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Original Article

Original Article

RP5-1148A21.3 (lncRP5) exerts oncogenic function in human ovarian carcinoma

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Abstract

Ovarian cancer (OC) is a fatal gynecological malignancy that is difficult to diagnose at early stages. Various long non-coding RNAs (lncRNAs) are aberrantly expressed in OC and exert regulatory effects on OC; however, the underlying mechanism requires in-depth investigation. This work is designed to explore the molecular regulatory axis of a newly identified lncRNA in OC, that is, lncRNA RP5-1148A21.3 (lncRP5). RT-qPCR shows lncRP5 is significantly upregulated in OC patients and cell lines, and it is mainly located in the cytoplasm of OC cells. The results of CCK-8, colony formation, and transwell assays demonstrate that overexpression of lncRP5 greatly contributes to malignant behaviors of OC cells, while inhibition of lncRP5 shows the opposite effects. Moreover, the binding relationship between lncRP5 and miR-545-5p is predicted by bioinformatics and is further verified by luciferase assay. Functionally, the regulatory effects of lncRP5 and miR-545-3p are negatively related; miR-545-5p serves as a tumor suppressor in OC. Further studies demonstrate that PTP4A1 is the target gene of miR-545-5p. Overexpression of PTP4A1 abrogates the inhibitory function of miR-545-5p on OC cell growth and metastasis. The lncRP5/miR-545-5p/PTP4A1 axis is subsequently demonstrated in vivo, and knockdown of lncRP5 notably inhibits tumor growth. This study provides a novel regulatory mechanism of OC, which may contribute to the diagnosis and therapy of OC.

Key words ovarian cancer, miR-545-5p, PTP4A1, RP5-1148A21.3 (lncRP5)

Introduction

Ovarian carcinoma (OC) is a common malignancy of female reproductive organs, ranking second following uterine corpus cancer in incidence among the female reproductive organ cancers [1,2]. OC can be pathologically classified into epithelial ovarian cancer (EOC) and ovarian germ cell malignancies. The former mainly includes serous carcinoma, mucinous carcinoma, clear cell carcinoma and endometrioid carcinoma [3]. OC, especially EOC, is of high mortality and threatens women's lives worldwide [2].

Most EOC patients are diagnosed at stage II/III with tumor cells migrating to the pelvic and abdominal organs because EOC presents limited symptoms at the early stage [4]. The overall five-year survival rate of the advanced EOC patients is only about 30%; however, the overall five-year survival rate of the patients diagnosed at the early stage can reach 70%–90% [5]. Biomarkers have been identified for early diagnosis for EOC, such as serum carbohydrate antigen 125 (CA125) and epididymis protein 4 (HE4). However, the sensitivity and specificity of these biomarkers are still not satisfactory. For instance, CA125 is barely detected at the early stage of ovarian cancers and is also reported to be highly expressed in other conditions like pregnancy or peritoneum inflammatory diseases. Similarly, HE4 is also found to be highly expressed in kidney diseases and tuberculosis. Therefore, exploring novel biomarkers with high sensitivity and specificity for EOC is urgent.

Thousands of long noncoding RNAs (lncRNAs) have been identified from the human genome, which are composed of more than 200 nt and incapable of protein-coding [6,7]. Multi-functions of lncRNAs in regulating gene expression have been reported, mainly through controlling DNA transcription, mRNA translation, and post-translational modifications [8,9]. Dysregulation of lncRNAs has been detected in various cancers, which involves multiple mechanisms. One of the molecular mechanisms is to sponge certain microRNAs (miRNAs) to affect the expression of the target mRNAs [10,11]. In OC, many lncRNAs have been reported to show regulatory effects. For instance, high level of lncRNA NEAT1 enhances cell proliferation and migration via sponging miR-506 and confers

cell paclitaxel resistance via sponging miR-194, denoting a poor prognosis in advanced ovarian cancer patients [12,13]. Similarly, lncRNA PTAR promotes serous OC cell invasion and metastasis by upregulating zinc finger E-box-binding homeobox 1 (ZEB1) via the endogenous competition with miR-101-3p [14]. Moreover, lncRNA MALAT1 enhances OC cell proliferation and inhibits apoptosis through mediating miR-503-5p/JAK2-STAT3 pathway [15]. In our previous study, we screened thousands of lncRNAs to investigate their relationship with epithelial ovarian cancer [16]. Among those lncRNAs, lncRNA RP5-1148A21.3 (lncRP5) attracted our attention due to its high level of conservation and its close regulatory relationship with phosphatase of regenerating liver 1 (PRL-1), also known as the protein tyrosine phosphatase 4A1 (PTP4A1). However, as a newly defined lncRNA, the role of lncRP5 is still a mystery.

