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Highly expressed long non-coding RNA DUXAP10 promotes proliferation of ovarian cancer

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Abstract. – OBJECTIVE: To investigate the expression of long non-coding RNA DUXAP10 in ovarian cancer and its effect on ovarian cancer cell lines HO8910 and A2780 cells.

MATERIALS AND METHODS: Search the microarray dataset from the Gene Expression Omnibus (GEO) database using the keywords "ovarian cancer" and "GPL570". The differentially expressed genes in ovarian cancer tissues and normal ovarian tissues were analyzed by bioinformatics. Normal ovarian epithelial cells IOSE386, ovarian cancer HEY, HO8910 and A2780 cell lines were cultured. Cell proliferation assay was detected by CCK8 method and cloning formation assay was done. Quantitative Real-time polymerase chain reaction (qRT-PCR) was used to detect the expression of RNA.

RESULTS: The results showed that the expression of DUXAP10 in ovarian cancer tissues was significantly higher than that in normal ovarian tissues, which was consistent with those of TCGA. Clinical data analysis showed that the expression level of DUXAP10 was correlated with tumor size and FIGO stage in clinical patients. Compared with the negative control group, the proliferation ability and cell cloning ability of HO8910 cells overexpressing DUXAP10 were significantly increased ($p<0.001$), while the proliferation and cell cloning ability of A2780 cells interfering with DUXAP10 were significantly decreased ($p<0.001$), indicating that DUXAP10 played a role in promoting the proliferation of ovarian cancer cells.

CONCLUSIONS: DUXAP10 was significantly overexpressed in ovarian cancer tissues, and its expression was positively correlated with tumor size and FIGO stage in clinical patients. DUXAP10 promoted the proliferation of ovarian cancer cells and was expected to be a predictor and a potential therapeutic target of ovarian cancer.

Key Words:
Long non-coding RNA, Ovarian cancer, Proliferation, GEO database.

Introduction

Ovarian cancer is a common malignant tumor of the female reproductive system. The 5-year survival rate of ovarian cancer is related to tumor stage, ovarian cancer with early stage can be as high as 70% to 90%, while advanced ovarian cancer is only 20% to 30%[1]. Early symptoms of ovarian cancer are concealed and effective early screening methods are laced, so it is important to look for biomarkers of early screening, diagnosis and prognosis associated with ovarian cancer. Long-non-coding RNA (lncRNA) is an RNA molecule with a length of over 200 nucleotides and does not encode a protein. It has a complex biological function involved in chromatin remodeling, gene imprinting, cell cycle regulation, RNA splicing, degradation and translation of the regulation, thus affecting the body's physiological and pathological process[2]. With the in-depth study on lncRNA, it was found that lncRNA expression levels in tumor cells significantly altered, so the relationship between lncRNA and tumor naturally aroused people's attention[3,4]. A large number of studies have found that lncRNA expression in different tumor cells such as bladder cancer, lung cancer and ovarian cancer varies a lot, lncRNA can be used as a biological indicator of disease diagnosis and prediction[5-8]. A large number of studies have revealed that lncRNA is closely related to the development and progression of ovarian cancer. Gao et al[9] reported that HOST2 was involved in the regulation of migration, invasion and proliferation of epithelial ovarian cancer cells. The discovery of OVAL by Akrami et al[10] in ovarian cancer may be specifically targeted by chromosome copy number amplification in somatic cells and was involved in the development and progression of tumors. In addition, PVT1[11,12], MEG3[13-15], LSINCT5[16,17],

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H19[18] were all reported to be closely related to the development and progression of ovarian cancer. Although lncRNA has great potential in studying the development and progression of tumors, the mechanism of lncRNA is very complex and has not yet been studied.

