

Original Research

# LncRNA *PART1* Regulates Ovarian Carcinoma Development via the miR-150-5p/*MYB* Axis

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## Abstract

**Background:** Over the past few years, there have been many reports on the abnormal expression and functional relevance of long non-coding RNAs (lncRNAs) in tumors. The role played by lncRNAs in epithelial ovarian carcinoma (EOC) remains poorly understood, however the goal of the present work was to study molecular mechanisms that underlie involvement of prostate androgen-regulated transcript 1 (*PART1*) lncRNA in EOC development. **Methods:** A total of 25 tumor and 17 normal specimens were obtained from women undergoing surgery between 2015 and 2019 in the Second Affiliated Hospital, Nanjing Medical University. Expression levels for *PART1* in EOC tissue and EOC cell lines were assessed using qRT-PCR. Assays for CCK-8, trans-well, colony forming and western blotting were used to investigate *PART1*, miR-150-5p and *MYB* (*MYB* proto-oncogene) for their involvement in EOC cell proliferation, migration and invasion. Luciferase reporter gene assay was also performed to investigate biological functions of *PART1*, miR-150-5p and *MYB* in EOC, and an animal xenograft model was employed to test tumorigenicity. **Results:** *PART1* expression was increased in EOC relative to normal cells and correlated with EOC cell proliferation, migration and invasion. *PART1* can sponge miR-150, thereby inhibiting growth of EOC by targeting *MYB*. The xenograft mouse model revealed that *PART1* can regulate tumorigenesis *in vivo*. **Conclusions:** The *PART1*/miR-150/*MYB* axis is involved in EOC pathogenesis and could represent a new target to use in diagnosis and therapy.

**Keywords:** ovarian carcinoma; long non-coding RNAs; *PART1*; ceRNA

## 1. Introduction

Epithelial ovarian carcinoma (EOC) accounts for a large proportion of gynecologic malignancies, with an annual incidence of 3–12 new cases per 100,000 women [1,2]. The most common treatment for EOC is maximum surgical cytoreduction combined with platinum chemotherapy. Despite the advances in surgery and chemoradiotherapy, 5-year survival rates for EOC patients are still poor due to the relatively late diagnosis of this disease and to acquired drug resistance [3–6]. It is therefore crucial to identify the molecular mechanism associated with the development of EOC.

A large-scale human genomic sequencing study showed that just 2% is transcribed into RNA that codes for protein. Most of the remaining RNA transcripts are non-coding (ncRNA) [7]. Long ncRNA (lncRNA) is defined as non-coding transcript >200 nucleotides(nt) long [8]. The use of lncRNA as possible biomarkers in different tumor types has been proposed [9,10]. Several studies have also reported an imbalance between lncRNAs and microRNAs (miRNAs) in lung, hepatic, pancreatic and gas-

tric cancers, and recently in EOC [11–16]. Other workers have shown that dysregulation between lncRNA and miRNA levels is closely correlated with several biological behaviors in cancer cells. These include aberrant proliferation, invasion and migration of cells, as well as chemical resistance. Such properties could be mediated through the signaling pathway for lncRNA-miRNA-mRNA in what is referred to as the competing endogenous RNA (ceRNA) hypothesis [17–19]. Expression of the lncRNA *HOXD-ASI*, is elevated in EOC, for example. This lncRNA was found to facilitate EOC cell invasion and migration, in addition to epithelial-mesenchymal transition, via *HOXD-ASI*/miR-186-5p/*PIK3R3* signaling pathways [20], such as the *ADAMTS9-AS2*/miR-182-5p/*FOXF2* pathway [21].

Here, we identified *PART1*/miR-150-5p/*MYB* axis in EOCs was identified, as well as the presence of a ceRNA mechanism. This axis was shown to have a major impact on EOC progression.



**Table 1. LncRNA *PART1* and the Clinicopathological Features of epithelial ovarian carcinoma (EOC) Patients.**

Variables	Cases (25)	LncRNA <i>PART1</i> expressional level		<i>P</i>
		Low/Negative	High	
		<3.994	≥3.994	
Age (years)				>0.05
≤55	11	6	5	
>55	14	6	8	
FIGO stage				<0.05
I–II	10	8	2	
III–IV	15	4	11	
Histological grade				<0.05
1–2	13	9	4	
3	12	3	9	

FIGO stage, International Federation of Gynecology and Obstetrics stage.

## 2. Material and Methods

### 2.1 Subjects and EOC Clinical Specimens

Tumor (n = 25) and normal (n = 17) tissue were obtained from women undergoing surgery for EOC between 2015 and 2019 at our institution. None had received pre-operative chemoradiotherapy. Histopathology was used to confirm specimens prior to storage in a liquid nitrogen tank at -196 °C until the extraction of RNA. All patients gave informed consent. The major clinicopathological features of cohort are indicated below (Table 1). For the relationship between *MYB*, miR-150-5p and clinicopathological features, see **Supplementary Tables 1,2**. Ethical approval for the study was given by the host institute (No. 2017AE02133, approval date December 3, 2017) and the Declaration of Helsinki guidelines were followed.

### 2.2 Cell Culture and Treatments

The human cell lines studied here were provided by the Chinese Science Academy (Shanghai, China) and Zhong Qiao Xin Zhou (Shanghai, China). These consisted of normal ovarian surface epithelial cells (IOSE80, ZQ0721, Zhong Qiao Xin Zhou, Shanghai, China) and three EOC cell lines ((A2780, ZQ0486, Zhong Qiao Xin Zhou, Shanghai, China), (SKOV3, TCHu185, Chinese Science Academy, Shanghai, China) and (OVCAR3, TCHu228, Chinese Science Academy, Shanghai, China)). SKOV3 was grown in McCoy's 5A medium (Gibco, New York, USA) with 10% fetal bovine serum (FBS), while remaining cell lines were grown in Dulbecco's modified Eagle medium (DMEM) with high-glucose and 10% FBS. Cells were kept at 37 °C in a humidified incubator with 5% CO<sub>2</sub> concentration. Mycoplasma testing has been done for the cell lines used. The cell lines used have been authenticated and Short Tandem Repeat (STR) was used for the authentication.