In the current study, we explored the function of lncRP5 in OC progression and the underlying regulatory mechanism of lncRP5, and identified lncRP5 as a potent target for molecular therapy of OC.

Materials and Methods

Bioinformatics analysis

The UCSC Genome Browser (http://genome.ucsc.edu/) was employed to identify multiz alignments of vertebrates, chromatin state segmentation, histone modifications, CpG methylation and the positional relationship between lncRP5 and PTP4A1.

Patients with ovarian cancer

Tissue samples from a total of 36 patients with OC (16 patients are < 50 years old and 20 patients are ≥50 years old) were collected from May 2017 to February 2018 in the Women's Hospital of Nanjing Medical University. All patients were diagnosed with OC by two clinical pathologists based on double-blind identification, and the clinical information was listed in Table 1. Tumor tissues were resected and frozen quickly in liquid N_2 and then stored at −80°C for usage. All patients provided informed written consent and the present study was approved by The Ethics Committee of Women's Hospital of Nanjing Medical University.

Cell culture

Human ovarian cancer cell lines (SK-OV-3, Caov-3, Ov90, and A2780) and normal human ovarian surface epithelium cell line (HOSE) used in the current study were from ATCC (Manassas, USA). All cells were cultured in commercial medium containing 10% FBS and 1% penicillin-streptomycin (Gibco, Carlsbad, USA) in humidified chambers with 5% CO_2 at 37°C. The medium used are shown in Table 2.

Cell transfection

Short hairpin RNAs (shRNAs) against lncRP5 which were named as sh-LNCRP5#1 and sh-lncRP5#2, were used to silence lncRP5 in cells and the negative control was denoted as sh-NC. miR-545-5p mimics, inhibitors, and the corresponding negative controls (miRNA NC) were synthesized by Biosyn Biotech Co., Ltd (Suzhou, China), and the sequences are as follows: sh-lncRP5#1 sense 5′-ACCTCGCGAGAGAGATTCCGATTTATTCAAGAGATAAATCGGAATCTCTCTCGCTT-3′ and antisense 5′-CAAAAAGCGAGAGAGATTCCGATTTATCTCTTGAATAAATCGGAATCTCTCTCGCG-3′; sh-lncRP5#2 sense 5′-ACCTCGACCAAACAGGACCTTTACCTTCAAGAGAGGTAAAGGTCCTGTTTGGTCTT-3′ and antisense 5′-CAAAAAGACCAAACAGGACCTTTACCTCTCTTGAAGGTAAAGGTCCTGTTTGGTCG-3′; sh-NC 5′-GTTCTCCGAACGTGTCACGTCAAGAGATTACGTGACACGTTCGGAGAATT-3′; miR-545-5p mimics sense 5′-UCAGCAAACAUUUAUUGUGUGC-3′ and antisense 5′-GCACACAAUAAAUGUUUGCUGA-3′; miR-545-5p inhibitor 5′-GCACACAAUAAAUGUUUGCUGA-3′; miRNA inhibitor NC 5′-CAGUACUUUUGUGUAGUACAA-3′; miRNA mimic NC sense 5′-UUCUCCGAACGUGUCACGUTT-3′ and antisense 5′-ACGUGACACGUUCGGAGAATT-3′. The whole sequences of lncRP5 and PTP4A1 were cloned into pcDNA3.1 vector (Gene-

Table 1. Clinical features of the enrolled OC patients

Clinical features	Patient number (*n*)	Ratio (%)
Age (years)		
<50	16	44.4
≥50	20	55.6
Histology		
Mucinous	6	16.7
Serous	19	52.8
Endometrioid	4	11.1
Clear cell	7	19.4
FIGO stage		
I–II	16	44.4
III–IV	20	55.6
CA125 (U/ml)		
<600	27	75.0
≥600	9	25.0

Table 2. The cell culture media used for different cell lines

Cell line	Medium
SK-OV-3/HOSE	RPMI-1640 + 15% FBS + 1% penicillin-streptomycin (Invitrogen)
Caov-3	89% DMEM (Gibcogen, Carlsbad, USA) + 10% FBS + 1% penicillin-streptomycin (Invitrogen) + 1% sodium pyruvate
A2780	DMEM (Gibco/Invitrogen) + 10% iron fortified bovine serum + 1% penicillin-streptomycin (Invitrogen)
OV-90	McCoy's 5A medium (Welgene, Kyungsan, Korea) with sodium pyruvate + 10% FBS + 1% penicillin-streptomycin (Invitrogen)

FBS, fetal bovine serum.

pharma, Shanghai, China) and transfected into the cells. The empty vectors were set as control. Cells were transfected with the small RNAs or the plasmid using Lipofectamine 3000 Reagent (Invitrogen) according to the manufacturer's instructions.

