LncRNA PAXIP1-AS1 is a Prognostic Biomarker and Correlated with Immune Infiltrates in Ovarian Cancer

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Abstract

Background: The long non-coding RNA (LncRNA) PAXIP1 antisense RNA 1 (PAXIP1-AS1) was found to promote proliferation, migration, EMT, and apoptosis of ovarian cancer (OC) cells in OC cell lines, but the relationship between PAXIP1-AS1 expression and clinical characteristics, prognosis, and immune infiltration of OC patients and its regulatory network are unclear.

Methods: QRT-PCR, Kruskal-Wallis test, Wilcoxon sign-rank test, logistic regression, Kaplan-Meier method, Cox regression analysis, Gene set enrichment analysis (GSEA), and immuno-infiltration analysis were used to evaluate the relationship between clinical characteristics and PAXIP1-AS1 expression, prognostic factors, and determine the significant involvement of PAXIP1-AS1 in function.

Results: Low PAXIP1-AS1 expression in OC was associated with age (P=0.045), histological grade (P=0.011), and lymphatic invasion (P=0.004). Low PAXIP1-AS1 expression predicted a poorer overall survival (OS) (HR: 0.71; 95% CI: 0.55–0.92; P=0.009), progression free interval (PFS) (HR: 1.776; 95% CI: 1.067–2.955; P=0.001) and disease specific survival (DSS) (HR: 0.67; 95% CI: 0.51–0.89; P=0.006). And PAXIP1-AS1 expression (HR: 0.711; 95% CI: 0.542-0.934; P=0.014) was independently correlated with PFS in OC patients. GSEA demonstrated that neutrophil degranulation, signaling by Interleukins, GPCR-ligand binding, G alpha I signaling events, VEGFVEGFR-2 signaling pathway, naba secreted factors, Class A 1 Rhodopsin-Like Receptors, PI3K-Akt signaling pathway, and Focal Adhesion-PI3K-Akt-mTOR-signaling pathway were differentially enriched in PAXIP1-AS1 high expression phenotype. PAXIP1-AS1 may inhibit the function of aDC, B cells, CD8 T cells, Cytotoxic cells, DC, iDC, Macrophages, Mast cells, Neutrophils, NK CD56dim cells, T cells, TFH, Tgd, Th1 cells, Th2 cells and Treg.

Conclusions: Low expression of PAXIP1-AS1 was significantly associated with poor survival and immune infiltration in OC. PAXIP1-AS1 could be a promising prognosis biomarker for OC.

Introduction

Ovarian cancer (OC) is one of the most deadly malignancies in the female reproductive system [1]. Nearly 295,000 women worldwide have been diagnosed with OC and 185,000 have died from the disease [2]. Over 70% of OC patients are diagnosed at an advanced stage (FIGO stage III or IV) due to nonspecific symptoms in the early stages and the lack of effective screening methods [3]. The 5-year survival rate for stage III-IV patients is approximately 30% and for stage I patients is approximately 92% [4]. Despite considerable progress in some of these areas, the treatment of this tumor remains a major challenge in gynaecological oncology, with little change in long-term survival rates for HGSOC and several issues hindering progress in clinical outcomes.

Long noncoding RNAs (lncRNAs) comprise a class of RNA transcripts >200 nucleotides in length that act as key regulators of target gene expression in a variety of biological processes including chromatin modification, gene transcription, RNA splicing, RNA transport and translation [5]. Aberrant lncRNA expression may be critical for cancer development and progression, and lncRNA-mediated biology may
be central in cancer development [6]. Unlike miRNAs and protein-encoding mRNAs, IncRNAs typically
display restricted tissue-specific and cancer-specific expression patterns [7]. Furthermore, their expression
is lower than that of protein-coding genes [8]. Given this IncRNA tissue specificity, they may be superior
biomarkers to many current protein-coding biomarkers [9]. Therefore, screening for molecular markers
associated with OC prognosis is important for the precise treatment of OC.

Changes in the expression of some IncRNAs have been reported in OC and in association with clinical
characteristics [10, 11]. Silencing of the IncRNA PAXIP1-AS1, a key mediator of cell death, was found to
contribute to cell survival [12]. Aberrant expression of the IncRNA PAXIP1-AS1 was evident in glioma cells
and tissues and was significantly associated with survival outcomes in glioma patients [13]. H3K27ac-
inducible IncRNA PAXIP1-AS1 promotes cell proliferation, migration, EMT, and apoptosis by targeting the
miR-6744-5p/PCBP2 axis in OC [14]. However, the relationship between PAXIP1-AS1 expression and
clinical characteristics, prognosis, and immune infiltration of OC patients and its regulatory network has
not been studied.

