PRPF6 promotes metastasis and paclitaxel resistance of ovarian cancer via SNHG16/CEBPB/GATA3 axis

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Research Article

Keywords: Ovarian cancer, PRPF6, SNHG16, paclitaxel resistance, GATA3.

Posted Date: March 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1471492/v1

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Abstract

Background: Metastasis and paclitaxel (PTX) resistance are the main reason for the poor prognosis of ovarian cancer (OC). Evidence has shown that RNA-binding proteins (RBPs) and long noncoding RNAs (lncRNAs) can modulate post-transcriptional regulation. The aim of this study was to determine the relationship among RBP, lncRNA and OC and to further guide clinical therapy.

Methods: The expression of pre-mRNA processing factor 6 (PRPF6) in OC tissues form 68 patients were detected by Immunohistochemistry. The expression of small nucleolar RNA host gene 16(SNHG16) transcripts-SNHG16-L/S in OC cell lines were analyzed by real-time PCR (RT-PCR). Transwell, CCK8 assays and flow cytometry analyses were used to detected effects of PRPF6 and SNHG16 on tumorigenesis and PTX-resistance. Molecular interactions were examined by dual-luciferase reporter gene assay, RNA immunoprecipitation and chromatin immunoprecipitation. OC cells that knockdown PRPF6 were injected subcutaneously in nude mice.

Results: The results showed that PRPF6 was upregulated in OC chemoresistant tissues and was closely related to advanced FIGO stages. PRPF6 promoted progression, and PTX resistance in vitro and in vivo. SNHG16-L/S were differentially expressed in OC cells and tissues as detected through real-time PCR (RT-PCR). SNHG16-L/S had opposite effects on progression and PTX resistance in OC. Mechanistically, SNHG16-L inhibited GATA-binding protein 3 (GATA3) transcription by binding to CCAAT/enhancer-binding protein B (CEBPB). Moreover, PRPF6 induced the alternative splicing of SNHG16, causing downregulation of SNHG16-L and, leading to the upregulation of GATA3 expression to further promote metastasis and PTX-resistance in OC.

Conclusions: Totally, these data unveiled that PRPF6 promotes metastasis and PTX resistance of OC through SNHG16-L/CEBPB/GATA3 axis, which provides a new direction for OC treatment.

Background

According to the global cancer statistics from the World Health Organization, 313,959 new cases of ovarian cancer (OC) and 207,252 OC-related deaths were reported in 2020 worldwide[1, 2]. Due to the lack of early symptoms and efficient screening methods, approximately 70% of patients reach an advanced stage at the time of diagnosis. The treatment of advanced OC involves surgery with platinum and paclitaxel (PTX) chemotherapy[3]. The high rate of recurrence and metastasis, and chemoresistance in patients with OC leads to a poor prognosis. The potential pathological mechanisms of PTX resistance in OC may be very complicated, and current research has not reached a consensus. It is generally accepted that the mutation of β-tubulin and interference with the polymerization of microtubules are the prominent mechanisms of PTX resistance in OC[4]. PTX inhibits depolymerization, causing cell cycle arrest, apoptosis and chemoresistance[5]. Many studies have confirmed that a high expression of β-tubulin III can reduce tubulin assembly and, decrease the binding rate of tumor cells to PTX[6]. Despite advances in OC diagnosis and treatment, the main obstacles to cure are still poor prognosis and high recurrence rates.
Therefore, it is essential to explore the key factors contributing to metastasis and PTX resistance in OC, and to seek novel strategies for the treatment of OC patients.

Long non-coding RNA (lncRNAs) are a class of non-coding RNAs with a length of more than 200 nucleotides, and can participate in various cancers[7-11]. Small nucleolar RNA host gene 16(SNHG16) is located in chromosome 17q25.1, has been shown to be involved in the mechanisms of various cancers. Studies have shown that SNHG16 can competitively combine with a variety of miRNAs to influence the expression of its target genes and promote the proliferation, invasion, and migration of various tumor cells such as nasopharyngeal carcinoma, breast cancer, cervical cancer, esophageal cancer and osteosarcoma[12-16]. However, the molecular mechanism of SNHG16 in OC is still in its early stage of development.

RNA-binding proteins (RBPs) can bind to RNA to further involved in RNA alternative splicing (AS), transcription and translation[17]. AS refers to the splicing of pre-mRNA to produce different transcripts[18]. Pre-mRNA processing factor 6 (PRPF6) is one of the components of small nuclear ribonucleoprotein (snRNP) and plays an important role in AS[19]. Mutations in C-terminal TPR domain of PRPF6 lead to synthesis disorders of the U4/U5/U6 snRNP complex and promote the occurrence of autosomal dominant retinitis pigmentosa[20]. PRPF6 can interact with targeted RNA, increase the expression of targeted transcripts with oncogenic function, and promote tumor proliferation and metastasis. For example, PPRF6 regulates the AS of the oncogenic transcript of ZAK to drive proliferation and metastasis in colon cancer[21]. PRPF6 activates AR/AR-Vs to promote the progression of hepatocellular cancer and prostate cancer[22, 23]. Our preliminary research found that PRPF6 may be a target gene of miR-134 mediating the regulation of PTX resistance in OC[24]. However, its specific function and mechanism in OC have not been studied.