Lentiviral transfection

sh-lncRP5 lentivirus vector and the empty vector were obtained from Cyagen Biosciences (Suzhou, China). To generate lncRP5-depleted cell lines, A2780 cells were transfected with lentivirus vector that contained sh-lncRP5 at a multiplicity of infection (MOI) of 20. A2780 cells were seeded into a 96-well plate at a density of 1×10^6 cells/well and grew to ~50% confluence before transfection. The transfection efficiency was tested by qPCR.

Xenograft mouse model

Female BALB/cA nude mice (6 weeks old) were obtained from Nanjing Biochemical Research Institute of Nanjing University (Nanjing, China) and maintained in a pathogen-free room under the controlled conditions (22–25°C, 40%–50% humidity, 12 h light/12 h night). The mice could obtain food and water freely. Totally, 1×10^6 A2780 cells stably expressing sh-lncRP5#1 or sh-lncRP5#2 were resuspended in PBS/Matrigel (1:1) and then the cells were subcutaneously injected into the armpit of mice ($n = 5$ in each group). Tumors were measured with a Vernier caliper, and the tumor volumes were estimated according to the formula $V = 1/2 \times LW^2$, in which L is longest dimension and W is shortest dimension. After 5 weeks, mice were humanely euthanized with 5% isoflurane followed by cervical dislocation. The xenograft tissues were removed for further analysis. The animal experiments were approved by The Ethics Committee of Women's Hospital of Nanjing Medical University.

qRT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's guidelines. The cDNA synthesis was conducted using the PrimeScript™ RT reagent Kit (TaKaRa, Tokyo, Japan). qRT-PCR was carried out using the SYBR Green PCR Kit (Applied Biosystems, Waltham, USA) on the 7900 real-time PCR System (Applied Biosystems). First, the polymerase was activated at 95°C for 2 min, followed by denaturation at 95°C for 5 s. Next, annealing and extension were at 60°C for 15 s. This amplification was run for 40 cycles. Fold changes of the RNAs were assessed using $2^{-\Delta\Delta Ct}$ method. *GAPDH* and *U6* were used as the controls for mRNA and miRNA, respectively. The sequences of the primers used in this study were listed in Table 3.

Western blot analysis

The total proteins of tissues or cells were collected using RIPA buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (Sigma-Aldrich, St Louis, USA). The protein concentrations were determined using the BCA assay kit (Abcam, Shanghai, China) and the protein samples were separated by 10% SDS-PAGE and transferred to PVDF membranes (Roche, Indianapolis, USA). After being blocked the membranes were incubated with the indicated primary antibodies: anti-PTP4A1 antibody (1:600, 11508-1-AP; Proteintech, Chicago, USA) and anti-GAPDH antibody (1:5000, ab8245; Abcam) at 4°C overnight. After extensive wash, the membranes were incubated with the corresponding secondary antibody (1:5000, ab6721; Abcam) for 1 h at room temperature. The protein bands were visualized using Pierce enhanced chemiluminescence (ECL) kit (Thermo Fisher Scientific, Waltham, USA).

CCK-8 assay

Cells were resuspended at the density of 2×10^3 cells/well in a 96-well plate and examined by CCK-8 assay (Dojindo Molecular Technologies, Tokyo, Japan) according to the manufacturer's illustration. The absorbance was determined on a microplate reader (MR-96A; Mindray, Shenzhen, China) at 450 nm.

Fluorescence in situ hybridization (FISH) assay

FISH assay was performed to examine the subcellular localization of lncRP5 using a Roche FISH Kit (Roche, Basel, Switzerland). Briefly, cells were fixed with 4% paraformaldehyde (Solarbio, Beijing, China) and incubated with hybridization solution containing lncRP5 probe (Sigma-Aldrich). DAPI (Sigma-Aldrich) was used for nucleus staining. The cells were examined under a confocal microscope (FV3000; Olympus, Tokyo, Japan). Five random fields were selected, and representative images were captured.