### 2.3 RNA Isolation for Quantitative RT-PCR (qRT-PCR)

RNA was extracted with Trizol (Invitrogen, Carlsbad, USA) and cDNA for qPCR was prepared using HiScript II

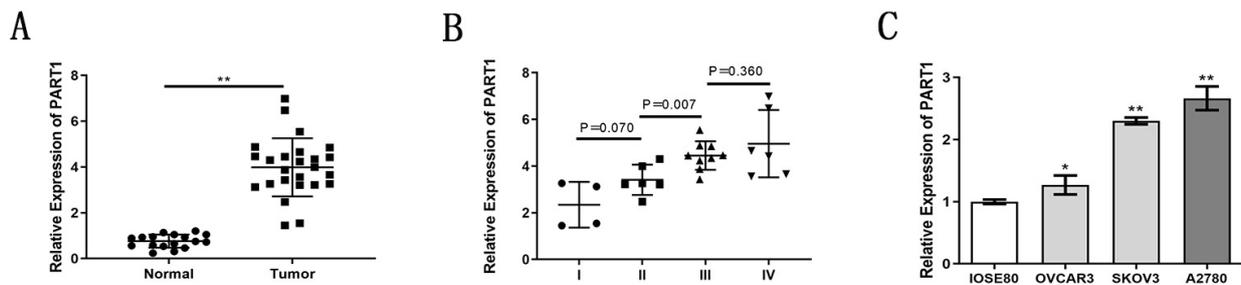
SuperMix (Vazyme, Nanjing, China). qRT-PCR (Q6, Life) was carried out with SYBR® Green PCR Master Mix (Applied Biosystems, Waltham, USA) and primer sequences as follow: *PART1* forward 5'CTCCTGCGGTTTCCCATT3', reverse 5'ATCTCACCAGACACCTGCCTAC3'; miR-150-5p forward 5'CGGGCTCTCCCAACCCTTGT3', reverse 5'CAGCCACAAAAGAGCACAAAT3'; *MYB* forward 5'GGCACACAAGAGACTGGGGA3', reverse 5'CGACCTTCCGACGCATTGTA3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH) forward 5'ACCCACTCCTCCACCTTTGAC3', reverse 5'TGTTGCTGTAGCCAAATTCGTT3'; U6 forward 5'CTCGCTTCGGCAGCACAA3', reverse 5'AACGCTTCACGAATTTGCGT3'. The cycling conditions were: stage 1 (pre-denaturation): 95 °C, 10 min; stage 2 (cyclic reaction, 40 times): 95 °C for 10 sec; 60 °C for 30 sec; stage 3 (dissolution curve): 95 °C for 15 sec; 60 °C for 60 sec; 95 °C for 15 sec. Expression levels for lncRNA, miRNA and mRNA were detected quantitatively by the 2<sup>-ΔΔCt</sup> method [22], with GAPDH the internal references.

### 2.4 Predicting Target Gene

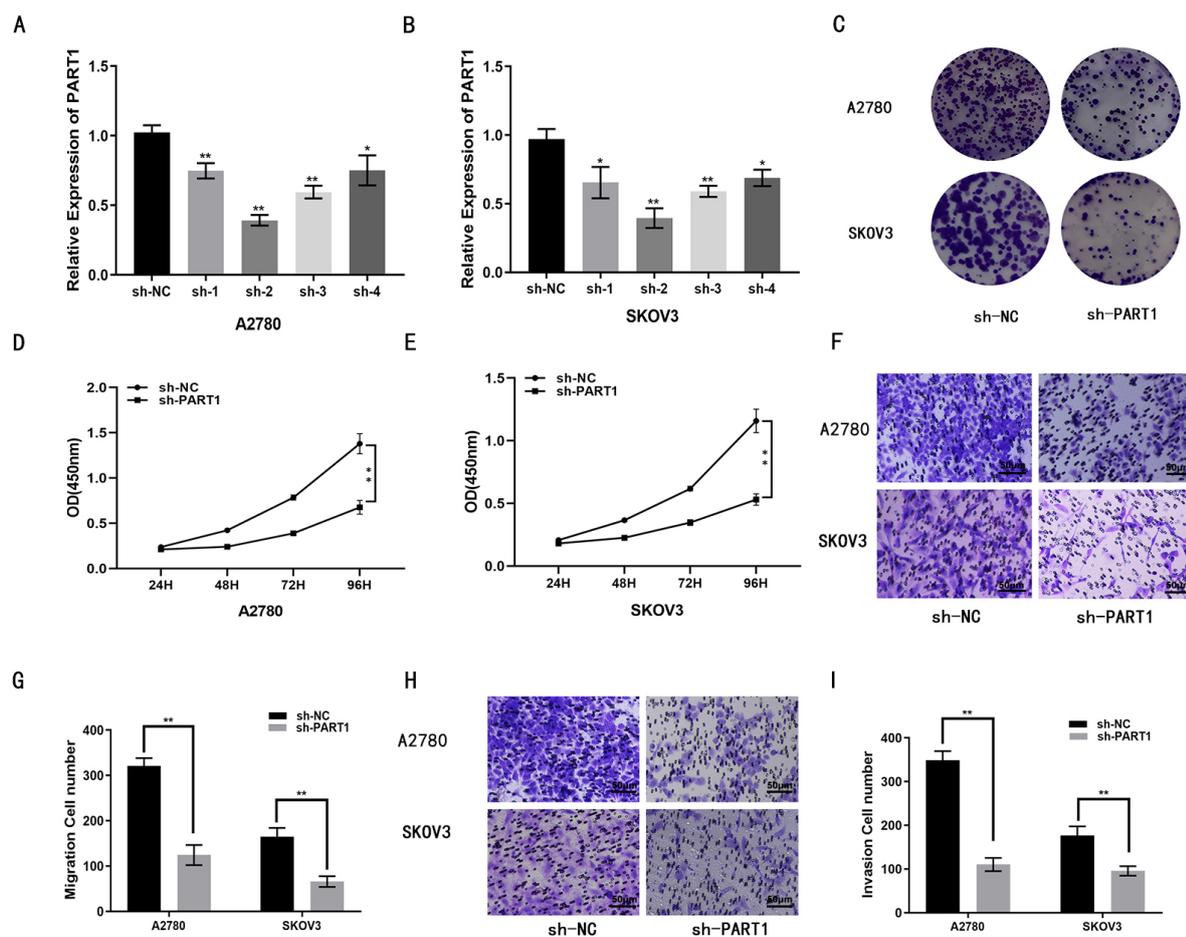
StarBase v3.0 (<https://starbase.sysu.edu.cn/index.php>) and miRcode (<http://www.mircode.org/index.php>) were employed to predict potential miRNAs that bind to *PART1* [23,24]. StarBase v3.0 was also used to identify candidate mRNAs that bind to miR-150-5p. This software integrates 5 different miRNA prediction softwares (TargetScan, PicTar, microT, miRmap, miRanda/mirSVR). The GEPIA database was used to analyze the expression levels of the target genes for miR-150-5p [25].