Studies have reported that DUXAP10 was highly expressed in non-small cell lung cancer and promoted the proliferation of it[19]. There are also reports suggesting that *in vivo* and *in vitro* experiments have shown that DUXAP10 was a carcinogenic lncRNA with pseudogenic expression, which promoted tumorigenesis through epigenetic genes silencing expressions of P21 and PTEN[20]. However, there were no reports on DUXAP10 in ovarian cancer. In this study, we first downloaded the common microarray (GSE40595 and GSE38666) from the GEO database. Through comprehensive analysis, we found that the expression level of DUXAP10 in ovarian cancer tissues was significantly higher than that in normal ovarian tissues. At present, there is no study of DUXAP10 in ovarian cancer. Therefore, the study suggested an important role of DUXAP10 in the development and progression of ovarian cancer.

Materials and Methods

Expression of lncRNA DUXAP10

The common ovarian cancer microarray datasets (GSE40595 and GSE38666) were downloaded from the GEO database, all primary data were from the GEO database. The probe was re-annotated with blast + 2.2.30, and a probe corresponding to multiple genes was removed. Maximum homogeneous signal is used to deal with the case where multiple probes correspond to one gene. The differential genes in the microarray datasets (GSE40595 and GSE38666) were calculated using the Limma function.

Cell Culture and siRNA, Plasmid Transfection

Normal ovarian epithelial cells IOSE386, ovarian cancer HEY, HO8910 and A2780 cell lines were cultured in RPMI 1640 medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (HyClone, South Logan, UT, USA), cells were cultured according to conventional cell culture methods in a sterile table, cells were placed in 37°C, 5% CO2 incubator. The cells were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at a cell density of about 80% in the 6-well plates, 5 μL Lipofectamine 2000 and 5 μL si-DUXAP10/4 μL Lipofectamine2000 and 2ug pcDNA-DUXAP10 were dissolved in 500 μL 1640 medium (Gibco, Rockville, MD, USA), the control group was added the same amount of Lipofectamine2000 and si-NC/pcDNA-NC as the experimental group, culture medium was replaced after transfection for 6 h. The sequence of si-DUXAP10 1 # was GGAACUUCCCAAACCUCCAUGAUUU, the sequence ofsi-DUXAP10 2 # was CAGCAUACUUCAAAUUCACAGCAAA.

RNA extraction and Quantitative Real-time polymerase Chain Reaction (qRT-PCR)

After 24 hours of transfection, the total RNA was extracted by 1 mL TRIzol. 1% agarose gel electrophoresis was used to detect the integrity of the RNA sample. The UV spectrophotometer was quantified and the cDNA was reverse transcript according to the instructions of the reverse transcription Kit (TaKaRa, Dalian, China). qRT-PCR was used to detect the expression level of DUXAP10, the reaction system was 5 μL, the qPCR conditions: 95°C 2 min; 95°C 10 s, 56°C 10 s, 68°C 12 s, 40 cycles; 95°C 1 min; 55°C 1 min; 70°C 6 s; 70°C 6 s; GAPDH gene was considered as the loading control, the upstream primer was GAPDH-F: 5'-CCCACTCCTCCACCTTTGAC-3', and the downstream primer was GAPDH-R: 5'-GGATCTCGCTCCTGGAAGATG-3'. PCR conditions were as the above, the measurements were repeated in triplicate.

Cell Proliferation Assay by CCK8 Method

The transfection time point was 0 h, medium was changed 6 h later, cells were inoculated into 96-well plates at a density of 2×10^3/100 μL at 24 h and cell counting kit-8 (CCK8) assay was performed after cultured for 24, 48, 72 and 96 h. The serum-free medium was replaced at the time of detection. 10 μL of CCK8 was added to each well. After incubation at 37°C and 5% CO_2 for 1 h, the OD value was measured at 450 nm. Each measurement was performed in quintuplicate.

Cloning Formation Assay

The transfection time point was 0 h, medium was changed 6 h later, cells were inoculated into the medium plate at a density of 3×10^3/100 μL at 24 h in an incubator at 37°C, 5% CO_2, medium was replaced every 2 d and the culture was terminated after 14 d. Medium was removed and cells

were washed with the phosphate buffered saline (PBS) twice, fixed with 5% paraformaldehyde for 30 min, remaining liquid was removed, add 1 mL of 0.1% crystal violet solution per well, removed the crystal violet solution after 30 min, cells were washed until the solution was clear with PBS, we counted the visible colonies.