Colony formation assay

Cells with various transfections were seeded into 6-well plates at

Table 3. The sequences of primers used in this study

Target	Primer sequence
miR-545-5p	Forward: 5′-AGCGCGTCAGTAAATGTTTATT-3′
	Reverse: 5′-GTTGTTGGTTGGTTGGTTGT-3′
miR-30a-5p	Forward: 5′-CGCGATGTTGAAACATCCTCGA-3′
	Reverse: 5′-ATCCAGTGCAGGGTCCGAGG-3′
miR-30b-5p	Forward: 5′-ACGGGCAAAAATACTCCAGCTCTCAAT-3′
	Reverse: 5′-CTCTGGAAAACTGGTGTCGACTGGTGTC-3′
miR-30c-5p	Forward: 5′-ACACTCCAGCTGGGTGTAAACATCCTACACTC-3′
	Reverse: 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGCTCAGAG-3′
lncRP5	Forward: 5′-CGCAATTCTGTGCCATAC-3′
	Reverse: 5′-TGATCCCTAAAGCCCTCT-3′
PTP4A1	Forward: 5′-ACCAATGCGACCTTAACAAA-3′
	Reverse: 5′-ATCTGGTTGGATGGTGGTG-3′
U6	Forward: 5′-GCTTCGGCAGCACATATACTAAAAT-3′
	Reverse: 5′-CGCTTCACGAATTTGCGTGTCAT-3′
GAPDH	Forward: 5′-GGGAGCCAAAAGGGTCAT-3′
	Reverse: 5′-GAGTCCTTCCACGATACCAA-3′

2×10^3 cells per well and cultured in fresh medium for seven days. The colonies were fixed with methanol, stained with hematoxylin (H69840; Acmec, Shanghai, China), and observed under a CX43 light microscope (Olympus, Tokyo, Japan).

Transwell assay

Cell invasion and migration were evaluated as described by Li *et al.* [17]. Briefly, cells transfected for 24 h were collected and re-suspended in 200 μL of serum-free medium. For invasion assay, 4×10^4 cells were seeded into the upper compartment pre-coated with Matrigel (Corning Co., Corning, USA) and 700 μl of culture medium containing 20% FBS was added into the lower compartment. For migration assay, 4×10^4 cells were seeded into the upper compartment without Matrigel, with the same lower compartment. After incubation for 48 h, cells on the upper surfaces were removed and cells on the lower membrane were fixed with 4% paraformaldehyde. The fixed cells were stained with 0.1% crystal violet and counted under an ECLIPSE TE 2000-U microscope (Nikon, Tokyo, Japan). Five random fields were selected, and representative images were captured. The metastatic cells were counted using Image J software (version 1.8.0.112).

Luciferase reporter assay

Sequences of lncRP5-Wt/Mut or PTP4A1 3′-UTR-Wt/Mut were cloned into pmirGLO vector (Promega, Mannheim, Germany). The vectors were co-transfected with miRNA mimic or miRNA NC into HEK293T cells. The luciferase activity was determined using the dual luciferase reporter assay system (Promega).

Statistical analysis

Statistical analysis was performed using the SPSS package. Statistical significance among multiple experimental groups was analyzed using ANOVA and Tukey's post hoc test. Student's *t*-test (two-tailed) was applied to assess the statistical significance between two groups. At least three independent experiments were carried out for each assay. $P < 0.05$ denoted statistical significance.

Results

Bioinformatics analysis of lncRP5

The UCSC database was employed to predict the potential biological functions of lncRP5. It was revealed that lncRP5 is located at 6q12 (chr: 64, 280, 910-64, 282, 313) and is 1404 bp in length (Figure 1A). In addition, lncRP5 is highly conserved among mammals, deletion and duplication of this chromosome are closely related to tumor invasion and metastasis (Figure 1B) [18–20]. The lncRP5-related chromosomal regions in K562 and HepG2 tumor cells, H1-hESC human embryonic stem cells and HMEC microvascular endothelial cells were activated, as revealed by chromatin status analysis (Figure 1C), suggesting that the lncRNAs may be related to tumor invasion and metastasis. Histone modification, enrichment and DNA methylation regulation were also found in this chromosome