In our study, we found that two SNHG16 transcripts, which are defined as SNHG16-L (SNHG16-001, ENST00000448136.5) and SNHG 16-S (SNHG16-002, ENST00000590435.5), were differentially expressed in PTX-sensitive OC cell line-SKOV3 and PTX-resistant OC cell line-SKOV3-TR30. And SNHG16-L/S were differential expressed in chemosensitive and chemoresistant OC tissues. Further functional experiments demonstrated the opposite effects of SNHG16-L/S and PRPF6 on progression and PTX resistance in OC. SNHG16-L could bind to CEBPB to further inhibit transcriptional activity of GATA3. In addition, PRPF6 was highly expressed in chemoresistant tissues and was closely related to the advanced FIGO stages. PRPF6 induced the AS of SNHG16 to upregulate GATA3 expression to promote metastasis and PTX resistance. This study may help in finding a valuable target for OC therapy.

**Methods**

**Patients and tissues**

Paraffin sections of OC tissue samples from 68 patients were collected and OC tissue samples for Real-Time PCR from 50 patients were obtained from the department of Gynecology and Obstetrics of
Shengjing Hospital of China Medical University from 2018-2020. At least two pathology experts jointly determined the postoperative pathology of in all the cases. Of these, 31 chemoresistant cases and 37 chemosensitive cases were determined according to NCCN guidelines. All patients have provided signed the informed consent and the experimental protocol was approved by the Institutional Medical Research Ethics Committee of the Shengjing Hospital of China Medical University(2020PS274K-X1).

**Cell culture**

The PTX-sensitive OC cell line, SKOV3, was acquired from the Tumor Cell Bank of the Chinese Academy of Medical Sciences (Beijing). The PTX-resistant OC cell line, SKOV3-TR30, was derived from SKOV3 and provided by Zhejiang University affiliated Obstetrics and Gynecology Hospital (Hangzhou)[25]. All the cells were cultured in RPMI/1640 (Hyclone, USA) medium contains 10% fetal bovine serum (FBS, Procell) and 1% penicillin/streptomycin at 37 °C with 5% CO2. SKOV3-TR30 cells were maintained with the addition of 20 nM of PTX (Sigma Aldrich, MO). 293T cells were cultured in DMEM (Hyclone, USA).

**Real-time PCR (RT-PCR)**

Total RNA was isolated using Trizol reagent (Invitrogen). cDNA was synthesized according to the manufacturer’s protocol (Takara). SYBR premix Ex TaqTM II (Takara) was used for PCR. The primers were synthesized by Sangong (Shanghai, China) and shown as follows: PRPF6: Forward: GAGGATGCTGACAGTTGTGTAG, Reverse: CCATGGTTCTTCTCGAAGTACG; SNHG16-L: Forward: CCAGTTACACAGGATGCGCTTCTTG, Reverse: AGCTGATTGCCTTGGTGAGTCAAC; SNHG16-S: Forward: GCCAAGGTGAAGCGAGCTGAG, Reverse: GCAAGAGACTTCCTGAGGCACAT; CEBPB: Forward: GCACAGCGACGAGTCAGA, Reverse: TGCTTGAACAGTTCCGCAG; GATA3: Forward: GTCTCTGTCCGACTGTGCA, Reverse: CGAGCTGTCTTTGGGAAGT. The expression of RNAs was normalized by 2^{-\Delta\Delta^{CT}} method.

**Cell transfection**

Cells were spread into 6-well plates with a density of 30-50%. After culturing for 24 hours, when cell fusion degree reaches 50-70%, change the medium into serum-free medium. Add 2ug plasmid DNA solution or 25nM siRNA solution and 4ul lipofectamine 3000 (Invitrogen) transfection reagent into new tubes. After mixing evenly for 10 minutes. Transfer the mixed solution to the 6-well plate, and add serum-free medium to a final volume of 2ml. After 4-6 hours, change to serum-containing medium. siRNAs were synthesized from Ribobio (Guangzhou, China). The sequences were as follows: si-PRPF6-001: GAAGCGGGTTCTTCCGAAA, si-PRPF6-002: GATCTAAATGACACCAAT, si-PRPF6-003: CTCGGAAACCTTGATCATGAA. The overexpression plasmids pHBLV-PRPF6, pcDNA3.1-SNHG16-L, pcDNA3.1-SNHG16-S, pcDNA3.1-CEBPB, and pHBLV-GATA3 were synthesized from Hanbio (Shanghai, China). Lipofectamine 3000 (Invitrogen) was used transfection according to the instructions.
Transwell assay

5*10^4 cells were suspended in a serum-free medium and plated on upper transwell migration chambers (Corning Costar). Transwell invasion assay was coated with Matrigel (BD). The lower chambers added medium with 10% FBS. After cultured 24 hours, the membranes were fixed with methanol and stained with 1% crystal violet. Five random fields (×400 magnifications) were counted and photographed under the light microscope.