This study compared PAXIP1-AS1 expression differences between tumor tissues and normal samples
based on The Cancer Genome Atlas (TCGA) database and OC RNA-seq data in GTEx, and assessed the
correlation between PAXIP1-AS1 expression levels and clinical features of OC, and the prognostic value of
PAXIP1-AS1 in OC. Genomic enrichment analysis (GSEA) was performed on the high and low PAXIP1-AS1
expression groups to reveal their possible functions. Correlation analysis between PAXIP1-AS1 expression
and immune infiltration was performed to explore the potential mechanisms by which PAXIP1-AS1
regulates OC occurrence and development. This study provided a new direction for the individualized and
precise treatment of OC.

Materials And Methods

Differential expression of PAXIP1-AS1

Unpaired samples analysis was carried out according to the references [15–17]. Molecules: PAXIP1-
AS1[ENSG00000273344].

Clinical Information

The analysis was carried out according to the references [15, 16]. Molecule: PAXIP1-AS1. Subgroup:
Median.

The relationship between PAXIP1-AS1 and clinical characteristics

Correlation analysis of gene expression with clinical characteristics was carried out according to the
references [15, 16]. Molecule: PAXIP1-AS1. Clinical variables: age, histological grade, and lymphatic
invasion.
Logistics analysis was carried out according to the references [15, 16]. Dependent variable: PAXIP1-AS1.

**The relationship between PAXIP1-AS1 and prognosis**

Kaplan-Meier method analysis was carried out according to the literatures [16, 18]. Molecule: PAXIP1-AS1. Subgroups: 0-50 vs 50-100. Prognosis type: overall survival (OS), progression free interval (PFS), and disease specific survival (DSS).

COX regression analysis was carried out according to the literatures [16, 18]. Prognosis type: PFS. Included variables: clinical characteristics and PAXIP1-AS1.

Forest plot. Software: R (version 3.6.3). R package: ggplot2 package.

Nomogram plot analysis was carried out according to the literatures [16, 18]. R package: rms package & survival package. Prognosis type: PFS. Included variables: FIGO stage; Primary therapy outcome; Tumor residual; PAXIP1-AS1.

**Gene set enrichment analysis (GSEA)**

Single gene differential analysis was carried out according to the references [16, 19]. Molecule: PAXIP1-AS1. Low expression group: 0-50%. High expression group: 50-100%.

GSEA analysis was carried out according to the references [16, 20, 21].

**Immune infiltration analysis by ssGSEA**

The analysis was carried out according to the literatures [15, 16, 22, 23]. Molecule: PAXIP1-AS1.

**QRT-PCR**

Tumor tissue and normal ovarian tissue samples were collected from 8 OC patients at the Obstetrics and Gynaecology Department of the Affiliated Hospital of Xuzhou Medical University. The study was approved by the Ethics Committee of the Affiliated Hospital of Xuzhou Medical University. All patients signed a written informed consent form. The clinical characteristics of the 8 OC patients were listed as shown in Table 1. PAXIP1-AS1 expression levels were identified in 8 OC tissue samples by qRT-PCR. The specific steps were performed according to the reference [24]. The primer sequences used are shown in Table 2.
Table 1
The clinical characteristics of the patients in this study.

| No. | Age | History                        | Stage | Grade |
|-----|-----|--------------------------------|-------|-------|
| 1   | 57  | High grade Serous ovarian cancer | IIIC  | 3     |
| 2   | 49  | High grade Serous ovarian cancer | IIIC  | 3     |
| 3   | 68  | High grade Serous ovarian cancer | IIIC  | 3     |
| 4   | 61  | High grade Serous ovarian cancer | IIIC  | 3     |
| 5   | 56  | High grade Serous ovarian cancer | IICc  | 3     |
| 6   | 49  | High grade Serous ovarian cancer | IIB   | 3     |
| 7   | 78  | High grade Serous ovarian cancer | IIC   | 3     |
| 8   | 66  | High grade Serous ovarian cancer | IIC   | 3     |

Table 2
The sequence of primers in the present study.

| Gene      | The sequence of primers (5’-3’) |
|-----------|----------------------------------|
| PAXIP1-AS1| Forward: GAAGTTGGGAGAAGAAAT      |
|           | Reverse: AGTGTACCGCAGAGTAAT      |
| U6        | Forward: CTCGCTTCGGCAGCACA       |
|           | Reverse: AACGCTTCACGAATTTGCGT     |