### 2.5 Lentivirus, siRNA, miR-150-5p Inhibitor and Transfection

*PART1*-shRNA-lentiviruses (sequence: sh1:GAAC TCAATTACGACTACATA; sh2:CCAGAUGAGACUAC GAUAATT; sh3:GAACAGAGUUGACUUUGUGTT; sh4:GCAAAGUAUCCAAGACCAATT) used for the



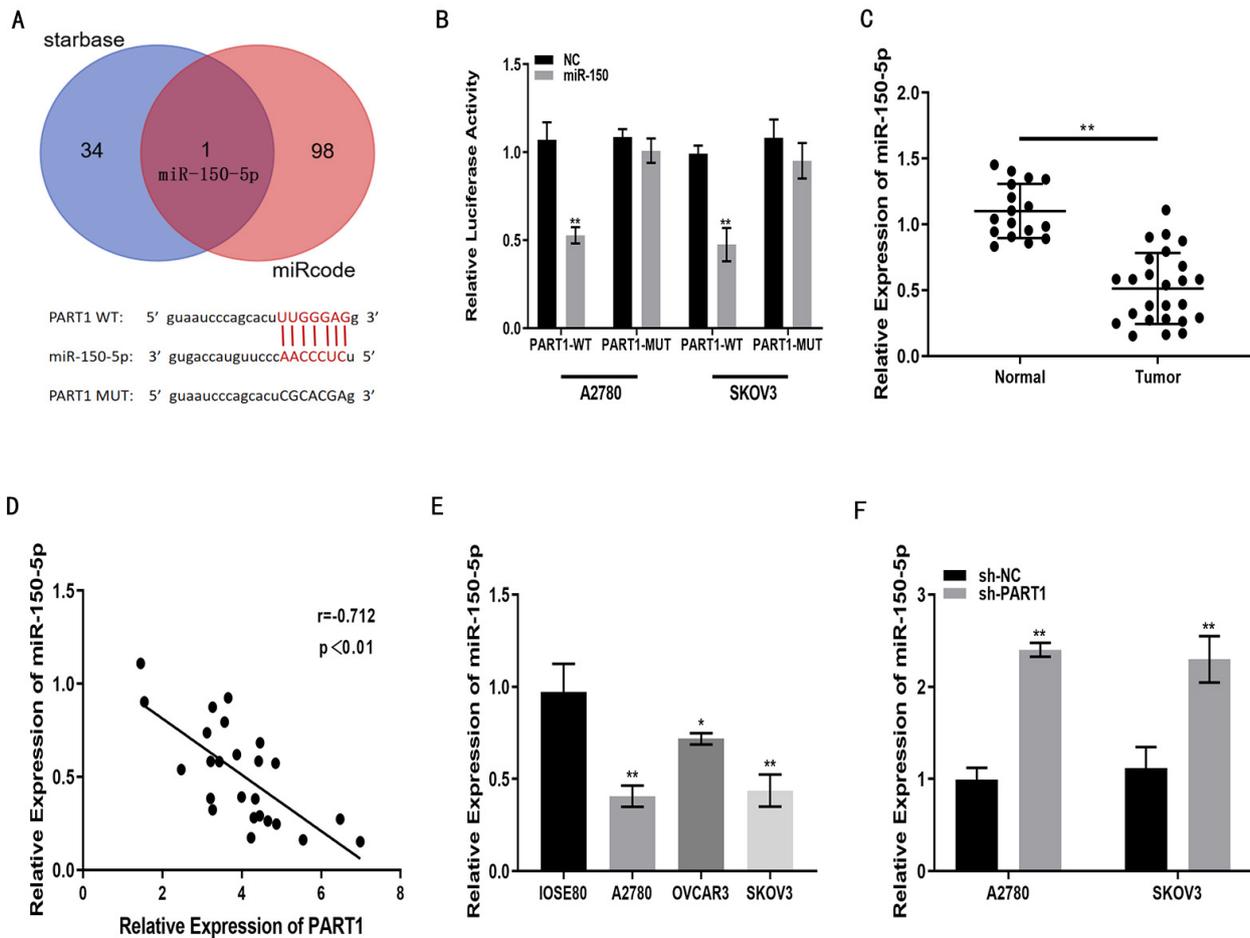
**Fig. 1. Elevated expression of lncRNA *PART1* in EOC detected with qRT-PCR.** (A) *PART1* expression in EOC and normal ovarian tissue. (B) *PART1* expression in EOCs at different FIGO stages. (C) *PART1* expression in EOC (OVCAR3, SKOV3, A2780) and ovarian surface epithelial (OSE) (IOSE80) cells. \* $p < 0.05$ , \*\* $p < 0.01$ .



**Fig. 2. LncRNA *PART1* knockdown reduces the proliferation, migration and invasion of A2780 and SKOV3 cells.** (A,B) *PART1* expression in cells transfected with sh-*PART1* (including sh-*PART1*-1, sh-*PART1*-2, sh-*PART1*-3 and sh-*PART1*-4) as measured by qRT-PCR. (C) Proliferation of cells following transfection with sh-*PART1* or sh-NC (control), as determined using colony-forming assay. (D,E) Proliferation of cells following transfection with sh-*PART1* or sh-NC, as determined by CCK8 assay. (F,G) Migration of cells following transfection with sh-*PART1* or sh-NC, as determined by transwell assay. (H,I) Invasion of cells following transfection with sh-*PART1* or sh-NC, shown using transwell assay. \* $p < 0.05$ , \*\* $p < 0.01$ .

*in vivo* experiments were provided by GenePharma (Shanghai, China), as were the siRNAs that target *PART1*. Transfection of cells was performed with Lipofectamine 3000 (Invitrogen, Carlsbad, USA). MiR-150-5p inhibitors

(sequence: CACUGGUACAAGGGUUGGGAGA) were also from GenePharma. Triplicate experiments were performed throughout.



**Fig. 3. LncRNA *PART1* can sponge miR-150-5p.** (A) Putative binding sites between miR-150-5p and *PART1* identified with online databases. (B) miR-150-5p reduces luciferase activity of *PART1*-WT, but not *PART1*-MUT, as shown by luciferase reporter gene assay. (C) miR-150-5p expression in EOC and normal ovarian tissue, as quantified by qRT-PCR. (D) Correlation analysis of relative expression levels for *PART1* and miR-150-5p in clinical specimens. (E) miR-150-5p expression levels in EOC cells (OVCAR3, SKOV3, A2780) and OSE cells (IOSE80), as measured with qRT-PCR. (F) miR-150-5p expression in A2780 and SKOV3 cells after transfection with sh-*PART1* or with sh-NC (negative control), as measured with qRT-PCR. \* $p < 0.05$ , \*\* $p < 0.01$ .

## 2.6 CCK-8 Assay

CCK-8 assay was used to quantify the proliferation rate of cells. Following inoculation into 96-well culture plates ( $1.5 \times 10^3$  cells/well), cells were grown in 100  $\mu$ L DMEM containing 10% FBS. CCK-8 solution (10  $\mu$ L) was added 0, 24, 48, 72 and 96 h after cellular attachment. The 450 nm absorbance was subsequently read following 2 h of incubation by Enzyme label (Multiskan FC, Thermo, Waltham, USA).