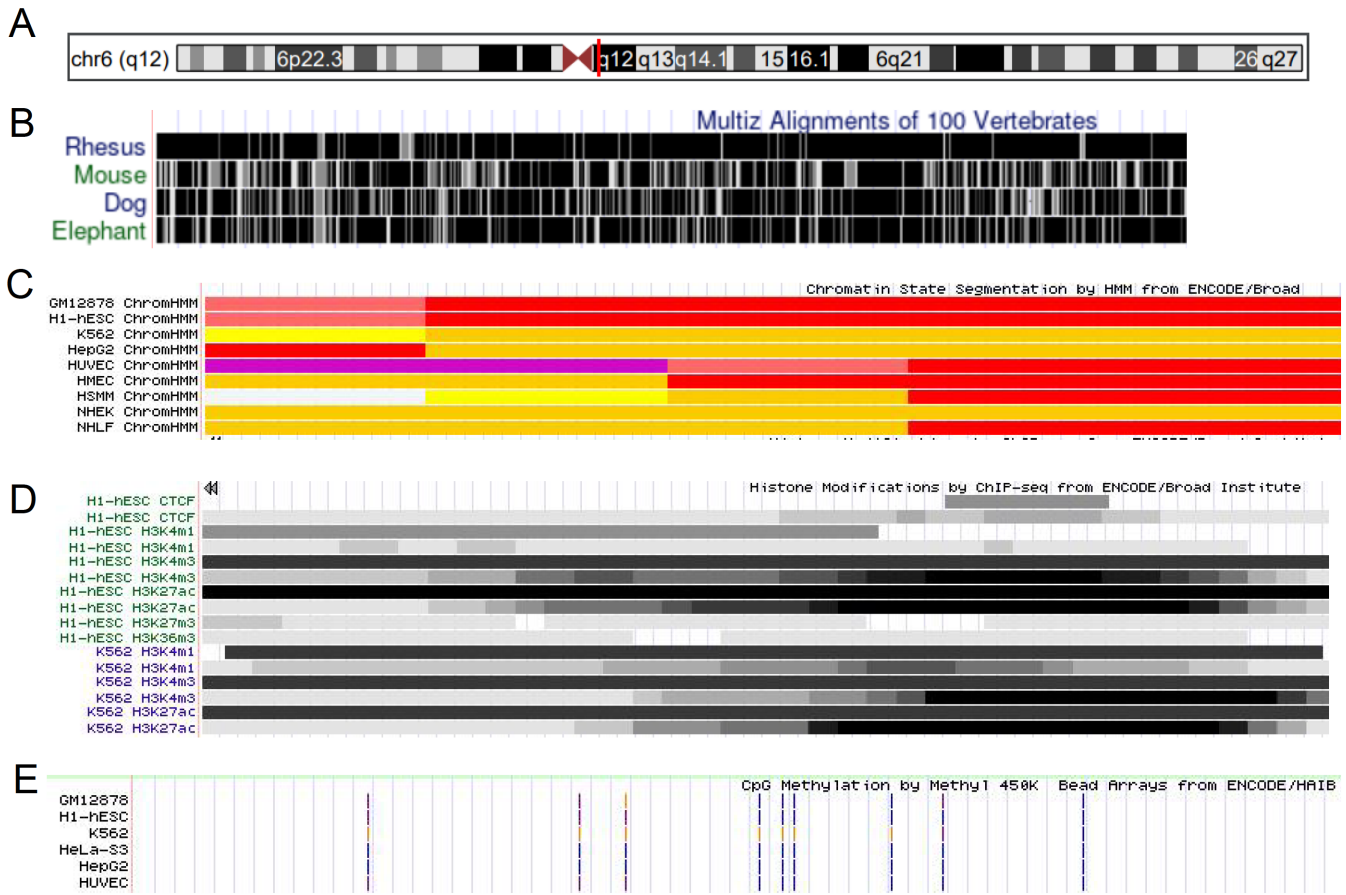

Figure 1. Genomic features of lncRP5 (A) Chromosome location of lncRP5. (B) Conservation analysis of lncRP5. (C) The chromatin status analysis of lncRP5. (D) Histone modification of lncRP5-related chromosome region. (E) Regulation of DNA methylation in lncRP5-related chromosome region.

region, such as H3K27ac, H3K4me1, H3K27me3, and H3K4me3, especially H3K27ac (Figure 1D,E), which indicates that the function of lncRP5 is important.