Results

Clinical characteristics

As shown in Table 3, the FIGO stage included 1 patient (0.3%), stage II in 23 (6.1%), stage III in 295 (78.5%), and stage IV in 57 (15.2%). The primary therapy outcome included 27 PD (8.8%), 22 SD (7.1%), 43 PR (14%), and 216 CR (70.1%). The race included 328 white patients, 12 Asian patients, and 25 Black or African American patients. The age included 208 patients (<=60, 54.9%) and 171 patients (>60, 45.1%). The histologic grade included 1 G1 (0.3%), 45 G2 (12.2%), 322 G3 (87.3%), and 1 G4 (0.3%). The anatomic neoplasm subdivision 102 included unilateral (28.6%) and 255 bilateral (71.4%). The venous invasion included 64 yes (61%) and 41 no (39%). The lymphatic invasion included 101 yes (67.8%) and 48 no (32.2%). The tumor residual included 67 NRD (20%) and 268 RD (80%). The age range was 51 to 68 years, with a median of 59 years.
Table 3
Clinical characteristics of patients with OC (TCGA).

| Characteristic                                      | levels     | Overall |
|-----------------------------------------------------|------------|---------|
| n                                                   |            | 379     |
| FIGO stage, n (%)                                   |            |         |
| Stage I                                             | 1 (0.3%)   |         |
| Stage II                                            | 23 (6.1%)  |         |
| Stage III                                           | 295 (78.5%)|         |
| Stage IV                                            | 57 (15.2%) |         |
| Primary therapy outcome, n (%)                      |            |         |
| PD                                                  | 27 (8.8%)  |         |
| SD                                                  | 22 (7.1%)  |         |
| PR                                                  | 43 (14%)   |         |
| CR                                                  | 216 (70.1%)|         |
| Race, n (%)                                         |            |         |
| Asian                                               | 12 (3.3%)  |         |
| Black or African American                           | 25 (6.8%)  |         |
| White                                               | 328 (89.9%)|         |
| Age, n (%)                                          |            |         |
| <=60                                                | 208 (54.9%)|         |
| >60                                                 | 171 (45.1%)|         |
| Histologic grade, n (%)                             |            |         |
| G1                                                  | 1 (0.3%)   |         |
| G2                                                  | 45 (12.2%) |         |
| G3                                                  | 322 (87.3%)|         |
| G4                                                  | 1 (0.3%)   |         |
| Anatomic neoplasm subdivision, n (%)                |            |         |
| Unilateral                                          | 102 (28.6%)|         |
| Bilateral                                           | 255 (71.4%)|         |
| Venous invasion, n (%)                              |            |         |
| No                                                  | 41 (39%)   |         |
| Yes                                                 | 64 (61%)   |         |
| Lymphatic invasion, n (%)                           |            |         |
| No                                                  | 48 (32.2%) |         |
| Yes                                                 | 101 (67.8%)|         |
| Tumor residual, n (%)                               |            |         |
| NRD                                                 | 67 (20%)   |         |
| RD                                                  | 268 (80%)  |         |
| Age, median (IQR)                                   |            | 59 (51, 68) |
PAXIP1-AS1 expression correlated with poor clinical characteristics of OC

PAXIP1-AS1 was low expressed in OC tissues (3.003±0.034 vs. 3.126±0.046, P=0.032), based on 427 OC tissues and 88 normal ovarian tissues of GTEx combined TCGA database (Figure 1A). The expression of PAXIP1-AS1 in OC were significantly lower than that in paired normal tissues (0.612±0.138 vs. 1.538±0.179, P<0.001) (Figure 1B).

The characteristics of OC patients were shown in Table 4, clinical and gene expression data were collected from TCGA database. According to the mean value of relative PAXIP1-AS1 expression, the patients with OC were divided into high (n=190) and low (n=189) expression groups. PAXIP1-AS1 expression was associated with age (P=0.002), histological grade (P=0.007), and lymphatic invasion (P=0.007). As shown in Figure 2 and Table 5, PAXIP1-AS1 was significantly related to age (P=0.045), histological grade (P=0.011), and lymphatic invasion (P=0.004).
Table 4
Correlation between PAXIP1-AS1 expression and clinical characteristics in OC.

