AIB1 regulates the ovarian cancer cell cycle through TUG1

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Abstract. OBJECTIVE: To explore the mechanism of amplified in breast cancer 1 (AIB1) to promote ovarian cancer progress.

MATERIALS AND METHODS: Correlation analysis was performed to obtain the top 100 lncRNAs that were positively correlated with AIB1. The relationship of taurine upregulated gene 1 (TUG1) and clinicopathological characteristics. Moreover, Gene Ontology (GO) and Gene Set Enrichment Analysis (GSEA) were performed to predict the biological process where TUG1 may be involved in. At last, Cell Counting Kit-8 (CCK-8), colony formation and flow cytometry were conducted to explore the biological process that TUG1 may influence. Meanwhile, Western blot was performed to explore the mechanism of TUG1.

RESULTS: In this study, it was found that P73 antisense RNA 1T (TP73-AS1), LINC00654 and TUG1 had the tumor-promoting effect in the top 100 lncRNAs that were positively correlated with AIB1. The expression level of TUG1 was significantly decreased after intervention of AIB1. Then, the clinical data were analyzed and the results showed that TUG1 was related to the tumor residue, tumor staging, tumor grade and lymph node metastasis. Moreover, the bioinformatics analysis revealed that TUG1 was mainly involved in the regulation of cell cycle. After intervention in TUG1, it was found that the cell proliferation capacity was significantly decreased, and the cell cycle was arrested in G1 phase. Finally, Western blot revealed that the expressions of G1 phase-related proteins were significantly changed. This study indicated that AIB1 regulates the cycle of ovarian cancer cells through TUG1.

CONCLUSIONS: This study proved that AIB1 can regulate the cell cycle through regulating TUG1.

Key Words: Amplified in breast cancer 1 (AIB1), Taurine upregulated gene 1 (TUG1), Ovarian cancer.

Introduction

Epithelial ovarian cancer is one of the three major gynecologic malignant tumors. Although the incidence rate ranks third, the mortality rate ranks first in the gynecologic malignant tumor. In spite the symptoms of most patients can be significantly alleviated via cytoreductive surgery combined with paclitaxel and carboplatin (TP) chemotherapy, the tumor will relapse in the vast majority of patients. Thus, the 5-year survival rate is about 40%. Epithelial ovarian cancer is a kind of major disease affecting the women’s health, so the research on it is pivotal.

The amplification and high expression of proto-oncogenes play important roles in the occurrence and development of a variety of tumors. Amplified in breast cancer 1 (AIB1) (also known as SRC-3, ACTR, TRAM-1, RAC3, NCoA3 and P/CIP), a kind of transcriptional coactivator of steroid hormone receptors, is a newly-defined proto-oncogene. Besides, it is involved in many biological processes in the body, such as cell proliferation, differentiation and migration. A large number of studies have shown that the amplification of AIB1 or its protein overexpression is related to the occurrence and development of various tumors, such as breast cancer, prostate cancer, colon cancer, gastric cancer and pancreatic cancer, so it is studied the most in breast cancer. At present, there is little research on AIB1 in ovarian cancer, and the study on the effect of AIB1 is of clinical importance.

Long non-coding RNA (lncRNA) is a class of molecule with more than 200 bp in length but without the coding capacity. It was considered as the transcriptional noise in the past; however, in recent years, works have found that lncRNAs are involved in regulating the cell differentiation, proliferation and apoptosis. In particular, they play important roles in tumor, thus they have become the research hotspot recently. Some studies have shown that lncRNA urothelial cancer associated 1 (UCA1) can up-regulate the matrix metalloproteinase (MMP)-14 and enhance the epithelial ovarian cancer cell invasion through the competitive binding to miRNA-485-5p. It is reported that HOX transcript antisense RNA (HOTAIR) can lead to the increased ex-
pression of human epidermal growth factor receptor-2 (HER-2) in gastric cancer and enhance the tumor activity. It is also reported in some articles\(^6\) that HOXA transcript at the distal tip (HOTTIP) can participate in the biological processes of breast cancer, colorectal cancer and other tumors. However, there are few studies on the lncRNAs in the ovarian cancer. Therefore, the research on the role of lncRNAs in ovarian cancer can help us better to investigate the occurrence mechanism of ovarian cancer. Several researches have shown that lncRNA can be used as a mediator to regulate the biological processes, but there has been no report on its correlation with AIB1. In this paper, the mechanism of AIB1 in regulating the biological processes via lncRNA was clarified through The Cancer Genome Atlas (TCGA) database analysis and biological experiments.