lncRP5 is highly expressed in OC

Our previous microarray and bioinformatic analysis revealed that lncRP5 may play a role in OC [16]. Therefore, we explored the specific role of lncRP5 in the regulatory mechanisms in OC. Firstly, we examined the expression of lncRP5 in tumor tissues and adjacent normal tissues by RT-qPCR and found that lncRP5 was highly expressed in the tumor tissues (Figure 2A). Consistently, there also exhibited an elevated lncRP5 expression in OC cell lines including SK-OV-3, Caov-3, OV90, and A2780, compared with that in the normal HOSE cell line (Figure 2B). Interestingly, a significant difference was observed in the expressions of lncRP5 between SK-OV-3 and A2780 cell lines (Figure 2B), therefore we employed SK-OV-3 and A2780 cell lines for further investigation.

lncRP5 promotes the proliferation and metastasis of OC cells

lncRNAs exert different functions based on the subcellular locations. To identify the potential role of lncRP5, we labeled lncRP5 and visualized the location by fluorescence *in situ* hybridization (FISH) analysis. The results showed that lncRP5 locates in the cytoplasm of SK-OV-3 cells (Figure 2C), implying that lncRP5 tends to play a regulatory role at the post-transcriptional level. We then detected the effects of lncRP5 on the viability, proliferation, and migration of ovarian cancerous cells. It was found that lncRP5 overexpression notably increased the lncRP5 level in SK-OV-3 cell line, and sh-lncRP5 markedly decreased the lncRP5 level in A2780 cell line (Figure 2D). Cell viability and proliferation were determined by CCK-8 assay and colony formation assay, respectively. It turned out that elevated expression of lncRP5 increased the cell