| Characteristic                        | Low expression of PAXIP1-AS1 | High expression of PAXIP1-AS1 | P value |
|--------------------------------------|------------------------------|-------------------------------|---------|
| n                                    | 189                          | 190                           | 0.562   |
| FIGO stage, n (%)                    |                              |                               |         |
| Stage I                              | 0 (0%)                       | 1 (0.3%)                      |         |
| Stage II                             | 9 (2.4%)                     | 14 (3.7%)                     |         |
| Stage III                            | 148 (39.4%)                  | 147 (39.1%)                   |         |
| Stage IV                             | 30 (8%)                      | 27 (7.2%)                     |         |
| Primary therapy outcome, n (%)       |                              |                               | 0.409   |
| PD                                   | 14 (4.5%)                    | 13 (4.2%)                     |         |
| SD                                   | 12 (3.9%)                    | 10 (3.2%)                     |         |
| PR                                   | 25 (8.1%)                    | 18 (5.8%)                     |         |
| CR                                   | 98 (31.8%)                   | 118 (38.3%)                   |         |
| Race, n (%)                          |                              |                               | 0.177   |
| Asian                                | 4 (1.1%)                     | 8 (2.2%)                      |         |
| Black or African American            | 9 (2.5%)                     | 16 (4.4%)                     |         |
| White                                | 168 (46%)                    | 160 (43.8%)                   |         |
| Age, n (%)                           |                              |                               | 0.057   |
| <=60                                 | 94 (24.8%)                   | 114 (30.1%)                   |         |
| >60                                  | 95 (25.1%)                   | 76 (20.1%)                    |         |
| Histologic grade, n (%)              |                              |                               | 0.007   |
| G1                                   | 1 (0.3%)                     | 0 (0%)                        |         |
| G2                                   | 14 (3.8%)                    | 31 (8.4%)                     |         |
| G3                                   | 170 (46.1%)                  | 152 (41.2%)                   |         |
| G4                                   | 1 (0.3%)                     | 0 (0%)                        |         |
| Anatomic neoplasm subdivision, n (%) |                              |                               | 0.120   |
| Unilateral                           | 58 (16.2%)                   | 44 (12.3%)                    |         |
| Bilateral                            | 120 (33.6%)                  | 135 (37.8%)                   |         |
| Characteristic                          | Low expression of PAXIP1-AS1 | High expression of PAXIP1-AS1 | P value |
|----------------------------------------|------------------------------|-------------------------------|---------|
| Venous invasion, n (%)                 |                              |                               | 0.107   |
| No                                     | 15 (14.3%)                   | 26 (24.8%)                    |         |
| Yes                                    | 35 (33.3%)                   | 29 (27.6%)                    |         |
| Lymphatic invasion, n (%)              |                              |                               | 0.007   |
| No                                     | 15 (10.1%)                   | 33 (22.1%)                    |         |
| Yes                                    | 57 (38.3%)                   | 44 (29.5%)                    |         |
| Tumor residual, n (%)                  |                              |                               | 0.107   |
| NRD                                    | 26 (7.8%)                    | 41 (12.2%)                    |         |
| RD                                     | 136 (40.6%)                  | 132 (39.4%)                   |         |
| Age, median (IQR)                      | 61 (53, 71)                  | 57 (48.25, 66)                | 0.002   |

Table 5
PAXIP1-AS1 expression associated with clinical characteristics (logistic regression).

| Characteristics                              | Total (N) | Odds Ratio (OR) | P value |
|----------------------------------------------|-----------|-----------------|---------|
| FIGO stage (Stage III & Stage IV vs. Stage I & Stage II) | 376       | 0.587 (0.241-1.353) | 0.220   |
| Primary therapy outcome (CR vs. PD&SD&PR)     | 308       | 1.498 (0.918-2.455) | 0.107   |
| Race (White vs. Asian & Black or African American) | 365       | 0.516 (0.247-1.033) | 0.067   |
| Age (>60 vs. <=60)                           | 379       | 0.660 (0.438-0.990) | 0.045   |
| Histological grade (G3 & G4 vs. G1 & G2)     | 369       | 0.430 (0.218-0.815) | 0.011   |
| Anatomic neoplasm subdivision (Bilateral vs. Unilateral) | 357       | 1.483 (0.935-2.364) | 0.095   |
| Venous invasion (Yes vs. No)                 | 105       | 0.478 (0.211-1.058) | 0.072   |
| Lymphatic invasion (Yes vs. No)              | 149       | 0.351 (0.166-0.715) | 0.005   |
| Tumor residual (RD vs. NRD)                  | 335       | 0.615 (0.353-1.057) | 0.082   |
Relationship between PAXIP1-AS1 and survival of HCC patients