**Materials and Methods**

**Data Collection**

The mRNA and clinical sample data of ovarian cancer were downloaded from TCGA web using the GDC tool. Data on 379 samples were obtained, including the age, tumor residue, International Federation of Gynecology and Obstetrics (FIGO) stage and lymph node metastasis. This investigation was approved by the Ethics Committee of Tengzhou Central People’s Hospital. Signed written informed consents were obtained from all participants before the study.

**Experimental Materials and Sources**

Epithelial ovarian cancer cells were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China); fetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI)-1640 culture solution were purchased from Gibco (Rockville, MD, USA); si-NC, si-AIB1 and si-TUG1 were purchased from RiboBio (Guangzhou, China); Total RNA extraction (TRIzol) kit, and Lipofectamine 2000 were purchased from In-vitrogen (Carlsbad, CA, USA); reverse transcription kit and Real-time quantitative polymerase chain reaction (PCR) kit were purchased from TaKaRa (Otsu, Shiga, Japan). Taurine upregulated gene 1 (TUG1); forward primer: 5’-CTGAAGAAAG-GCAACATC-3’; reverse primer: 5’-GCTCCGT-GAACCACTCG-3’; reverse primer: 5’-GACCTGC-CAAGGAACACTCT-3’; LINC00654: forward primer: 5’-AACTCCTGCCAGTTGTTTC-3’, reverse primer: 5’-TCACCTCCTGACACGAAT-3’; si-AIB1: 5’-GGACCGUUGUAACACAAGUTT-3’; si-NC: 5’-UUCUCCGAACGUUCAGUTT-3’; si-TUG1: 5’-CCCAGAAGUUGUAAGUUCACCUU- GA-3’; pl6, p21, cyclin-dependent kinase 2 (CDK2) and CDK4 were purchased from Cell Signaling Technology (CST, Danvers, MA, USA).

**Detection of Changes in Ovarian Cancer Cell Proliferation After Transfection Via Cell Counting Kit-8 (CCK-8) and Plate Clone Assay**

Cells were digested and collected at 24 h after transfection, and were inoculated into the 96-well plate (2×10^3/well). 100 μL single cell suspension was added into each well, and five control wells were set for each group. After 1-4 d, the detection was performed and the cell proliferative activity was detected by CCK-8 (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) after cell adherence. 2 h before detection, 10 μL CCK-8 solution were added into each well for incubation at 37°C for 2 h. The absorbance value of each well at the wavelength of 450 nm was measured using the microplate reader. Cells were digested and collected at 24 h after transfection, and 1000 cells were taken from each group and inoculated into the 60 mm dish. The medium was replaced for 4 d, followed by observation every other day. After 14 d, cells were fixed with paraformaldehyde (Gibco, Rockville, MD, USA) and stained with crystal violet (Solarbio, Beijing, China).