CCK8 assay

2000 cells were seeded in 96-well plate with 100ul medium per well. We added 10ul CCK8 reagent (Sigma) at 0, 24, 48, 72, and 96 hours. The optical density was measured at 450 nm. To test differing PTX sensitivities, we added various concentrations of PTX after seeded for 24 hours. Then, added 10ul CCK8 reagent at 48 hours and examined in 2 hours.

Colony Formation Assay

Cells were plated in 6-well plates and incubated for 10 days (1000 cells/well). Then fixed with methanol and stained with 0.1% of crystal violet and calculated the number of visible colonies.

Western blotting

Total protein was extracted via RIPA lysis (Beyotime) with phenyl-methane-sulfonyl fluoride and protease inhibitor. 10% SDS-PAGE gel electrophoresis with 30μg protein per well was performed and then we transferred PVDF membranes. Antibodies PRPF6 (1:2000, Abcam), CEBPB (1:2000, Abcam), GATA3(1:2000, Abcam), Vimentin (1:1000, Elabscience), E-cadherin (1:1000, Elabscience), N-cadherin (1:1000, Elabscience), β-tubulin III (1:2000, Immunoway), β-actin (1:5000, Bioworld) were incubated overnight at 4°C.

RNA immunoprecipitation (RIP)

The experiment was conducted followed the manufacturer's protocol of Magna RIP kit (Millipore). The antibodies were PRPF6(10ug per reaction, Abcam) and CEBPB (10ug per reaction, Abcam). RNA was extracted after detachment from the bead using protease K. The expression of SNHG16 pre-mRNA, SNHG16-L and SNHG16-S was determined using RT-qPCR.

Chromatin immunoprecipitation (ChIP)
ChIP was performed according to the instruction of EZ ChIP KIT (Millipore). The antibody was CEBPB (10ug, Abcam). The possible binding sites of CEBPB and GATA3 promoter were predicted via Jaspar, and specific primers were synthesized by Sangong (Shanghai, China), sequences were as follows: GATA3 promoter: Forward: CAAGCCCTTTGCCCCAT, Reverse: CAGGTAGAGTTTTCCCTTCACAA. The enrichment of GATA3 promoter was detected by RT-PCR.

**Dual-luciferase reporter assay**

The luciferase plasmids pSI-Check2-GATA3 wild type/mutant type (wt-GATA3/mut-GATA3) were synthesized by Hanbio (Shanghai, China). According to the instruction of Dual-Luciferase® Reporter Assay System (Promega), the luciferase activity was detected.

**Immunohistochemistry (IHC)**

The paraffin sections were deparaffinized and antigen retrieval was performed by adding citrate buffer (pH 6.1). The sections were then incubated with PRPF6 antibodies (Abcam, 1:250) were diluted in 5% BSA, followed by DAB staining (Elabscience) and observation under microscope.

**Immunofluorescence (IF)**

Cells growing on coverslips in 6-well plates were removed and fixed with 4% paraformaldehyde. Then, permeabilized in 0.5–1.0% Triton X-100 for 10 min and blocked with 5% BSA for 30 minutes. The cells were then incubated with antibodies PRPF6 (1:150, Abcam), CEBPB (1:150, Abcam), GATA3 (1:150, Abcam) overnight at 4°C. Fluorescent-labelled secondary antibody (1:100, Proteintech) was added and incubated in the dark for 2h. Cells were stained with DAPI and observed under the fluorescent microscope.

**Fluorescence in situ Hybridization (FISH)**

Cells growing on coverslips in 6-well plates were removed. The SNHG16 probe were synthesized by Servicebio. The experiment was operated according to the protocol of Fluorescent in Situ Hybridization Kit (Ribobio). The slips observed under the fluorescence microscope.

**TCGA and GTEx database analysis**

The transcriptome profile containing clinical information from TCGA-OV database using R package TCGA biolinks through R (version 4.0.2). HTSeq-Counts data was collected for identification of DEGs and HTSeq-FPKM data was processed by log2(FPKM+1) for further analysis. GTEx (Genotype-Tissue
Expression) gene expression data were obtained from UCSC Xena project, normalized via affy Bioconductor library Normalize Between Arrays.

**Xenografts in nude mice**

The lentivirus containing siPRPF6 sequence was synthesized by Gene-Pharma (Shanghai, China). The SKOV3-TR30 cells were infected with lentivirus and obtained stably transfected cells. 4-weeks-old female BALB/cA-nu Mice (N=3/group) were purchased from Huafukang (Beijing, China). The mice were randomly divided into four groups. The mice subcutaneously inoculated with cell suspension (200 µL, 5 × 10^6 cells) into dorsal part to observe tumor growth. After 1 week, PTX (20 mg/kg) or saline was injected into tumor every 3 days for 3 weeks when tumor size reached 80-100mm^3. Animal experiments were performed according to the ethical guidelines for animal experiments and were approved by China Medical University Animal Welfare and Ethical Community (CMU2020341).