As shown in Figure 3, expression of PAXIP1-AS1 was positively correlated with poor OS (HR: 0.71; 95% CI: 0.55–0.92; P=0.009), PFS (HR: 1.776; 95% CI: 1.067–2.955; P=0.001), and DSS (HR: 0.67; 95% CI: 0.51–0.89; P=0.006) of OC patients. As shown in Table 6 and Figure 4, the results of univariate analysis showed low PAXIP1-AS1 expression levels were associated with worse PFS (HR: 1.776; 95% CI: 1.067–2.955; P=0.001), primary therapy outcome (HR: 0.401; 95% CI: 0.304-0.528; P<0.001), and tumor residual (HR: 1.695; 95% CI: 1.219-2.358; P=0.002). The result of multivariate analysis showed that PAXIP1-AS1 expression (HR: 0.711; 95% CI: 0.542-0.934; P=0.014) and primary therapy outcome (HR: 0.496; 95% CI: 0.369-0.667; P<0.001) were independently correlated with PFS in multivariate analysis (Table 6). The results suggested that decreased expression of PAXIP1-AS1 level is associated with poor PFS. A nomogram was constructed to predict the 1-, 3-, and 5-year survival probability of OC patients by combining the expression level of PAXIP1-AS1 with clinical variables, as shown in Figure 5.
Table 6
Associations with PFS and clinical characteristics in TCGA OC patients (Cox regression).

| Characteristics                                      | Total (N) | Univariate analysis | Multivariate analysis |
|------------------------------------------------------|-----------|---------------------|-----------------------|
|                                                      |           | Hazard ratio (95% CI) | P value | Hazard ratio (95% CI) | P value |
| FIGO stage (Stage III & Stage IV vs. Stage I & Stage II) | 374       | 1.573 (0.918-2.694)  | 0.099    | 1.516 (0.760-3.024)  | 0.237   |
| Primary therapy outcome (CR vs. PD&SD&PR)            | 307       | 0.401 (0.304-0.528)  | <0.001   | 0.496 (0.369-0.667)  | <0.001  |
| Race (White vs. Asian & Black or African American)    | 364       | 0.843 (0.561-1.266)  | 0.409    |                     |         |
| Histologic grade (G3&G4 vs. G1&G2)                   | 367       | 1.188 (0.835-1.688)  | 0.338    |                     |         |
| Age (>60 vs. <=60)                                   | 377       | 1.076 (0.848-1.366)  | 0.547    |                     |         |
| Anatomic neoplasm subdivision (Bilateral vs. Unilateral) | 356       | 1.134 (0.865-1.488)  | 0.363    |                     |         |
| Venous invasion (No vs. Yes)                         | 105       | 0.890 (0.547-1.448)  | 0.638    |                     |         |
| Lymphatic invasion (Yes vs. No)                      | 148       | 1.115 (0.729-1.704)  | 0.615    |                     |         |
| Tumor residual (RD vs. NRD)                          | 334       | 1.695 (1.219-2.358)  | 0.002    | 1.345 (0.928-1.949)  | 0.117   |
| PAXIP1-AS1 (High vs. Low)                            | 377       | 0.668 (0.527-0.847)  | <0.001   | 0.711 (0.542-0.934)  | 0.014   |

PAXIP1-AS1-related pathways based on GSEA

A dataset of 111 significant differences was enriched in PAXIP1-AS1 high expression phenotype. As shown in Table 7 and Figure 6, the top 9 low P-value datasets included neutrophil degranulation, signaling by Interleukins, GPCR-ligand binding, G alpha I signaling events, VEGFAVEGFR-2 signaling pathway, naba secreted factors, Class A 1 Rhodopsin-Like Receptors, PI3K-Akt signaling pathway and Focal Adhesion-PI3K-Akt-mTOR-signaling pathway.
Table 7
Enrichment of gene sets in PAXIP1-AS1 high and low expression groups in OC (GSEA).

| Gene set name                                      | NES   | P adjust | FDR  |
|---------------------------------------------------|-------|----------|------|
| REACTOME_NEUTROPHIL_DEGRANULATION                 | -2.055| 0.036    | 0.030|
| REACTOME_SIGNALING_BY_INTERLEUKINS                | -1.940| 0.036    | 0.030|
| REACTOME_GPCR_LIGAND_BINDING                      | -1.839| 0.036    | 0.030|
| REACTOME_G_ALPHA_I_SIGNALLING_EVENTS              | -1.563| 0.036    | 0.030|
| WP_VEGFAVEGF2_SIGNALING_PATHWAY                   | -1.570| 0.036    | 0.030|
| NABA_SECRETED_FACTORS                             | -1.962| 0.036    | 0.030|
| REACTOME_CLASS_A_1_RHODOPSINLIKE_RECEPTORS_       | -2.020| 0.036    | 0.030|
| WP.PI3KAKT_SIGNALING_PATHWAY                      | -1.840| 0.036    | 0.030|
| WP_FOCUSONADHESIONPI3KAKTMTORSIGNALING_PATHWAY    | -1.928| 0.036    | 0.030|