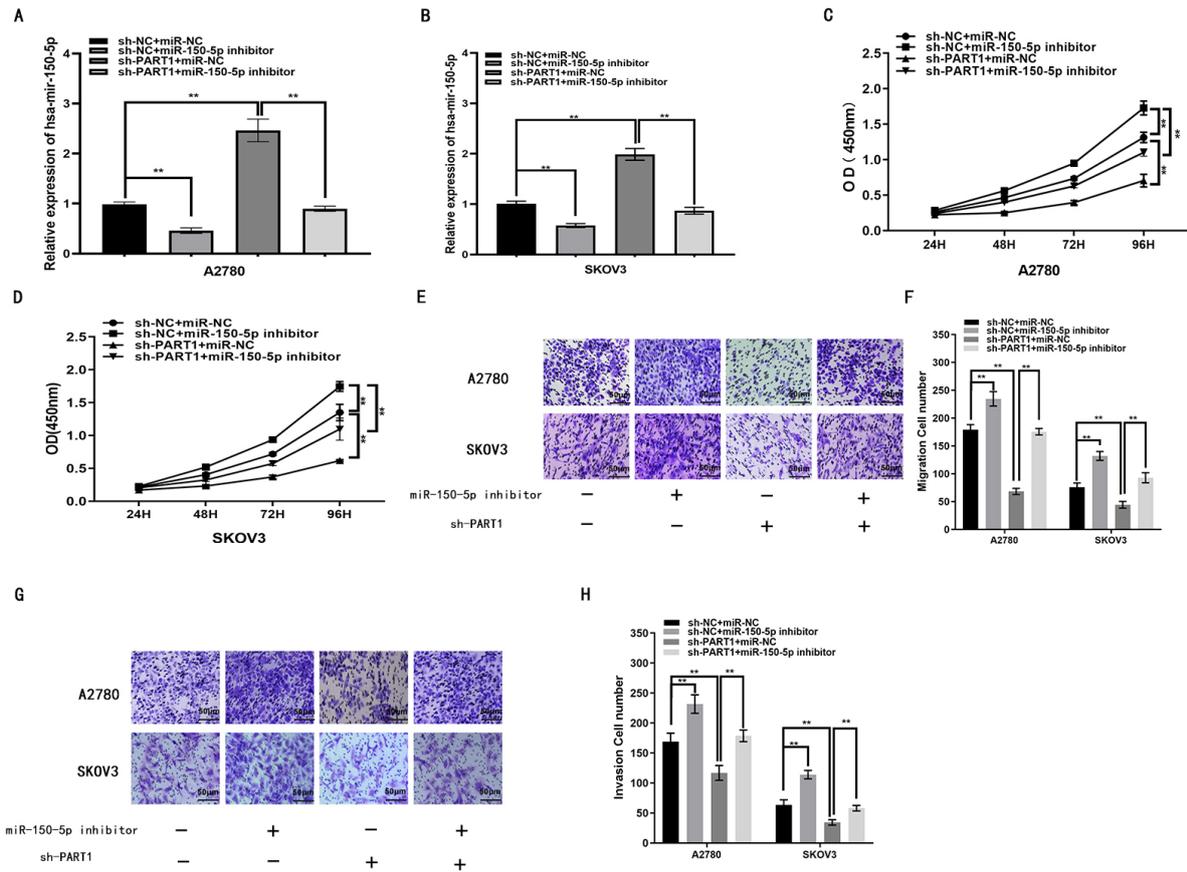
## 2.7 Transwell Assays

Transwell chambers (Millipore, Darmstadt, Germany) used for cell invasion assays were first pretreated with 50  $\mu$ L of a 1:9 Matrigel/DMEM solution (BD, New Jersey, USA). Subsequently,  $1 \times 10^5$  cells were dispersed in DMEM without FBS (1 mL) and 200  $\mu$ L of cellular solution was placed in the upper chamber. Following this, DMEM containing 10% FBS (600  $\mu$ L) was placed into the lower

chamber to act as a chemotactic agent. 48 h later, residual cells in the upper chamber were scraped off, while invading cells were immobilized in 4% paraformaldehyde then dyed in 2% crystal violet. The invading cells were counted by light microscopy (D-35578, Leica, Weztlar, Germany). The experimental procedure used to quantify the migration of cells was identical to the above, but with no Matrigel pretreatment.

## 2.8 Colony Formation Assay

Cells were dispersed into a single-cell suspension at 48 h after transfected. The colony forming assay was carried out by incubating  $1 \times 10^3$  cells at 37  $^{\circ}$ C for two weeks in a culture dish containing 10% FBS medium. The cells were then stabilized, dyed using 0.1% crystal violet, and the colonies counted manually. Experiments were repeated three times for all groups.



**Fig. 4. LncRNA *PART1* enhances the progression of EOC by binding miR-150-5p.** (A,B) miR-150-5p expression in A2780 and SKOV3 cells after transfection with miR-150-5p inhibitor, sh-*PART1* or the corresponding controls, as determined with qRT-PCR. (C,D) A2780 and SKOV3 cell proliferation after transfection with miR-150-5p inhibitor, sh-*PART1* or the corresponding controls, as measured with the CCK8 assay. (E,F) Migration of A2780 and SKOV3 cells following transfection with miR-150-5p inhibitor, sh-*PART1* or the corresponding controls, as measured with the transwell assay. (G,H) Invasion of A2780 and SKOV3 cells following transfection with miR-150-5p inhibitor, sh-*PART1* or the corresponding controls, as measured with the transwell assay. \* $p < 0.05$ , \*\* $p < 0.01$ .