Statistical Analysis

Statistic Package for Social Science (SPSS) 22.0 statistical software (IBM, Armonk, NY USA) was used for data analysis, GraphPad Prism 5.0 (Version X; La Jolla, CA, USA) was used for picture editing. Measurement data were compared with *t* test and presented as mean ± standard deviation ($\bar{x} \pm s$), categorical data were compared with x^2 test. $p<0.05$ indicated significant difference; $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$.

Results

Relationship Between Expression of DUXAP10 in Ovarian Tissue of Ovarian Cancer and Clinical Data

Two microarray datasets (GSE40595 and GSE38666) were calculated by the Limma function, and found that DEXAP10 was highly expressed in ovarian tissue of patients with ovarian cancer compared with normal ovarian tissues (Figure 1B, $p<0.001$ and Figure 1C, $p<0.01$). We then selected 60 ovarian cancer tissues of patients with ovarian cancer and 38 normal tissues to perform qRT-PCR to detect the expression of DUXAP10. The results further confirmed that DUXAP10 was highly expressed in ovarian cancer tissues (Figure 2A, $p<0.001$). The clinical data of patients were analyzed and found that the overall survival of patients with ovarian cancer whose DCEAP10 was overexpressed was significantly lower than that in the DUXAP10 low expression group (Figure 2B). In clinical data analysis, expression of DUXAP10 increased in patients with advanced tumors and large tumor volumes (Figure 2C and Figure 2D). To investigate the correlation between DUXAP10 expression and clinical data, patients were divided into high expression group and low expression group based on the median expression of DUXAP10. x^2-test results showed that tumor stage in DUXAP10 high expression group was higher, and the tumor volume was larger. However, the expression of DUXAP10 was not associated with age, tissue subtype, tissue typing, lymphatic metastasis and distant metastasis (Table I).

Cell Lines Screening

The normal cell line IOSE-386 was used as the control cell line, the total RNA was extracted from cell lines (IOSE-386, HO8910, HEY, A2780) and then the relative expression of DUXAP10 was de-

Table I. Correlation between expression of lncRNA DUXAP10 and clinicopathological features in patients with ovarian cancer (n = 60).

Clinicopathologic features	Number of cases	DUXAP10 expression		*p*-value
		Low (n=30)	High (n=30)	
Age (years)				0.4383
< 50	31	14	17	
≥ 50	29	16	13	
Histologicalsubtype				0.3711
Serous	44	24	21	
Others	16	6	9	
Tumor size				0.0321*
<8 CM	38	23	15	
≥ 8 CM	22	7	15	
FIGO stage				0.0006*
I-II	27	25	12	
III-IV	33	5	18	
Histological grade				0.5921
G1-G2	38	20	18	
G3	22	10	12	
Lymph node metastasis				0.5839
Absent	40	21	19	
Present	20	9	11	