Correlation of PAXIP1-AS1 expression with immune infiltration

For aDC, the mean level of PAXIP1-AS1 in the high expression group (0.398±0.147) was significantly lower than that in the low expression group (0.436±0.123) (P=0.006) (Figure 7A). The correlation analysis (r=-0.110, P=0.025) showed a negative correlation between PAXIP1-AS1 and aDC (Figure 8A and Figure 9). For B cells, the mean level of PAXIP1-AS1 in the high expression group (0.174±0.07) was significantly lower than that in the low expression group (0.195±0.07) (P=0.004) (Figure 7B). The correlation analysis (r=-0.190, P<0.001) showed a negative correlation between PAXIP1-AS1 and B cells (Figure 8B and Figure 9). For CD8 T cells, the mean level of PAXIP1-AS1 in the high expression group (0.606±0.021) was significantly lower than the mean level in the low expression group (0.611±0.021) (P=0.013) (Figure 7C). The correlation analysis (r=-0.130, P=0.01) showed a negative correlation between PAXIP1-AS1 and CD8 T cells (Figure 8C and Figure 9). For Cytotoxic cells, the mean level of PAXIP1-AS1 in the high expression group (0.358±0.108) was significantly lower than that in the low expression group (0.408±0.097) (P<0.001) (Figure 7D). The correlation analysis (r=-0.260, P<0.001) showed a negative correlation between PAXIP1-AS1 and Cytotoxic cells (Figure 8D and Figure 9). For DC, the mean level of PAXIP1-AS1 in the high expression group (0.315±0.106) was significantly lower than that in the low expression group (0.34±0.108) (P=0.027) (Figure 7E). The correlation analysis (r=-0.160, P=0.002) showed a negative correlation between PAXIP1-AS1 and DC (Figure 8E and Figure 9). For iDC, the mean level of PAXIP1-AS1 in the high expression group (0.401±0.064) was significantly lower than that in the low expression group (0.423±0.069) (P=0.001) (Figure 7F). The correlation analysis (r=-0.230, P<0.001) showed a negative correlation between PAXIP1-AS1 and iDC (Figure 8F and Figure 9). For Macrophages, the mean level of PAXIP1-AS1 in the high expression group (0.518±0.065) was significantly lower than that of the low
expression group (0.541±0.064) (P<0.001) (Figure 7G). The correlation analysis (r=-0.260, P<0.001) showed a negative correlation between PAXIP1-AS1 and Macrophages (Figure 8G and Figure 9). For Mast cells, the mean level of PAXIP1-AS1 in the high expression group (0.122±0.062) was significantly lower than that of the low expression group (0.138±0.068) (P=0.013) (Figure 7H). The correlation analysis (r=-0.160, P=0.002) showed a negative correlation between PAXIP1-AS1 and Mast cells (Figure 8H and Figure 9). For Neutrophils, the mean level of PAXIP1-AS1 in the high expression group (0.233±0.075) was significantly lower than that of the low expression group (0.259±0.076) (P=0.001) (Figure 7I). The correlation analysis (r=-0.200, P<0.001) showed a negative correlation between PAXIP1-AS1 and Neutrophils (Figure 8I and Figure 9). For NK CD56dim cells, the mean level of PAXIP1-AS1 in the high expression group (0.12±0.09) was significantly lower than that of the low expression group (0.157±0.089) (P<0.001) (Figure 7J). The correlation analysis (r=-0.250, P<0.001) showed a negative correlation between PAXIP1-AS1 and NK CD56dim cells (Figure 8J and Figure 9). For T cells, the mean level of PAXIP1-AS1 in the high expression group (0.281±0.124) was significantly lower than that of the low expression group (0.328±0.113) (P<0.001) (Figure 7K). The correlation analysis (r=-0.230, P<0.001) showed a negative correlation between PAXIP1-AS1 and T cells (Figure 8K and Figure 9). For TFH, the mean level of PAXIP1-AS1 in the high expression group (0.311±0.038) was significantly lower than that of the low expression group (0.321±0.038) (P=0.015) (Figure 7L). The correlation analysis (r=-0.140, P=0.006) showed a negative correlation between PAXIP1-AS1 and TFH (Figure 8L and Figure 9). For Tgd, the mean level of PAXIP1-AS1 in the high expression group (0.203±0.035) was significantly lower than that of the low expression group (0.219±0.039) (P<0.001) (Figure 7M). Correlation analysis (r=-0.260, P<0.001) showed a negative correlation between PAXIP1-AS1 and Tgd (Figure 8M and Figure 9). For Th1 cells, the mean level in the PAXIP1-AS1 high expression group (0.337±0.059) was significantly lower than the mean level in the low expression group (0.355±0.059) (P=0.002) (Figure 7N). Correlation analysis (r=-0.180, P<0.001) showed a negative correlation between PAXIP1-AS1 and Th1 cells (Figure 8N and Figure 9). For Th2 cells, the mean level of PAXIP1-AS1 in the high expression group (0.355±0.045) was significantly lower than that of the low expression group (0.369±0.04) (P=0.002) (Figure 7O). Correlation analysis (r=-0.210, P<0.001) showed a negative correlation between PAXIP1-AS1 and Th2 cells (Figure 8O and Figure 9). For TReg, the mean level in the PAXIP1-AS1 high expression group (0.297±0.138) was significantly lower than the mean level in the low expression group (0.341±0.135) (P=0.002) (Figure 7P). Correlation analysis (r=-0.170, P=0.001) showed a negative correlation between PAXIP1-AS1 and TReg (Figure 8P and Figure 9).