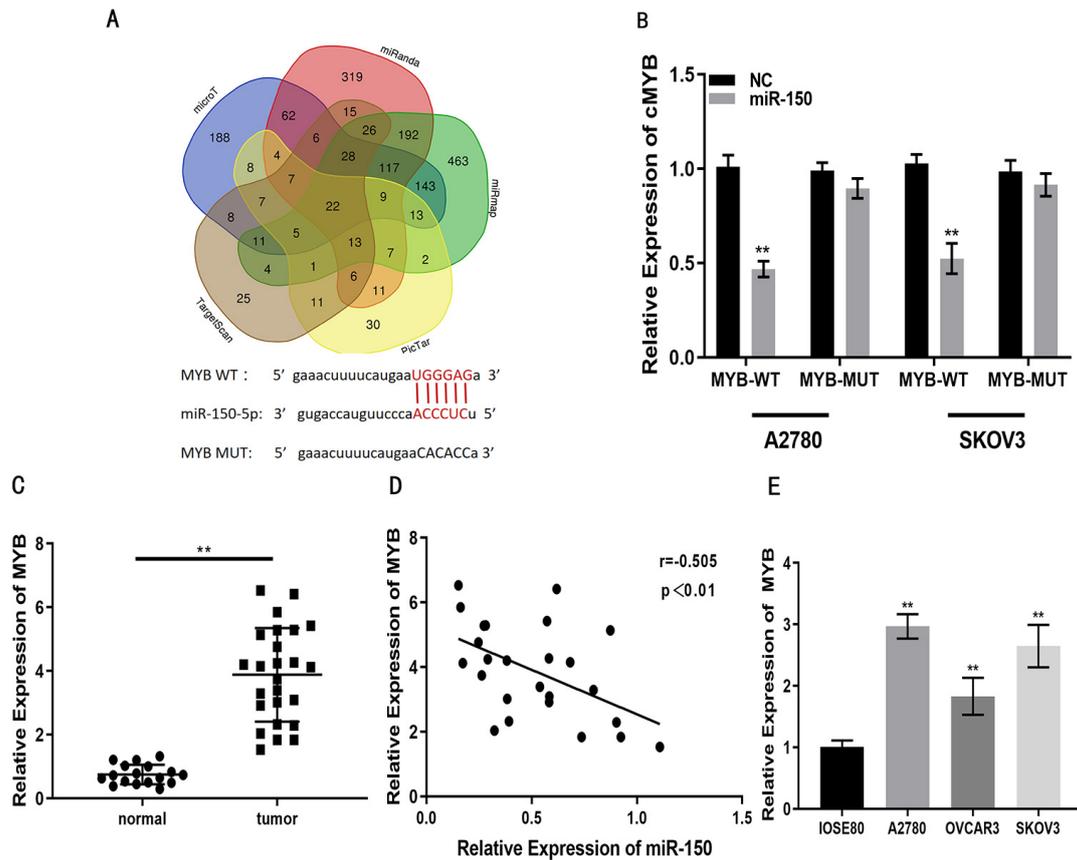
### 2.9 Western Blotting Assay

Five  $\times 10^5$  cells per well were inoculated in 6-well plates, grown for 48 h, then rinsed in PBS and subsequently immersed in ice-cold lysis buffer. The concentration of protein in the lysate was quantified with the bicinchoninic acid (BCA) method (P0012S, Beyotime, Shanghai, China). Protein from each sample was separated with 10% SDS-PAGE then transferred to polyvinylidene fluoride film (162017, Bio-Rad, Hercules, USA). The film was subsequently incubated for 2 h with a solution of 5% non-fat dried milk in TBS with 0.1% Tween-20 to block non-specific proteins. *MYB*- (1:2000, ab109127, abcam, Cambridgeshire, UK) or GAPDH-specific antibody (1:5000, 60004-1-Ig, Proteintech, Wuhan, China) was then incubated with the polyvinylidene fluoride film for 24 h at 4 °C. The film was subsequently rinsed before incubation for 2 h with horseradish peroxidase (HRP)-conjugated sec-

ondary antibody (1:5000, Proteintech, 60004-1-Ig, Wuhan, China) at 37 °C. Immunoblot analysis was performed using an enhanced chemiluminescence reagent (34579, Invitrogen, Carlsbad, USA) and the film was subsequently irradiated with X-rays by WB Imaging Instrument (5260, Tanon, Shanghai, China). Image J software (1.8.0, NIH, USA) was used to analyse protein expression levels, with GAPDH used as the reference.

### 2.10 Dual-Luciferase Reporter Gene Assay

A2780 and SKOV3 cell lines were grown in 6-well plates. Wild-type (WT) and mutant fragments (MUT) from the 3'-untranslated region of *PART1* (*PART1*- WT: GUAAUCCAGCACUUUGGGAGG; *PART1*-MUT: GUAAUCCAGCACUCGCA CGAG) and *MYB* (*MYB*-WT: GAAACUUUCAUGAAUGGGAGA; *MYB*-MUT: GAAACUUUCAUGAACACACCA) that were related



**Fig. 5. MYB is a target for miR-150-5p.** (A) Putative binding sites for miR-150-5p on MYB, identified with online databases. (B) miR-150-5p reduces luciferase activity of MYB-WT in EOC cell lines, but not for MYB-MUT, as shown by luciferase reporter assay. (C) MYB expression levels in EOC and normal ovarian tissue, as measured with qRT-PCR. (D) Correlation between MYB and miR-150-5p expression in clinical specimens, as determined using Pearson's analysis. (E) MYB expression in EOC (OVCAR3, SKOV3, A2780) and OSE (IOSE80) cells, as measured with qRT-PCR.  $**p < 0.01$ .

to the binding site for miR-150-5p were synthesized and added to pMIR-REPORT vectors. Reporter gene assay was conducted as per our previous study [26]. Normalization of luciferase activity was carried out in relation with Renilla luciferase activity.

### 2.11 Xenograft Model

Female BALB/c nude mice aged 4–5 weeks were obtained from Animal Core Facility, Nanjing Medical University. Each mouse (5 mice per group) received a subcutaneous injection in the right armpit with a 200  $\mu$ L suspension containing  $5 \times 10^5$  A2780 ovarian cancer cells. Tumor growth rate was evaluated at regular intervals starting from the 6th day. Tumor size was quantified with vernier calipers, where L was the longest diameter and W the longest transverse diameter perpendicular to the longest diameter when the tumor is viewed as an ellipse. Tumor volume was subsequently estimated with the formula:  $V = \pi/6 \times L \times W \times W$ . Animal experimentation conformed with the guidelines for “Animal Research: Reporting of *In Vivo* Experiments (ARRIVE)”. The animal ethics approval number was 1811051, and the approval date was April 2018.