**Statistical analysis**

The statistics were analyzed with SPSS 22.0. The results represented as the mean± standard deviation (SD). Data with normal distribution and homogeneity of variance were compared by paired sample t-test or non-paired t-test. One-way analysis of variance (ANOVA) was used for comparison among multiple groups. Repeated measures ANOVA, followed by the Bonferroni post hoc test, were used to analyze multiple groups at different time points. Significantly difference was set as P<0.05. (*P < 0.05, **P < 0.01, ***P < 0.001).

**Results**

**SNHG16-L/S had a different effect on the tumorigenesis, and PTX resistance in OC**

ENSEMBL annotation showed that the difference in SNHG16-L/S was the existence of exon 1 (Figure.1A). Through RT-PCR analysis, we found that the expression of SNHG16-L in the PTX-resistant cell line, SKOV3-TR30, was lower compared with that in the PTX-sensitive cell line, SKOV3. The expression of SNHG16-S was higher in SKOV3-TR30 (Figure 1B). We then detected the expression in OC tissues. The results revealed that compared with chemosensitive tissues, SNHG16-L had lower expression in chemoresistant tissues while SNHG16-S had higher expression (Figure 1C). Therefore, full-length of SNHG16-L/S were cloned into specific vectors (pcDNA3.1) for overexpression studies. RT-PCR analysis confirmed that the SNHG16-L/S vectors significantly elevated SNHG16-L/S levels in SKOV3-TR30 and SKOV3 cells, respectively (Supplementary Figure 1).

Further functional experiments were performed to determine the effects of their expression levels. Transwell assays were performed to assess cell migration and invasion abilities. The results confirmed
that the presence of OE-SNHG16-L decreased cell migration and invasion, whereas OE-SNHG16-S increased cell migration and invasion (Figure 2A). We then used a colony formation assay to analyze cell growth and found that SNHG16-L overexpression inhibited cell growth, whereas SNHG16-S overexpression induced it (Figure 2B). The results of the CCK8 assay also showed that overexpression of SNHG16-L and SNHG16-S had decreased and increased cell growth, respectively (Figure 2C). Subsequently, SNHG16-L overexpression inhibited PTX-resistance, while SNHG16-S overexpression promoted PTX resistance in the CCK8 assay (Figure 2D).

In addition, western blotting was used to analyze the effects of SNHG16-L/S on the expression of EMT-related proteins (N-cadherin, vimentin, and E-cadherin) and PTX resistance-related proteins (β-tubulin III). The results showed that overexpression of SNHG16-L decreased the expression of N-cadherin, β-tubulin III, and vimentin, and increased E-cadherin expression, while overexpression of SNHG16-S present the opposite trend (Figure 2E).

**SNHG16-L bound to CEBPB and inhibited the transcriptional activity of GATA3 to further inhibit metastasis and PTX resistance of OC**

LncRNAs can interact with DNA-binding protein to regulate transcription activity of target genes to mediate development of various cancer. Previous study shows that SNHG16 can combine with transcription factor SPI1 to upregulate PARP9 transcription to further promote tumorigenicity of cervical cancer[26]. To further explore the mechanism of SNHG16-L/S in OC, we used the LncMAP database (http://bio-bigdata.hrbmu.edu.cn/LncMAP) to find related transcription factors and targets genes. The results suggested that SNHG16 may regulate GATA3 via the transcriptional factor CEBPB in OC (Supplementary Figure 2A). We then predicted the binding sites of SNHG16 and CEBPB, and found that SNHG16-L combined with the CEBPB protein using CatRAPID-omics (http://service.tartaglialab.com/page/catrapid_omics_group) program (Supplementary Figure 2B).

First, we determined whether the cellular localization of SNHG16, CEBPB, and GATA3 was consistent. SNHG16 was located in the nucleus, as detected by FISH (Supplementary Figure 2C). Immunofluorescence analysis showed that CEBPB and GATA3 were expressed in the nucleus (Supplementary Figure 2D). Previous studies have shown that GATA3 can be used as an independent risk factor to promote cell migration, invasion, and PTX resistance in OC[27, 28]. We then overexpressed GATA3 in SKOV3 cells to examine the expression levels of N-cadherin, vimentin, β-tubulin III and E-cadherin using western blotting. The results showed that overexpression of GATA3 upregulated N-cadherin, vimentin, and β-tubulin III and downregulated E-cadherin (Figure 3A). To determine whether SNHG16-L can regulate cell migration, invasion, EMT, and PTX resistance, we performed transwell, CCK8, and western blot assays after the overexpression of SNHG16-L and GATA3. These results indicated that SNHG16-L overexpression inhibited PTX resistance, migration, and invasion (Figure 3B&C). It also downregulated GATA3, N-cadherin, vimentin and β-tubulin III, and upregulated E-cadherin expression.
Overexpression of both SNHG16-L and GATA3 reversed the effects caused by the overexpression of SNHG16-L alone (Figure 3D).