**Discussion**

LncRNAs have been implicated in the molecular mechanisms of carcinogenesis [25]. As regulators of the flow of genetic information interacting with epigenetic, transcriptional, and post-transcriptional pathways, LncRNAs promote tumor formation, progression, and metastasis in many human malignancies [26]. Understanding the specific molecular events that underpin OC tumorigenesis can lead to early detection and improved outcomes. LOXL1-AS1 expression correlates with poor clinical outcome in EOC patients and can be used as an independent prognostic indicator as well as a new diagnostic biomarker [27].
LINC00472 may be a potential tumor suppressor in OS by interacting with miR-300 and FOXO1 [28]. High plasma levels of IncRNA ROR can be used as a potential biomarker for the diagnosis of OC [29]. Therefore, it is important to study IncRNAs as new prognosis OC biomarkers and therapeutic targets in the future.

PAXIP1-AS1 was significantly related to age (P=0.045), histological grade (P=0.011), and lymphatic invasion (P=0.004). Expression of PAXIP1-AS1 was positively correlated with poor OS (P=0.009), PFS (P=0.001), and DSS (P=0.006) of OC patients. PAXIP1-AS1 expression (HR: 0.711; 95% CI: 0.542-0.934; P=0.014) was an independently correlated with PFS in OC patients.

Overexpression of PAXIP1-AS1 advances glioma development by recruiting the transcription factor ETS1 to increase KIF14 expression [13]. PAXIP1-AS1 may modulate smooth muscle cell function by affecting multiple IPAH-specific transcriptional programs [30]. Based on GSEA, PAXIP1-AS1 was found to be involved in the pathways including neutrophil degranulation, signaling by Interleukins, GPCR-ligand binding, G alpha I signaling events, VEGFAVEGFR-2 signaling pathway, naba secreted factors, Class A 1 Rhodopsin-Like Receptors, PI3K-Akt signaling pathway, and Focal Adhesion-PI3K-Akt-mTOR-signaling pathway.

Immune infiltration of OC is currently a hot topic and understanding of immune infiltration will facilitate the development of immunotherapy for OC. The results of this study showed a modest relationship between PAXIP1-AS1 expression and immune cells in OC. These correlations may suggest that PAXIP1-AS1 may inhibit the function of aDC, B cells, CD8 T cells, Cytotoxic cells, DC, iDC, Macrophages, Mast cells, Neutrophils, NK CD56dim cells, T cells, TFH, Tgd, Th1 cells, Th2 cells and Treg, which in turn exert a suppressive effect on OC through a potential mechanism.

Although there are some limitations, this is the first study to explore the relationship between PAXIP1-AS1 and OC. This study was mainly based on bioinformatic analysis and could be further strengthened by experimental studies. The mechanism of PAXIP1-AS1-mediated ovarian carcinogenesis needs to be further investigated.