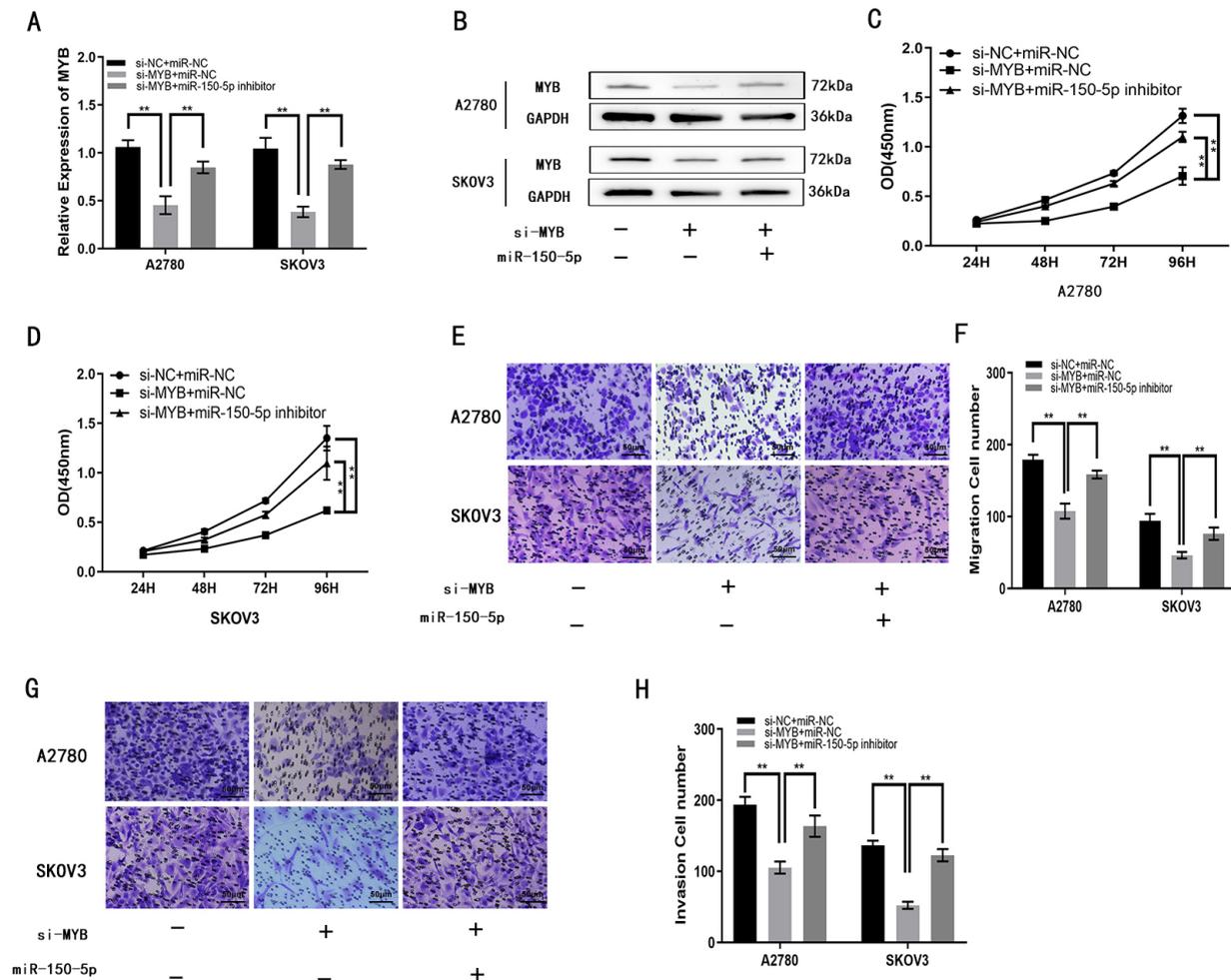
### 2.12 Statistical Analyses

Experiments were conducted in triplicate. SPSS 15.0 (IBM, Chicago, USA) was used for statistical analyses, with values expressed as the mean  $\pm$  standard deviation (SD). One-way-ANOVA analysis of variance was used to evaluate the differences among at least three groups. Least Significance Difference (LSD) was used with ANOVA. Student's *t* test was used to determine the differences between groups. Pearson correlation analysis was used to analyse correlations between data. The normal distribution was tested using the Shapiro-Wilk (S-W test) by SPSS. *p*-values  $< 0.05$  were considered to show statistical significance.

## 3. Results

### 3.1 Increased LncRNA PART1 Expression in Ovarian Carcinoma

LncRNA PART1 expression in clinical specimens was quantified by qRT-PCR. Fig. 1A shows the expression was significantly higher in EOC relative to normal ovarian surface epithelial (OSE) tissue. LncRNA PART1 expression was also higher in advanced stage EOC relative to lower



**Fig. 6. MYB enhances the proliferation, migration and invasion of A2780 and SKOV3 cells *in vitro*.** (A,B) MYB expression in A2780 and SKOV3 cells following transfection with si-MYB, or si-MYB plus miR-150-5p inhibitor, as measured with qRT-PCR and Western blotting. (C,D) Proliferation of A2780 and SKOV3 cells following transfection with si-MYB, or si-MYB plus miR-150-5p inhibitor, as measured with the CCK8 assay. (E,F) Migration of A2780 and SKOV3 cells following transfection with si-MYB, or si-MYB plus miR-150-5p inhibitor, as determined using the transwell assay. (G,H) Invasion by A2780 and SKOV3 cells following transfection with si-MYB, or si-MYB plus miR-150-5p inhibitor, as measured using transwell assays. \*\* $p < 0.01$ .

stage tumors (Fig. 1B). Finally, the expression level in three EOC cell lines (OVCAR3, SKOV3, A2780) was greater relative to the OSE cell line (IOSE80), particularly in SKOV3 and A2780 cells (Fig. 1C).

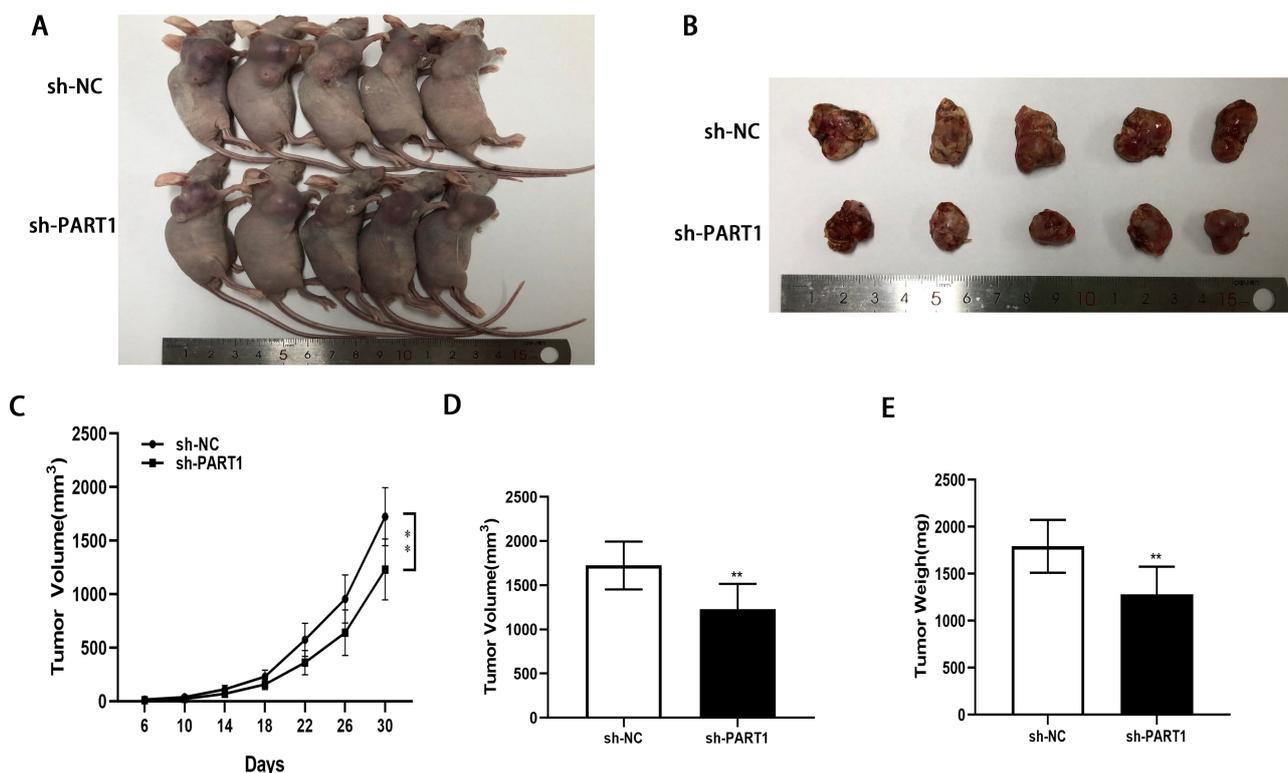
### 3.2 LncRNA PART1 Knockdown Reduces EOC Cell Proliferation, Migration and Invasion