$^{*}p<0.05$

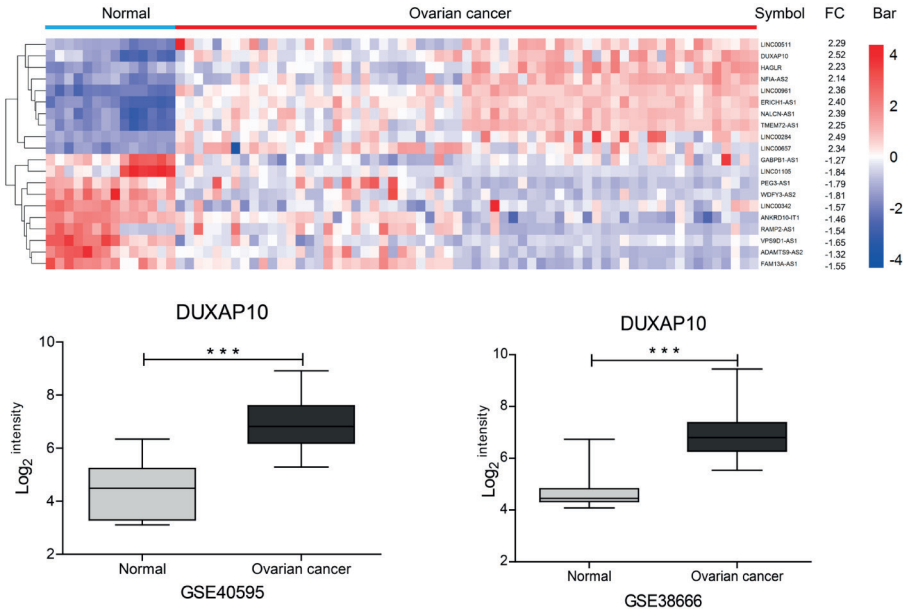

Figure 1. Long non-coding RNA DUXAP10 was highly expressed in ovarian cancer. *A,* Differential expressions in ovarian cancer tissue and normal ovarian tissue. ***B,*** Analysis in the GSE40595 data set showed that DUXAP10 was highly expressed in ovarian cancer tissues. ***C,*** Analysis in the GSE38666 data set showed that DUXAP10 was highly expressed in ovarian cancer tissues.

tected by qRT-PCR. Results were shown in Figure 3A, the expression level of DUXAP10 was slightly lower in HO8910 cell line and was significantly higher in A2780 cell line. Therefore, HO8910 cell line was selected for subsequent overexpression experiment and A2780 cell line was used for knockout experiment. Corresponding plasmid and interference RNA were constructed and transferred to the corresponding ovarian cancer cell lines, transfection results were shown in Figure 3B, 3C.

Effects of DUXAP10 on the Proliferation and Cloning of HO8910 Cells After Overexpression

CCK8 results showed that D450 increased with pcDNA-DUXAP10 transfected with HO8910 cells compared with negative control transfected with pcDNA-NC, which indicated that overexpression of DUXAP10 could promote the proliferation of HO8910 cells, the difference was statistically significant (Figure 3D). Cloning formation assay results showed that the number of clones formed by HO8910 cells in the normal control (pcDNA-NC) group was much less than that in overexpression (pcDNA-DUXAP10). This indicated that the ability of a single cell to form a cell mass was enhanced after DUXAP10 overexpression in HO8910 cells (Figure 3E). Cloning formation assay and CCK8 assay for detecting cell proliferation showed that DUXAP10 could promote the proliferation of ovarian cancer cells.

Inhibitory Effect of DUXAP10 Knockdown on the Proliferation and Cloning ability of A2780 Cells

CCK8 results showed that D450 decreased as si-DUXAP10 transfected with A2780 cells compared with negative control transfected with si-NC, which indicated that knockdown of DUXAP10 inhibited the proliferation of A2780 cells, the difference was statistically significant (Figure 3F). The cloning formation results showed that the number of clones formed by A2780 cells in the normal control (si-NC) group was much larger than that in the overexpression group (si-DUXAP10). This suggested that the ability of a single cell to form a cell mass was reduced after DUXAP10 in A2780 cells was knocked down (Figure 3G). This further demonstrated that LncRNA DUXAP10 could promote the proliferation of ovarian cancer cells.

Discussion

Ovarian cancer is one of the three major malignant tumors of female reproductive system, of which epithelial tumor is the most common one,

