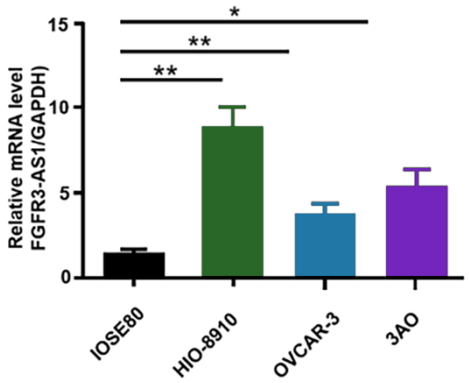

Fig. 4: The endogenous expression of LncRNA FGFR3-AS1 in human ovarian epithelial cells and ovarian cancer cells was detected by qPCR. Data were shown as mean with SD. $*P < 0.05$, $**P < 0.01$

Discussion

Ovarian cancer is one of the gynecological malignancies with the highest morbidity and mortality. However, because of the lack of specific diagnostic markers, rapid progress and easy metastasis, the treatment of ovarian cancer is still a major problem in the research field of ovarian cancer. Recently, the emergence and development of molecular-targeted therapy has brought a new approach to the treatment of ovarian cancer, which is based on the understanding of the molecular mechanism of ovarian cancer. Therefore, more and more attention has been paid in the exploration of novel molecules involved in the development and progression of ovarian cancer. For example, nucleolar spindle-associated protein 1 (NuSAP1) was significantly upregulated in ovarian cancer, knockdown of which could induce inhibition of cell proliferation and migration, arrest of cell cycle, and promotion of cell apoptosis (12). TCF12 might be involved in the growth and metastasis of ovarian cancer, which could be used as a potential prognostic indicator for ovarian cancer (13). Recently, SNCG was identified as a key regulator in the progression and metastasis of high-grade serous ovarian cancer, of which PI3K/Akt signaling pathway acted as the potential downstream mechanism (14).

In this study, our group discerned LncRNA FGFR3-AS1 as a novel regulator of ovarian cancer, thus a potential therapeutic target for treatment and prognostic indicator. The role of LncRNA FGFR3-AS1 in ovarian cancer was further confirmed by in vitro verifications, which showed that LncRNA FGFR3-AS1 promoted ovarian cancer cell proliferation and inhibited cell apoptosis. Moreover, several PI3K related proteins such as CCND1, CDK6, MAPK9 and PIK3CA were involved in the regulation of cell apoptosis by LncRNA FGFR3-AS1 overexpression.

Conclusion

LncRNA FGFR3-AS1 was identified as a potential therapeutic target and prognostic indicator of ovarian cancer. We demonstrated the upregulation of LncRNA FGFR3-AS1 in ovarian cancer, whose expression was positively related to cell proliferation, and negatively correlated with cell apoptosis. The study also proved the involvement of LncRNA FGFR3-AS1 in ovarian cancer development through regulating PI3K-related proteins.

Journalism Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This work was supported by Natural Science Foundation of Liaoning Province in 2019 (2019-MS-187).

Conflict of Interest

The authors declare that there is no conflict of interest.

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