**Conclusions**

PAXIP1-AS1 was lowly expressed in OC relative to normal tissue and related to poor OS, PFS, and DSS. PAXIP1-AS1 might participate in the development of OC by pathways including neutrophil degranulation, signaling by Interleukins, GPCR-ligand binding, G alpha I signaling events, VEGFAVEGFR-2 signaling pathway, naba secreted factors, Class A 1 Rhodopsin-Like Receptors, PI3K-Akt signaling pathway, and Focal Adhesion-PI3K-Akt-mTOR-signaling pathway. PAXIP1-AS1 expression was associated with immune infiltrating cells. This study partly revealed the role of PAXIP1-AS1 in OC, providing a potential prognosis biomarker for OC.

**Declarations**
**Ethics approval and consent to participate**

Approval was obtained from the ethics committee of the Affiliated Hospital of Xuzhou Medical University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. BZC and GLL contributed to conceptualization. XYL, QMZ, QC, SYZ, and HL contributed to data curation. BZC and XYL contributed to conceptualization. QC, XYL, and GLL contributed to methodology. XYL, QMZ, QC, SYZ, and HL contributed to software. BZC and XYL contributed to writing–original draft. HL and GLL contributed to writing – review & editing.

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**Availability of data and materials**

The data within the article are available from the corresponding author upon request.

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Figures
Figure 1

Expression of PAXIP1-AS1 in OC and normal ovarian tissues. (A) OC and unpaired normal ovarian tissues (B) OC and paired normal ovarian tissues. *, P < 0.05; ***, P < 0.001.

Figure 2

Association with PAXIP1-AS1 expression and clinical stage. (A) age. (B) histologic grade. (C) lymphatic invasion. *, P < 0.05; **, P < 0.01.
Figure 3

Low expression of PAXIP1-AS1 was associated with poor OS, PFS and DSS in OC patients. (A) OS, overall Survival. (B) PFS, progress Free Interval. (C) DSS, disease Specific Survival.

| Characteristics                              | Total (N) | Hazard ratio (95% CI) | Multivariate analysis | P value Multivariate analysis |
|----------------------------------------------|-----------|-----------------------|-----------------------|------------------------------|
| FIGO stage (Stage III & Stage IV vs. Stage I & Stage II) | 374       | 1.516 (0.760-3.024)   |                       | 0.237                        |
| Primary therapy outcome (CR vs. PD&SD&PR)     | 307       | 0.496 (0.369-0.667)   |                       | <0.001                       |
| Tumor residual (RD vs. NRD)                   | 334       | 1.345 (0.928-1.949)   |                       | 0.117                        |
| PAXIP1-AS1 (High vs. Low)                     | 377       | 0.711 (0.542-0.934)   |                       | 0.014                        |

Figure 4

Forest plot of the multivariate Cox regression analysis in OC.
Figure 5

Nomogram for predicting the probability of patients with 1-, 3- and 5-year overall survival.
Figure 6

GSEA analysis of PAXIP1-AS1 in OC. Enrichment plots from gene set enrichment analysis (GSEA). (A) neutrophil degranulation, (B) signaling by Interleukins, (C) GPCR-ligand binding, (D) G alpha I signaling events, (E) VEGFAVEGFR-2 signaling pathway, (F) Class A 1 Rhodopsin-Like Receptors, (G) naba secreted factors, (H) PI3K-Akt signaling pathway, and (I) Focal Adhesion-PI3K-Akt-mTOR-signaling pathway, were enriched in PAXIP1-AS1-related OC. NES, normalized ES; FDR, false discovery rate.
Figure 7

Correlation between PAXIP1-AS1 expression and 24 immune cells in OC (grouped comparison plots). (A) aDC, (B) B cells, (C) CD8 T cells, (D) cytotoxic cells, (E) DC, (F) iDC, (G) Macrophages, (H) Mast cells, (I) Neutrophils, (J) NK CD56dim cells, (K) T cells, (L) TFH, (M) Tgd, (N) Th1 cells, (O) Th2 cells, and (P) TReg.
Figure 8

Correlation between PAXIP1-AS1 expression and 24 immune cells in OC (scatter plots). (A) aDC, (B) B cells, (C) CD8 T cells, (D) cytotoxic cells, (E) DC, (F) iDC, (G) Macrophages, (H) Mast cells, (I) Neutrophils, (J) NK CD56dim cells, (K) T cells, (L) TFH, (M) Tgd, (N) Th1 cells, (O) Th2 cells, and (P) TReg.
Figure 9

Correlation between PAXIP1-AS1 expression level and 24 immune cells in OC (lollipop chart). The size of the dots indicates the absolute value of Spearman r.