To confirm the binding between SNHG16-L and CEBPB proteins, the RIP test was conducted in SKOV3-TR30 cells using the CEBPB antibody. Using RT-PCR, we found that CEBPB could bind to SNHG16-L but not to SNHG16-S (Figure 3E). Furthermore, sequence analysis through Jaspar website (http://jaspar.genereg.net/analysis) revealed a potential binding site for CEBPB in the promoter region of GATA3 (Figure 3F). ChIP detection using an antibody against CEBPB in SKOV3-TR30 was performed to explore whether CEBPB could bind to the GATA3 promoter. RT-PCR was conducted using specific primers of predicted sites, we found that CEBPB-bound complexes significantly enriched the 898-907bp region of the GATA3 promoter (Figure 3G). In addition, we constructed GATA3 luciferase mutant plasmids on this region and further validated our findings by using dual-luciferase reporter assay. The results showed overexpression of CEBPB enhanced the luciferase activity of the GATA3-wt group, but not the GATA3-mut group (Figure 3H). This enhancement was weakened after the overexpression of SNHG16-L (Figure 3I). Thus, SNHG16-L could downregulate the transcriptional activity of GATA3 via binding to CEBPB, further inhibiting cell migration, invasion, and PTX resistance of OC.

**PRPF6 was upregulated in chemo-resistant tissues and was related to the advanced FIGO stages of OC**

The differential function of SNHG16-L/S in OC indicated that SNHG16 may be mediated by AS. To find possible splicing factors binding to SNHG16, catRAPID omics analysis found that the 413-419bp segment of the SNHG16 pre-mRNA exon 1 may be a potential binding site for PRPF6 (Figure 4A). To clarify the expression level of PRPF6 in OC, Wilcoxon rank-sum analysis was conducted using the RNA-seq data of TCGA-OV and GTEx database. We found that its expression was higher than that in normal tissues (Figure 4B). We used RT-PCR and IHC to detect the PRPF6 expression in OC cells and tissues, respectively. We then analyzed PRPF6 expression in OC tissues using IHC and found that PRPF6 was located in the nucleus and was relatively higher in chemoresistant OC tissues (N=31) than that in chemosensitive tissues (N=37, Figure 4C). We then used the median relative expression of PRPF6 in OC tissues, divided the patients into high- and low-expression groups, and analyzed the correlation between the expression level and clinicopathological characteristics of patients using the chi-square test. The results showed that PRPF6 was closely related to advanced FIGO stages but was not related to patient age, differentiation, or lymph node metastasis (Table 1). Furthermore, RT-PCR analysis revealed that PRPF6 was relatively higher in SKOV3-TR30 cells than that in SKOV3 cells (Figure 4D).

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<td>Correlation between PRPF6 and clinicopathological characteristics in OC patients.</td>
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PRPF6 promoted tumorigenesis and PTX resistance in vitro and in vivo

Three siRNAs were designed for knockdown studies and pc-DNA3.1-PRPF6 vector for overexpression studies. RT-PCR analysis showed that si-PRPF6-003 significantly downregulated PRPF6 in SKOV3-TR30 cells and was named si-PRPF6, and the vector markedly upregulated PRPF6 expression in SKOV3 cells (Supplementary Figure 1B). CCK8 assays showed that si-PRPF6 and upregulation of PRPF6 significantly inhibited and promoted cell growth, respectively (Figure 5A). Transwell assays were performed to assess cell migration and invasion. The results indicated that si-PRPF6 resulted in a decrease in invasion and migration, while PRPF6 overexpression resulted in an increase (Figure 5B). Moreover, the cell viability
assays after treatment with various doses of PTX indicated that si-PRPF6 inhibited PTX resistance, while PRPF6 overexpression promoted PTX resistance (Figure 5C). The colony formation assay showed si-PRPF6 inhibited cell growth, whereas overexpression of PRPF6 promoted growth (Figure 5D). si-PRPF6 transfected in SKOV3-TR30 cells inhibited GATA3, N-cadherin, vimentin, and β-tubulin III expression and upregulated E-cadherin expression, as detected by western blotting (Figure 5E).

To investigate function of PRPF6 in vivo, we constructed stable cell lines by transfecting lentiviruses containing the si-PRPF6 sequence in SKOV3-TR30 cells, and injected cells into 4-week-old female BALB/cA-nu mice (N=3/group). Mice were divided into four groups (Figure 5F). After a week, PTX (20 mg/kg) or saline was injected into the tumor every 3 days for 3 weeks. The results showed that knockdown of PRPF6 inhibited tumor expansion and weight (Figure 5G&H).