PART1 expression in SKOV3 and A2780 cells was reduced following transfection with a knockdown vector (sh-PART1). A control vector (sh-NC) was also used, and transfection efficiency confirmed using qRT-PCR. Among them, sh-1, sh-2, sh-3 and sh-4 were compared with sh-NC, respectively, and sh-2 had the best transfection efficiency (Fig. 2A,B). Results from colony forming assays and CCK-

8 assays demonstrated that lncRNA PART1 knockdown inhibited EOC cell proliferation (Fig. 2C–E). Furthermore, transwell assay revealed that lncRNA PART1 knockdown also inhibited the migration and invasion ability of cells (Fig. 2F–I). Hence, the above results imply that PART1 enhances the proliferation, migration and invasion of EOC cells *in vitro*.

### 3.3 LncRNA PART1 Sponges miR-150-5p

The findings from starBase revealed the presence of binding sites between lncRNA PART1 and miR-150-5p (Fig. 3A). Luciferase reporter gene assay also demonstrated that miR-150-5p decreased luciferase activity in lncRNA PART1-WT cells, but not lncRNA PART1-MUT



**Fig. 7. *MYB* promotes *in vivo* growth of EOC.** (A,B) EOC growth in mice injected with A2780 cells that had been transfected with sh-*PART1* or with sh-NC. (C–E) *PART1* downregulation resulted in a lower tumor growth rate (volume and weight) than observed in controls. \*\* $p < 0.01$ .

cells (Fig. 3B). miR-150-5p expression was significantly reduced in EOC tissue relative to normal tissue (Fig. 3C). Pearson's analysis demonstrated an inverse correlation between *PART1* and miR-150-5p levels in EOC tissue (Fig. 3D). Similar results to EOC tissues were obtained for miR-150-5p expression in OSE and EOC cells. The expression levels in the three EOC cell lines (OVCAR3, SKOV3, A2780) were lower than those in the OSE80 cell line, especially in SKOV3 and A2780 cells (Fig. 3E). miR-150-5p expression was increased in SKOV3 and A2780 cells in which lncRNA *PART1* was down-regulated (Fig. 3F). Together, these results indicate lncRNA *PART1* is able to sponge miR-150-5p in EOC cells.

#### 3.4 LncRNA *PART1* Enhances EOC Progression by Binding to miR-150-5p

Functional assays were performed to determine whether lncRNA *PART1* acts in A2780 and SKOV3 cells by binding to and therefore effectively removing miR-150-5p. Transfection efficiencies for miR-150-5p inhibitor, sh-*PART1* and corresponding controls were confirmed using qRT-PCR (Fig. 4A,B). CCK-8 assays revealed that lncRNA *PART1* down-regulation decreased EOC cell proliferation. This was partially reduced by miR-150-5p inhibitor (Fig. 4C,D). Similarly, results from transwell assays revealed miR-150-5p inhibitor can attenuate inhibition of migration and invasion caused by lncRNA *PART1*

downregulation (Fig. 4E–H). These findings suggest that lncRNA *PART1* can enhance EOC cell proliferation, migration and invasion through binding and thus inactivation of miR-150-5p.

#### 3.5 MiR-150-5p Inhibits OEC Cell Proliferation, Migration and Invasion by Targeting *MYB*

We investigated miR-150-5p target genes to test the ceRNA hypothesis. 22 genes were screened out by Star-Base v3.0. Then three of these genes, *MTCH2*, *MYB* and *NDC1*, were found to be elevated in ovarian cancer tissues relative to normal ovarian tissues through the GEPIA database (Supplementary Fig. 1). *MYB* is one of the more classical, malignant progression-promoting oncogenes in a variety of tumors, such as: breast, liver, colon and lung cancers [27–30]. Next, *MYB* was selected as the object of study (Fig. 5A). The luciferase reporter gene assay revealed that miR-150-5p decreased luciferase activity for *MYB*-WT, but there was no significant effect for *MYB*-MUT (Fig. 5B). The expression of *MYB* was elevated in EOC compared to OSE tissue (Fig. 5C). Moreover, *MYB* expression was inversely associated miR-150-5p expression in EOC specimens, as shown by Pearson's correlation analysis (Fig. 5D). Three EOC cell lines (OVCAR3, SKOV3, A2780) were compared with OSE cell line (OSE80), respectively. qRT-PCR also demonstrated that *MYB* expression in EOC cells was higher than in OSE cells (Fig. 5E). Together, the data indi-