**Detection of Cell Cycle Via Flow Cytometry**

After transfection, the cells in each group were prepared into single cell suspension and fixed with 75% ethanol. Then, 200 μL RNase A (1 mg/mL) were added for incubation at 37°C for 30 min. After being mixed with 800 μL propidium iodide staining solution, cells were stained in a dark place at 4°C for 30 min (Dojindo Molecular Technologies Inc., Kumamoto, Japan). The cell cycle was detected using the FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The transfected cells were collected and the sediments were washed once with phosphate-buffered saline (PBS). Finally, the cell sediments were collected and using BD instrument.
Detection of the Protein Expression Level After Transfection Via Western Blot

The protein extraction buffer was added into the transfected ovarian cancer cells, and the cell lysis buffer was collected. After the protein concentration was measured, the loading amount of each well was adjusted. The protein was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Thermo Fisher Scientific, Waltham, MA, USA), followed by membrane transfer. Then, the membrane was placed in the 5% skim milk powder for sealing and it was added with anti-1 antibody for incubation (Cell Signaling Technology, Danvers, MA, USA). Finally, the membrane was washed, and the secondary antibody was incubated, followed by exposure.

Statistical Analysis

The correlation of mRNA was analyzed via Cor function. The experiment was repeated for three times. The measurement data were presented as mean ± standard deviation and Statistical Product and Service Solutions (SPSS) 22.0 (Version X; IBM, Armonk, NY, USA) was used for data statistics. t-test or x² test was used for the difference between the two groups, and Graphpad Prism (La Jolla, CA, USA) was used for drawing. All experiments were repeated three times. p<0.05 suggested that the difference was statistically significant.

Results

AIB1 was Positively Correlated with the IncRNA TUG1 Expression, and TUG1 was the Downstream Gene of AIB1

The raw RAN-seq vcount of ovarian cancer downloaded from TCGA database was converted using the logarithmic method. Next, first 100 IncRNAs that were positively correlated with AIB1 were calculated via Cor package. After searching PubMed, it was found that among the top 100 IncRNAs, TP73-AS1, LINC00654, and TUG1, are reported to have the tumor-promoting effect (Figure 1A-C). After intervention in AIB1, it was found that the expression level of TUG1 was decreased significantly, but TP73-AS1 and LINC00654 had no significant changes (Figure 1D).

Figure 1. TUG1 is a target gene of AIB1. A-C, The Pearson correlation of AIB1 and TP73-AS1, LINC00654 and TUG1. D, TUG1 was down-regulated significantly after AIB1 was silenced.
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Relationship Between TUG1 Expression Level and Clinical Data

According to the median of TUG1, patients were divided into high-expression group and low-expression group. Next, the X²-test was performed and it was found that the tumor residue, tumor grading, tumor staging and lymph node metastasis were significantly increased in high-expression TUG1 group, suggesting that TUG1 may be involved in regulating the malignant behavior of ovarian cancer (Table I).

TUG1 could be mainly involved in the regulation of cell cycle

The first 500 mRNAs that were positively and negatively related to AIB1 were obtained through the Cor function. These mRNAs received the gene ontology (GO) analysis and it was found that the 500 genes that were positively correlated with AIB1 were mainly enriched in the cell cycle regulation process, while the 500 genes that were negatively correlated with AIB1 were also enriched in the cell cycle regulation process (Figure 2A-B). After that, gene set enrichment analysis (GSEA) showed that the biological process of cell cycle was significantly activated in high- and low-expression TUG1 groups (Figure 2C), while Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that the cell cycle pathway was activated (Figure 2D), suggesting that TUG1 may regulate the cell cycle, thus affecting the biological behaviors of tumors.

Discussion

Ovarian cancer is one of the three common malignant tumors in the female reproductive system. Its early symptoms are not obvious and not easy to be detected, so 70% patients have been in the advanced stage when being treated. In particular, the incidence rate of epithelial ovarian cancer is the highest, accounting for about 90%. Many studies have shown that the abnormal expression of AIB1 protein is closely related to the occurrence and development of breast cancer, prostate cancer, etc. AIB1 is a kind of nuclear

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**Table I.** Association between TUG1 expression and clinicopathological characteristics of patients with EOC (n=379).