**PRPF6 induced the AS of SNHG16 to upregulate GATA3 expression to promote metastasis and PTX resistance**

PRPF6 as a splicing factor, can mediate AS in various diseases[20, 29]. To investigate molecular mechanism between PRPF6 and SNHG16, we first detected effects of PRPF6 differing expression on SNHG16-L/S. The results showed that knockdown of PRPF6 upregulated SNHG16-L and downregulated SNHG16-S, while overexpression of PRPF6 had the opposite effects (Figure 6A). We then conducted the RIP assay in SKOV3-TR30 cells. RT-PCR analysis showed that PRPF6 could bind to SNHG16-L and SNHG16 pre-mRNA, but not to SNHG16-S (Figure 6B). Transwell and CCK8 assays were conducted to test the migration, invasion, PTX resistance of cells. The results indicated that the overexpression of SNHG16-L and PRPF6 weakened the promotion of migration, invasion, and PTX resistance caused by PRPF6 overexpression (Figure 6C&D).

To determine whether PRPF6 regulates GATA3 expression through SNHG16-L, IHC was used to detect GATA3 expression in the previous s xenograft tumor tissues. We found that knockdown of PRPF6 downregulated GATA3 in vivo (Figure 6E). Western blot analysis showed that upregulation of PRPF6 increased GATA3, N-cadherin, β-tubulin, III and vimentin expression and decreased E-cadherin expression. However, SNHG16-L overexpression reversed this effect (Figure 6F). Taken together, these data implicate that PRPF6 promotes metastasis and PTX resistance through SNHG16/CEBPB/GATA3 axis in OC.

**Discussion**

More than 28,000 lncRNAs have been discovered, and there are still many lncRNAs to be discovered and annotated. lncRNAs are involved in the malignant biological behavior of tumors and the regulation of chemoresistance[30]. SNHG16 has been upregulated and downregulated in multiple cancers and participates in cell proliferation, migration, invasion and EMT[31]. SNHG16 also plays an important role among gynecological tumors. SNHG16 is highly expressed in cervical cancer tissues, and is closely
related to the TNM stage, tumor size, distant metastasis and survival prognosis. SNHG16 can activate Wnt/β-catenin pathway and EMT in cervical cancer[32]. In OC, there is one research showing that SNHG16 can promote cell proliferation, migration and invasion[33]. In our study, we first illustrate the different effects of SNHG16-L/S in OC. SNHG16-L overexpression inhibited tumorigenesis, EMT and PTX resistance in OC, whereas SNHG16-S induced the opposite effect. SNHG16 may therefore participate in the regulation of OC, the molecular mechanisms need to be illustrated.

EMT is the process by which epithelial cells transform into mesenchymal cells, which promotes tumor metastasis[34]. EMT shows a variety of gene expression changes, including the downregulation of epithelial genes such as E-cadherin and cytokeratin, and the upregulation of mesenchymal genes, such as N-cadherin and vimentin[35]. A decrease in E-cadherin expression is closely associated with peritoneal metastasis, and inhibits progression-free survival, and overall survival rate[36]. In addition, recent studies have shown that EMT plays a key role in chemoresistance. PTX-resistant cells can exhibit some characteristics of mesenchymal cells, including cell polarity and cell adhesion loss[37]. Genes associated with EMT, like E-cadherin, vimentin, N-cadherin, and ZEB1, are involved in regulating response of OC cells to PTX resistance[38, 39]. Therefore, our study on EMT indicated SNHG16-L/S were of great significance for metastasis and PTX resistance of OC.

Increasing evidences indicate that lncRNAs can mediate transcriptional regulation by affecting the binding of transcription factors and target genes. For example, SNHG16 can recruit the SPI1 protein to promote the transcriptional activation of the PARP9 promoter in cervical cancer[26]. Mechanistically, we first report that SNHG16-L downregulates transcription activity of GATA3 through CEBPB, and inhibits migration, invasion and PTX resistance of OC. CEBPB plays a vital role in cell proliferation and tumor development. For example, CEBPB, a DNA-binding protein, can enhance the activity of the H3K79 methyltransferase, DOT1L, and regulate the methylation of H3K79 to promote cisplatin resistance in OC[40]. Moreover, CEBPB can block the transcriptional regulation of GDF15 and this effect can be inhibited by the GAS5 in OC[41]. GATA3 is highly expressed in multiple OC cell lines, and is the induction of EMT and having a poor prognosis. GATA3 promotes the arrest of the cell cycle in the G2/M phase, and induces EMT and cisplatin resistance in OC[27]. Moreover, GATA3 can interact with HIF1A to prevent its ubiquitination and proteasomal degradation, thereby promoting progression and metastasis in OC[42]. High GATA3 expression increases the ratio of p-p38MAPK/p-ERK, and promotes stemness and PTX resistance in OC[43]. Here, our findings first expose SNHG16/CEBPB/GATA3 axis in OC, which provides novel evidences for lncRNAs participating in RNA transcription.