## LncRNA PART1/miR-150-5p/MYB Axis

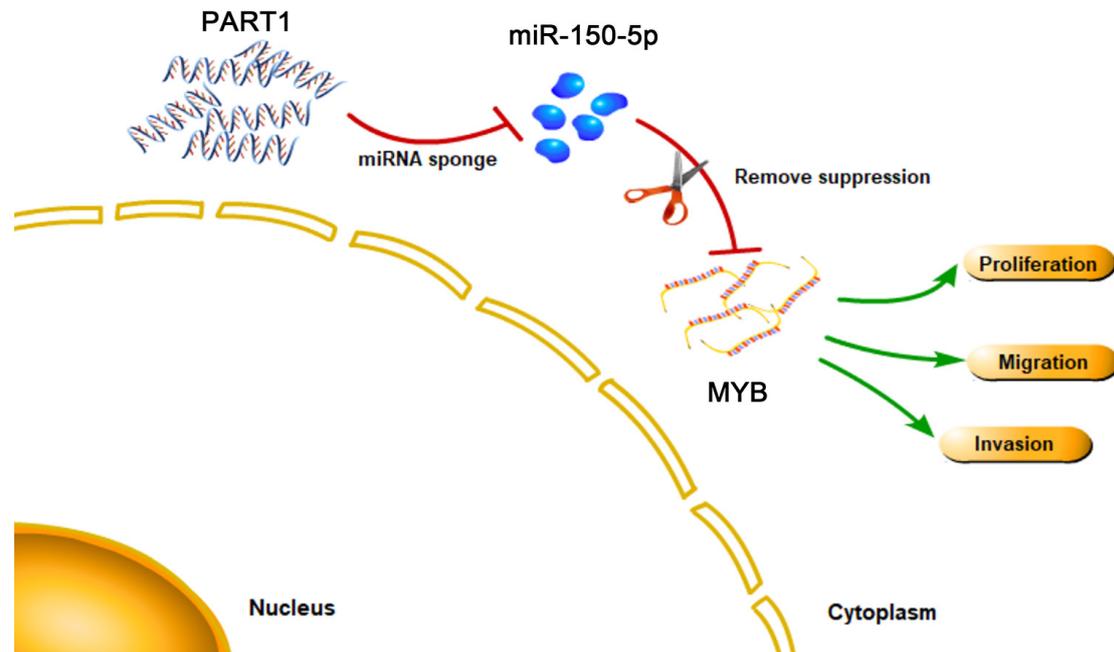


Fig. 8. Schematic model showing the putative role for lncRNA *PART1* in the proliferation, migration and invasion of EOC.

cate that *MYB* is a target for miR-150-5p. Functional assays showed the impacts of si-*MYB* on the proliferation, migration and invasion of EOC cells were partly attenuated by miR-150-5p inhibitor (Fig. 6A–H). Overall, these findings demonstrate that miR-150-5p can reduce proliferation, migration and invasion of EOC cells through targeting *MYB*.

### 3.6 Knockdown of lncRNA *PART1* Suppresses the Growth of Ovarian Carcinoma In Vivo

To investigate whether lncRNA *PART1* is involved in EOC growth *in vivo*, female nude mice received subcutaneous injections of A2780 cells following transfection with sh-*PART1* or sh-NC (negative control). Tumor size was estimated every four days and the tumor growth rate determined after 30 days. sh-*PART1* was found to suppress cancer growth in the nude mice compared to controls (Fig. 7A–C). In addition, mean tumor volume and weight were smaller with sh-*PART1* (Fig. 7D,E). Overall, these findings indicate that lncRNA *PART1* can enhance the growth of EOC *in vivo*.

## 4. Discussion

EOC shows a high degree of malignancy in female patients [31]. The lack of obvious symptoms during the initial stages of EOC mean that is easily be ignored by patients. As a consequence, most women are diagnosed with middle and late stages of this cancer, thus greatly increasing the difficulty of treatment [32,33]. A large body of research has revealed a major role for lncRNAs in the patho-

genesis of human malignancies [34,35]. Novel transcriptome sequencing technology has resulted in the discovery of an ever-increasing number of lncRNAs. There is now strong research evidence showing the involvement of lncRNAs in several biological behaviors including the regulation of gene transcription, epigenetic regulation, ontogenetic regulation, and chromatin modification [36–38]. Several research groups have demonstrated that progression of several tumor types, including EOC, correlates with aberrant expression of lncRNAs [39]. Some lncRNAs have also been suggested as markers for early cancer detection and diagnosis [40–42]. *PART1* is increasingly recognized to be associated in the pathogenesis of many cancer types. Recently, Zhao *et al.* [43] published that lncRNA *PART1* can suppress the growth of esophageal cancer, while Chen *et al.* [44] reported it could also increase proliferation of lung cancer cells. Presently, we confirmed that lncRNA *PART1* expression was elevated in EOC compared to normal tissue. Knockdown of *PART1* expression in EOC cells markedly inhibited EOC cell proliferation, migration and invasion *in vitro*. An EOC xenograft animal model was also established and was used to show that lncRNA *PART1* knockdown lowered the growth rate of xenografts.

Salmena *et al.* [19] demonstrated that lncRNAs could act as a sponge to absorb miRNAs, thereby indirectly regulating mRNA expression. An increasing number of interrelationships in the lncRNA-miRNA-mRNA ceRNA axis have recently been found. Several investigators have suggested a role for ceRNA during progression of multiple tu-

mor types including bladder, colorectal and cervical cancer [45–47]. With major advances in sequencing technology, bioinformatics now plays an important role in both foundational and clinical medicine [48,49]. In the current study, the online databases starbase V3.0 and miRcode were used to identify miR-150-5p as being a downstream target of lncRNA *PART1*. LncRNA *PART1* expression was higher in EOC tissue and cells, but this was decreased by miR-150-5p. Functional studies also revealed that lncRNA *PART1* had oncogene-like properties in EOCs. Both *PART1* sh-RNA and miR-150-5p mimic reduced *MYB*-WT luciferase activity in a reporter gene assay, while the inhibitory action of *PART1* sh-RNA was restored with miR-150 inhibitor. Furthermore, qRT-PCR and Western blot revealed that lncRNA *PART1* knockdown reduced *MYB* expression. This effect was partly attenuated with miR-150 inhibitor. In addition, functional assays showed the effect of sh-*PART1* on the proliferation, migration and invasion ability of EOC cells was abolished with miR-150 inhibitors. These results indicate *PART1* can act like a ceRNA, thus regulating the miR-150/*MYB* axis and hence EOC progression.

This study has several limitations. The study cohort of EOC tissues was relatively small and hence the five different EOC subtypes (low grade plasmacytosis, high grade plasmacytosis, endometrioid, clear cell, mucinous) could not be evaluated individually. The fallopian tube is the main source of high-grade serous ovarian carcinoma (HGSOC), which is the most frequent type of ovarian cancer. Therefore clinical tissue selection cannot be limited to normal ovarian tissue alone. Moreover, the development of ovarian cancer involves complex molecular regulation pathways that need further investigation.

## 5. Conclusions

Our work suggests a tumorigenic role for lncRNA *PART1* in EOC. It has revealed a potential mechanism by which lncRNA *PART1* binds competitively to miR-150-5p, thereby upregulating the downstream oncogene *MYB* (Fig. 8). This research confirms a role for lncRNA *PART1* in the development and pathogenesis of EOC, as well as identifying the likely molecular pathway.

## Availability of Data and Materials

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

## Author Contributions

JW, YH and TZ performed the *in vivo* studies. JW and YH performed the *in vitro* studies. JL and BX contributed to the design and analysis of the study and revised the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the

work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

## Ethics Approval and Consent to Participate

All patients were provided with written informed consent. This study obtained ethical approval from the Second Affiliated Hospital of Nanjing Medical University. All tests were carried out in accordance with the Declaration of Helsinki. Ethics Committee of the Second Affiliated Hospital of Nanjing Medical University, number 2017AE02133.

All animal experiments complied with the Animal Research: Reporting of *In vivo* Experiments (ARRIVE) guidelines. AND all experiments involving use of mice were conducted in accordance with animal protocol (certificate number: 1811051) approved by the Animal Care and Use Committee of the Model Animal Research Center of Key Lab of Reproductive Medicine.

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## Conflict of Interest

The authors declare no conflict of interest.

## Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2810270>.

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