<table>
<thead>
<tr>
<th>Clinicopathologic features</th>
<th>Number of cases</th>
<th>TUG1 expression</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Low (n=189)</td>
<td>High (n=190)</td>
<td></td>
</tr>
<tr>
<td>Age (Year)</td>
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<td></td>
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</tr>
<tr>
<td>&lt;60</td>
<td>132</td>
<td>65</td>
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<tr>
<td>≥60</td>
<td>247</td>
<td>124</td>
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<td>Residual tumor</td>
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</tr>
<tr>
<td>No</td>
<td>318</td>
<td>168</td>
<td>0.012*</td>
</tr>
<tr>
<td>Yes</td>
<td>61</td>
<td>21</td>
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<td>FIGO stage</td>
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<tr>
<td>I–II</td>
<td>155</td>
<td>83</td>
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<tr>
<td>III–IV</td>
<td>224</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Histological grade</td>
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<tr>
<td>G1–G2</td>
<td>151</td>
<td>87</td>
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</tr>
<tr>
<td>G3</td>
<td>228</td>
<td>102</td>
<td></td>
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<tr>
<td>Lymph node metastasis</td>
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</tr>
<tr>
<td>Absent</td>
<td>131</td>
<td>78</td>
<td>0.040*</td>
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<tr>
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</table>

*p <0.05; **p<0.01; FIGO, International Federation of Gynecology and Obstetrics.
hormone receptor coactivator and widely exists in various malignant tumors. The AIB1 overexpression is found in a number of hormone-related tumors, such as ovarian cancer, breast cancer and endometrial cancer17,18, suggesting that AIB1 may be involved in the tumor formation through estrogen receptor. At the same time, the AIB1 protein overexpression is also found in some non-hormone-dependent tumors, such as esophageal squamous cell carcinoma19, bladder cancer20, colorectal cancer21, non-small cell lung cancer22, and ER-negative and progesterone receptor (PR)-negative breast cancer, ovarian cancer and prostate cancer23. Therefore, it is believed that AIB1 can also change the gene or protein expression levels, such as p5324, CK125 and MMPs26, through the interaction with many transcription factors, instead of the estrogen pathway, thus playing a role in the formation and progression of tumors.

TUG1 a kind of lncRNA with 7.1 kb in length, which was first found in the detection of the up-regulated genes in mouse retinal cells after taurine treatment27. Researches28,29 have shown that TUG1 can promote the cell proliferation in osteosarcoma, pancreatic cancer and other tumors, while some30 have revealed that TUG1 is down-regulated in non-small cell carcinoma and is an important regulator of p53. More-
over, some studies have shown that TUG1 can promote the ovarian cancer cell proliferation, inhibit the apoptosis and promote the invasion\(^5\), but there is no research on the effect of TUG1 on cell cycle. In this experiment, the correlation between AIB1 and IncRNA was analyzed. It was found in literature review that among the first 100 positively-correlated IncRNAs, TUG1, LINC00654 and TP73-AS1 had the tumor-promoting effect. After intervention in AIB1, the expression level of TUG1 was decreased significantly. Then, the clinical data were analyzed and the results revealed that TUG1 was related to the tumor residue, tumor staging, tumor grading and lymph node metastasis, indicating that TUG1 plays an important role in the progression of ovarian cancer. GO analysis performed for 500 mRNAs that had the strongest positive and negative correlations with AIB1 showed that the main biological pathway was mainly in regulating the cell cycle. Finally, GSEA analysis revealed that the genes were significantly enriched in the cell cycle-related pathways. After intervention in TUG1, the proliferation of ovarian cancer cells was significantly decreased. It was found in flow cytometry that the ovarian cancer cell cycle was mainly arrested in G1 phase after intervention in TUG1. In addition, Western blotting showed that the expressions of CDK2 and CDK4, the G1 phase-promoting proteins, were significantly decreased after TUG1 knockout, but the expressions of p16 and p21 were increased, suggesting that TUG1 may regulate the cell cycle through regulating the G1 phase-related proteins.

**Conclusions**

We showed that AIB1 can regulate the cell cycle through regulating TUG1 and provided an important basis for AIB1 as a therapeutic target of ovarian cancer.

**Conflict of Interest:**
The authors declared no conflict of interest.
References


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