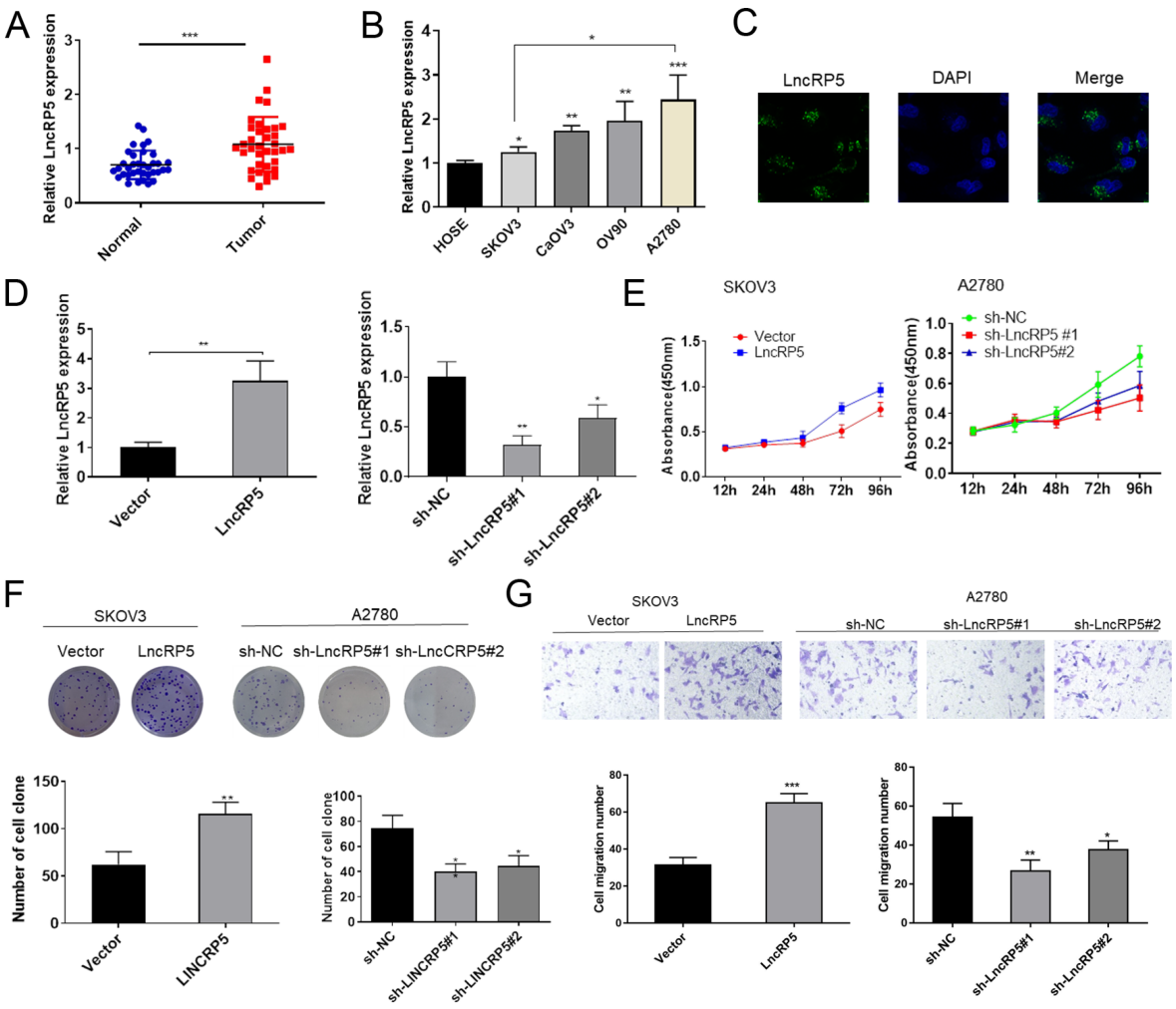

Figure 2. The effects of lncRP5 on ovarian cancer progression (A) The expression of lncRP5 in ovarian tumor tissues and the adjacent normal tissues. (B) The expression of lncRP5 in various ovarian cancer lines. qRT-PCR were used to detect the mRNA levels of lncRP5. (C) FISH assay of lncRP5. lncRP5 were detected with green fluorescent probe, and the nucleus were stained the with DAPI. At least three independent replicates were carried out, and the representative images were shown. (D) qRT-PCR analysis of lncRP5 under different transfections in different cell lines. Cancerous cell viability, proliferation, and migration were detected by (E) CCK-8 assay, (F) colony formation assay, and (G) transwell assay. Each experiment was performed with at least three independent biological replicates. For CCK-8 assay, each point on the line graph is the average value of OD, and the error bar indicates SD. For colony formation assay and transwell assay, representative images are shown. $*P<0.05$, $**P<0.01$, $***P<0.001$.

viability and proliferation of SK-OV-3 cells, whereas inhibition of lncRP5 expression impaired the cell growth of A2780 cells (Figure 2E,F). Similarly, the results of transwell assay showed that overexpression of lncRP5 increased the cell metastasis of SK-OV-3 cells, while silencing of lncRP5 decreased the cell migration ability of A2780 cells (Figure 2G). These data indicated that lncRP5 plays an oncogenic role in OC.

lncRP5 serves as a competing RNA for miR-545-5p

lncRP5 was proved to mainly locate in the cytoplasm of SK-OV-3 cells. To our knowledge, the cytoplasmic lncRNAs could sponge miRNA to block the inhibitory effects of miRNA on its downstream target mRNAs to regulate target gene expression [21]. We therefore investigated whether there is an miRNA responsible for this lncRP5-mediated pathway. The target miRNAs were predicted using DIANA-LncBase v3 (https://diana.e-ce.uth.gr/lncbasev3/home) [22] and starBase (http://starbase.sysu.edu.cn/) [23]. These two databases provided four overlapping miRNA candidates (Figure 3A). We then tested the expressions of these miRNAs in ovarian tumors, and found that miR-545-5p was the most prominently downregulated miRNA in tumor tissues (Figure 3B). Further analysis revealed that miR-545-5p was markedly downregulated in OC cell lines compared with that in HOSE cells (Figure 3C). Next, cells were transfected with luciferase reporter plasmid harboring WT or MUT miR-545-5p binding site in lncRP5 (Figure 3D). Luciferase reporter assay results showed that miR-545-5p overexpression caused a significant reduction in the relative luciferase activity of WT-HEK293T cells. On the contrary, miR-545-5p inhibitor significantly elevated the luciferase activity of WT-HEK293T cells. However, the relative luciferase activity was not significantly changed in the lncRP5-MUT group (Figure 3E). These data implied that there is an interaction between lncRP5 and miR-545-5p.