accounting for 85% to 90% of ovarian malignancies. Incidence of ovarian cancer is hidden, without obvious symptoms and signs in early stage, effective screening and diagnostic methods are lacked, which has seriously threatened the majority of women's health and life[21]. Despite the efforts made in clinical and basic research, the overall survival of ovarian cancer has not improved significantly. The occurrence and development of ovarian cancer is a multi-step, multi-factor, the pathological mechanism is not yet fully clear. It is an important clinical prospect to find a new target for early diagnosis, treatment and prognosis of ovarian cancer. Long non-coding RNAs have become a hot topic in many tumor researches, and play an important role in the development of tumor. LncRNAs are a class of RNA molecules whose transcript is over 200 nt, and they are initially considered to be "noises" of genomic transcription because of their lack of open reading frames without coding protein potential, a by-product of RNA polymerase II transcription without biological function[22]. However, several studies have shown that lncRNA was involved in genotypic markers, chromatin modification, transcriptional activation, post-transcriptional regulation and other multidimensional regulation, and its transcription and dysfunction were involved in tumorigenesis[23]. Some known lncRNAs, such as HOST2[9], OVAAL[10], HOTAIR[24-27], LSINCT5[16,27], MEG3[13-15], H19[28], Xist[29] were reported in large numbers of literatures which played important roles in the ovarian cancer. Research found that HOST2 was involved in the regulation of epithelial ovarian cancer cell migration, invasion and proliferation process. Further mechanism studies have shown that HOST2 was a "bait" of lncRNA; it had binding site of miRNA let-7b, which can capture miRNA let-7b and antagonized its function. MiRNAlet-7b can induce silence of target genes at post-transcriptional levels by mRNA degradation due to complementary pairing with target mRNA[9]. Overexpression of HOTAIR can increase expressions of epithelial cell mesenchymal transition, cell metastasis-related genes, thus increasing the invasive ability of ovarian cancer cells, low expression of HOTAIR can up-regulate the cell cycle regulation and expressions of

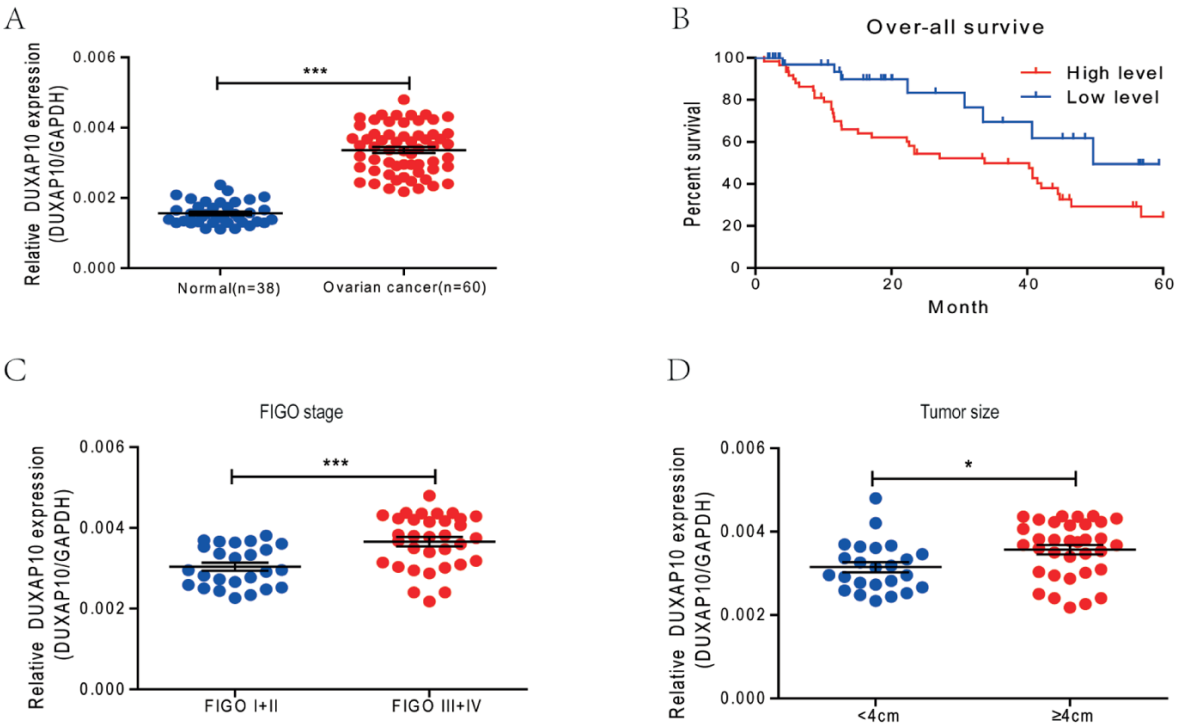

Figure 2. Long non-coding RNA DUXAP10 in clinical samples. ***A,*** Expression of lncRNA DUXAP10 was significantly higher in 60 ovarian cancer tissues than in 38 normal ovarian tissues. ***B,*** The overall survival rate of DUXAP10 overexpressing ovarian cancer patients was significantly lower than that of DUXAP10 low expression group. ***C,*** The expression level of DUXAP10 was positively correlated with FIGO staging. ***D,*** The expression level of DUXAP10 was positively correlated with tumor size.