Notably, recent studies have demonstrated that different isoforms of lncRNAs exhibit opposite functions in tumorigenesis. For example, MBNL3 regulates the AS of the lncRNA PXN-AS1 and promotes the inclusion of exon 4 to upregulate PXN in hepatocellular cancer[44]. This study provides a novel direction for future research on RBPs and lncRNAs. PRPF6, as a splicing protein, acts as an oncogene in many cancers. In this study, we first report that PRPF6 is upregulated in chemoresistant OC tissues, and closely related to advanced FIGO stages. PRPF6 promotes cell progression, metastasis, and PTX resistance in OC. PRPF6 is closely related to the advanced stage of hepatocellular cancer, which is consistent with our
findings[22]. PRPF6 could therefore be a promising target for the treatment of OC. However, the relationship between PRPF6 and the survival of patients with OC still needs further exploration by expanding sample size of the cohort.

The opposing effects of SNHG16 transcripts indicated that SNHG16 might be induced by AS. AS has seven basic patterns, such as having skipped exons, retained introns, and alternate donor sites. RBPs bind to adjacent splice sites and promote the recruitment of other spliceosomes[45]. Interestingly, our study suggests that PRPF6 preferentially binds to the SNHG16 pre-mRNA and SNHG16-L, instead of SNHG16-S, indicating that PRPF6 may be possibly recruited to bind to exon 1. These data indicated that the splicing pattern of SNHG16 mediated by PRPF6 may be exon 1 skipping. However, the patterns and sites of AS remain to be investigated in-depth in future studies. In our study, we only used SKOV3 and SKOV3-TR30 cell lines. Thus, it’s necessary for us to validate the results in other OC cell lines to increase the reliability of our findings.

**Conclusion**

Collectively, our study demonstrated that PRPF6 promotes metastasis and PTX resistance via the SNHG16-L/CEBPB/GATA3 axis in OC. Our findings demonstrated the significant role of PRPF6, as a novel biomarker for OC treatment.

**Abbreviations**
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<tr>
<td>AS</td>
<td>Alternative splicing</td>
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<tr>
<td>β-tubulin III</td>
<td>Tubulin Beta 3 Class III</td>
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<td>β-actin</td>
<td>Beta Actin</td>
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<td>CEBPB</td>
<td>CCAAT enhancer binding proteins</td>
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<td>Immunohistochemistry</td>
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<tr>
<td>IF</td>
<td>Immunofluorescence</td>
</tr>
<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>Calcium dependent adhesion protein, Neuronal</td>
</tr>
<tr>
<td>PRPF6</td>
<td>Pre-mRNA processing factor</td>
</tr>
<tr>
<td>PTX</td>
<td>Paclitaxel</td>
</tr>
<tr>
<td>RIP</td>
<td>RNA Immunoprecipitation</td>
</tr>
<tr>
<td>SNHG16</td>
<td>Small nucleolar RNA host gene 16</td>
</tr>
<tr>
<td>siRNA</td>
<td>Small-interfering RNA</td>
</tr>
</tbody>
</table>

**Declarations**

**Ethics approval and consent to participate**

All patients have provided signed the informed consent and the experimental protocol was approved by the Institutional Medical Research Ethics Committee of the Shengjing Hospital of China Medical University (2020PS274K-X1).

**Consent for publication**

Not applicable
Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work is supported by The Outstanding Scientific Fund of Shengjing Hospital (Grant No.201705).

Author Contributions

Han Wang, Yingying Zhou and Min Wang designed the experiments. Han Wang analyzed and interpreted the data of the experiments. Han Wang, Siyang Zhang and Ya Qi performed the experiments. The first draft was written by Han Wang. All authors read and approved the final manuscript.

Acknowledgments

Not applicable.

References


Figures
Figure 1

SNHG16-L/S had differential expression in OC cells and tissues

A. ENSEMBL annotation showed that the difference in SNHG16-L/S. B. The expression of SNHG16-L in SKOV3-TR30 cells was lower than that in SKOV3 (0.41±0.05, P=0.000). The expression of SNHG16-S in SKOV3-TR30 was higher than that in SKOV3 (1.70±0.28, P=0.016). C. Compared with chemosensitive tissues, SNHG16-L had lower expression in chemoresistant OC tissues (P=0.006), while SNHG16-S had higher expression (P=0.000).
SNHG16-L/S had a different effect on the tumorigenesis, and PTX resistance in OC

A. Transwell assays showed that OE-SNHG16-L in SKOV3-TR30 inhibited cell migration and invasion (p = P=0.007, P=0.001). OE-SNHG16-S in SKOV3 promoted cell migration and invasion (P=0.003, P=0.002). B. Colony formation assay showed that OE-SNHG16-L inhibited cell growth (0.50±0.05, P = 0.005). OE-SNHG16-S could promoted cell growth (2.57±0.18, P=0.000). C. CCK8 assays showed that OE-SNHG16-L
could inhibit cell proliferation (P < 0.05). OE-SNHG16-S promoted cell proliferation (P < 0.05). D. Compared with the NC group, OE-SNHG16-L decreased PTX resistance activity (P < 0.05). OE-SNHG16-S increased PTX resistance activity detected by CCK8 assays (P < 0.05). E. Western blotting showed that OE-SNHG16-L in SKOV3-TR30 cells decreased the expression of N-cadherin, β-tubulin III, and vimentin, and increased E-cadherin expression, P < 0.05. OE-SNHG16-S in SKOV3 cells increased the expression of N-cadherin, β-tubulin III, and vimentin, and decreased E-cadherin expression, P < 0.05.
SNHG16-L bound to CEBPB and inhibited the transcriptional activity GATA3 to further inhibit metastasis and PTX resistance of OC