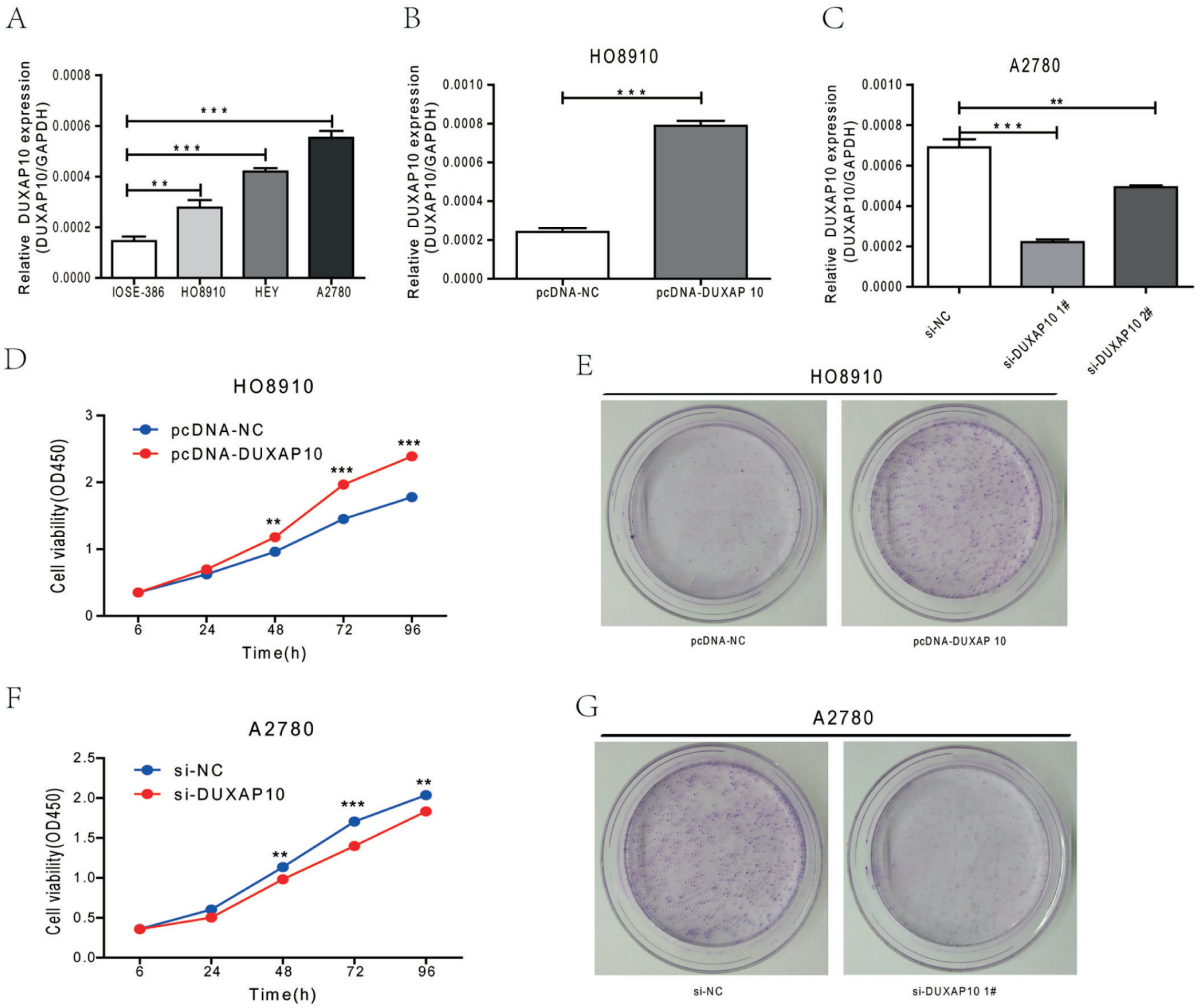

Figure 3. Long non-coding RNA DUXAP10 promoted ovarian cancer cell proliferation. ***A,*** Expression of lncRNA DUXAP10 in ovarian normal cells and cancer cell lines (IOSE-386, HO8910, HEY, A2780). ***B,*** Transfection of pcDNA-DUXAP10 in HO8910. **C,** Interference efficiency of si-DUXAP10 in A2780 cells. ***D,*** CCK8 assay results showed that overexpression of DUXAP10 in HO8910 cells promoted cell viability. ***E,*** Overexpression of DUXAP10 in HO8910 cells increased cloning ability. ***F,*** CCK8 assay results showed that knockdown of DUXAP10 inhibited the cell viability of A2780 cells. ***G,*** Knockdown of DUXAP10 in A2780 cells reduced the ability of cells cloning.

apoptosis-related genes, thus inducing cell cycle arrest and apoptosis, as well as the proliferation, migration and invasion of ovarian cancer cells *in vitro*[30]. Guan et al[31] found that PVT1 expression in ovarian cancer was upregulated, and can promote cell proliferation and inhibit cell apoptosis; inhibition of PVT1 overexpression by siRNA can promote cell apoptosis and inhibit cell proliferation; in addition, ovarian cancer cells also found that PVT1 overexpression can antagonize chemokines induced by paclitaxel-induced apoptosis. Sheng et al[32] found that MEG3 was significantly lower in epithelial ovarian cancer tissues and cells, whereas overexpression of MEG3 inhibited the growth and proliferation of OVCAR3 cells *in vitro* and *in vivo* and promoted apoptosis. In summary, lncRNA involved in tumor formation, metastasis and other links. DUXAP10 have been reported in bladder cancer, colorectal cancer and other tumors, but its relationship with ovarian cancer has not been studied. This paper was based on the data downloaded from the GEO database (GSE40595 and GSE38666). The results showed that the expression of lncRNA DUXAP10 in ovarian cancer was significantly higher than that in normal ovarian tissue. TCGA data analysis results were consistent. In order to further explore the functional significance of DUXAP10 in ovarian cancer,