A. Western blotting showed that the OE-GATA3 upregulated N-cadherin, β-tubulin III, and vimentin and downregulated E-cadherin (P<0.05). B. Overexpression of SNHG16-L and GATA3 in SKOV3 cells, CCK8 assays showed that overexpression of SNHG16-L and GATA3 could reverse the inhibition of PTX resistance caused by overexpression of SNHG16-L (P<0.05). C. Transwell assays showed that overexpression of SNHG16-L and GATA3 could reverse the inhibition of cell migration and invasion caused by overexpression of SNHG16-L (P<0.05). D. Western blotting showed that overexpression of SNHG16-L could reverse the inhibition of cell migration and invasion caused by overexpression of SNHG16-L downregulated GATA3, N-cadherin, vimentin, and β-tubulin III, and upregulated E-cadherin. Overexpression SNHG16-L and GATA3 reversed the effects (P<0.05). E. the RIP assays confirmed that CEBPB proteins bound to SNHG16-L, not SNHG16-S. F. A schematic illustration of the proximal region between CEBPB and GATA3 promoter. G. ChIP detection in SKOV3-TR30 showed that CEBPB can bind to the GATA3 promoter. H. Dual-luciferase reporter assay were performed in SKOV3 cells which were transfected with OE-CEBPB, GATA3-WT and GATA3-MUT. I. Dual-luciferase reporter assay was performed after transfected with OE-SNHG16-L, OE-CEBPB, GATA3-WT and GATA3-MUT.
Figure 4

PRPF6 was upregulated in chemo-resistant tissues and was related to the advanced FIGO stages in OC.

A. The analysis of RNA interaction predicted on catRAPID omics the putative binding sequence of SNHG16 pre-mRNA to PRPF6 protein. B. PRPF6 was higher expressed in OC tissues than that in normal tissues through TCGA and GTEx data. C. PRPF6 expressed higher in SKOV3-TR30 cells than that in SKOV3, analyzed by RT-PCR. D. PRPF6 expression was higher in chemoresistant OC tissues (N=31) than that in chemosensitive tissues (N=37), examined by IHC.
Figure 5

PRPF6 promoted tumorigenesis, and PTX-resistance *in vitro* and *in vivo*

A. CCK8 assays showed si-PRPF6 in SKOV3-TR30 inhibited cell growth and OE-PRPF6 in SKOV3 cells promoted cell growth (P<0.05). B. Transwell assays indicated that siPRPF6 inhibited cell migration and invasion (P=0.000, P=0.000), while OE-PRPF6 had the opposite effects (P=0.001, P=0.001). C. PTX
resistance were examined by CCK8 cell viability assays. D. The colony formation analysis showed that si-PRPF6 inhibited cell growth (0.04±0.02 P=0.000), while OE-PRPF6 was the opposite (2.08±0.25 P=0.002). E. GATA3, N-cadherin, E-cadherin, β-tubulin III, and vimentin were detected by western blot. F. SKOV3 cells were stably transfected with lentivirus containing the siPRPF6 sequence. The cells were injected in 4-week-old female BALB/cA-nu mice (N=3/group). PTX (20 mg/kg) or saline was injected into the tumor every 3 days for 3 weeks. Images of tumors were pictured. G. Tumor expansion was measured every 3 days (tumor volume=length ×width2/2). H. Tumor weight was measured. G. IHC was used to detect PRPF6 expression in tumor tissues.
Figure 6

PRPF6 induced the AS of SNHG16 to upregulate GATA3 expression to promote metastasis and PTX resistance.

A. siPRPF6 in SKOV3-TR30 and OE-PRPF6 in SKOV3 affect SNHG16-L/S expression. B. The RIP test using PRPF6-specific antibody was conducted in SKOV3-TR30 cells and the co-precipitated RNAs were
determined by RT-PCR. C. Transwell assay was used to detect cell migration and invasion after transfection of OE-PRPF6 and OE-SNHG16-L. D. CCK8 assay to detect PTX resistance. E. IHC was used to detect GATA3 expression in mice tumor tissues. F. GATA3, N-cadherin, E-cadherin, β-tubulin III, and vimentin expression was examined by western blotting after overexpression of PRPF6 and SNHG16-L. G. A scheme of the proposed mechanisms, PRPF6 induced AS of SNHG16 to promote EMT and PTX resistance via SNHG16-L/CEBPB/GATA3 axis in OC.

**Supplementary Files**

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- SupplementaryFigure.2.tif