the differential expressions of DUXAP10 in 60 ovarian cancer tissues and 38 normal ovarian tissues were detected by qRT-PCR. The results were consistent with the above analysis. This suggested that DUXAP10 may act as a cellulase lncRNA involved in the pathophysiology of organelles. The clinical data of the patients were analyzed and the results of chi-square test showed that the expression of DUXAP10 was related to tumor size and staging of ovarian cancer. To further study whether DUXAP10 was involved in the proliferation of ovarian cancer in the development of ovarian cancer, proliferation and cell cloning ability in ovarian cancer HO8910 cells were significantly increased ($p<0.001$) when DUXAP10 expression was up-regulated in the cell model, and interfered DUXAP10 expression, the proliferation and cell cloning ability of ovarian cancer A2780 cells were significantly decreased ($p<0.001$), indicating that DUXAP10 may play an important role in the proliferation of ovarian cancer. Compared with the negative control group, the proliferation ability and cell cloning ability of HO8910 cells overexpressing lncRNA DUXAP10 were significantly decreased ($p<0.001$). After the expression of DUXAP10 was interfered, the result was opposite, which indicated that overexpression of DUXAP10 played a role in promoting the proliferation of ovarian cancer cells.

Conclusions

We showed that DUXAP10 expression in ovarian cancer was upregulated, which was positively associated with tumor size, FIGO stage in clinical patients, overexpression of DUXAP10 can significantly promote tumor cell proliferation, interfered DUXAP10 can significantly inhibit the proliferation of tumor cells, which indicated that DUXAP10 played an important role in the development of ovarian cancer. It can be used as a molecular marker and potential therapeutic target for predicting prognosis of ovarian cancer; however, its transcriptional regulation mechanism still needs further investigations.

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Conflict of Interest

The Authors declare that they have no conflict of interest.

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