lncRP5 promotes OC cell proliferation and metastasis via miR-545-5p

Next, we examined whether miR-545-5p is the responsible regulator for lncRP5-mediated OC cell proliferation and migration. Since lncRP5 expression level was significantly different between SK-OV-3 and A2780 cell lines, SK-OV-3 cell line transfected with lncRP5 and A2780 cell line transfected with sh-lncRP5 were employed for further study. In SK-OV-3 cells, miR-545-5p overexpression increased the level of miR-545-5p, whereas overexpression of lncRP5 reduced the level of miR-545-5p, which could be rescued by miR-545-5p co-transfection (Figure 4A, left). Similarly, transfection of miR-545-5p inhibitor decreased miR-545-5p content in A2780 cells, whereas silencing of lncRP5 promoted the level of miR-545-5p, whose impact could be inhibited by the co-transfection with miR-545-5p inhibitor (Figure 4A, right). We then examined the role of miR-545-5p in this lncRP5-regulated cell viability, proliferation, migration, and invasion. Reductions in cell viability, proliferation, migration, and invasion were observed in the OC cells transfected with miR-545-5p, which were restored by the co-transfection with lncRP5. Consistently, the promoted cell viability, proliferation, migration, and invasion induced by miR-545-5p inhibitor were reversed by silencing of lncRP5 (Figure 4B–D), indicating the involvement of miR-545-5p in this lncRP5-mediated cell growth. These data indicated that lncRP5 regulates the behaviors of OC cells by

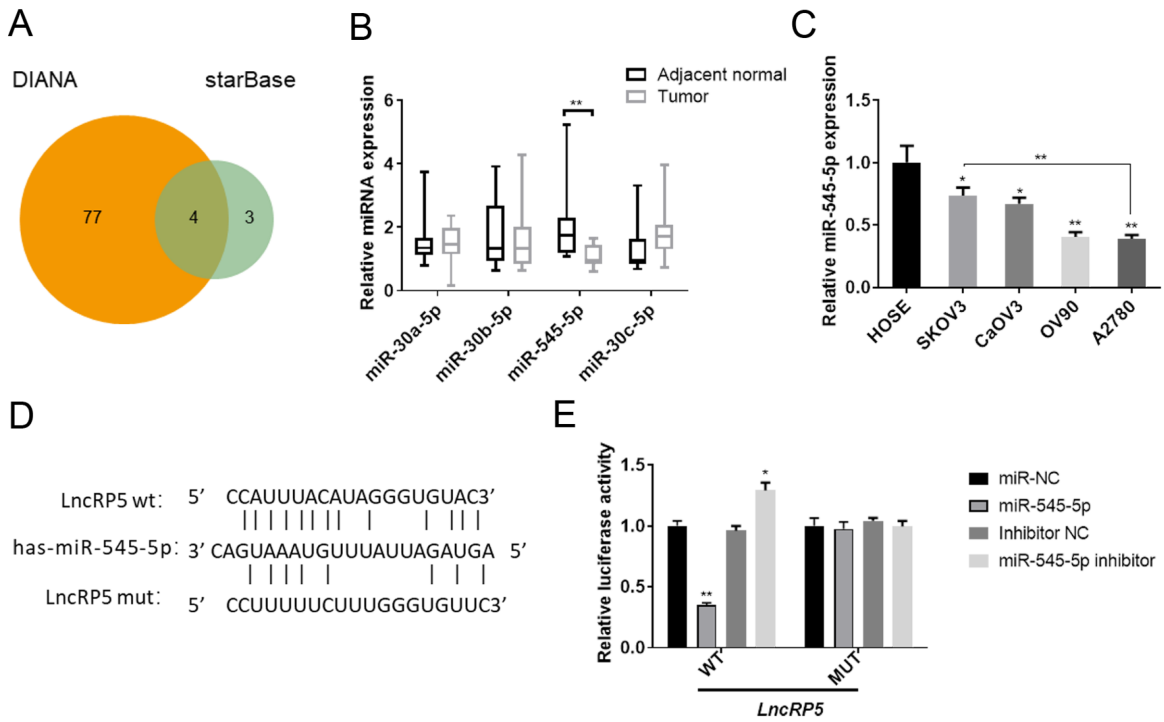

Figure 3. miR-545-5p targeted lncRP5 in ovarian cancerous cells (A) Screening of potential miRNAs targeting lncRP5. The target miRNAs were predicted using DIANA-LncBase v3 (https://diana.e-ce.uth.gr/lncbasev3/home) and starBase (http://starbase.sysu.edu.cn/), and shown in Venn graph. The expression levels of potential targeting miRNAs in (B) ovarian cancer tissues and in (C) various ovarian cancerous cell lines. (D) lncRP5 and miR-545-5p binding site. (E) Dual luciferase reporter gene assay for lncRP5 and miR-545-5p. Data are presented as the mean ± SD, $n = 3$. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs miR-NC group.

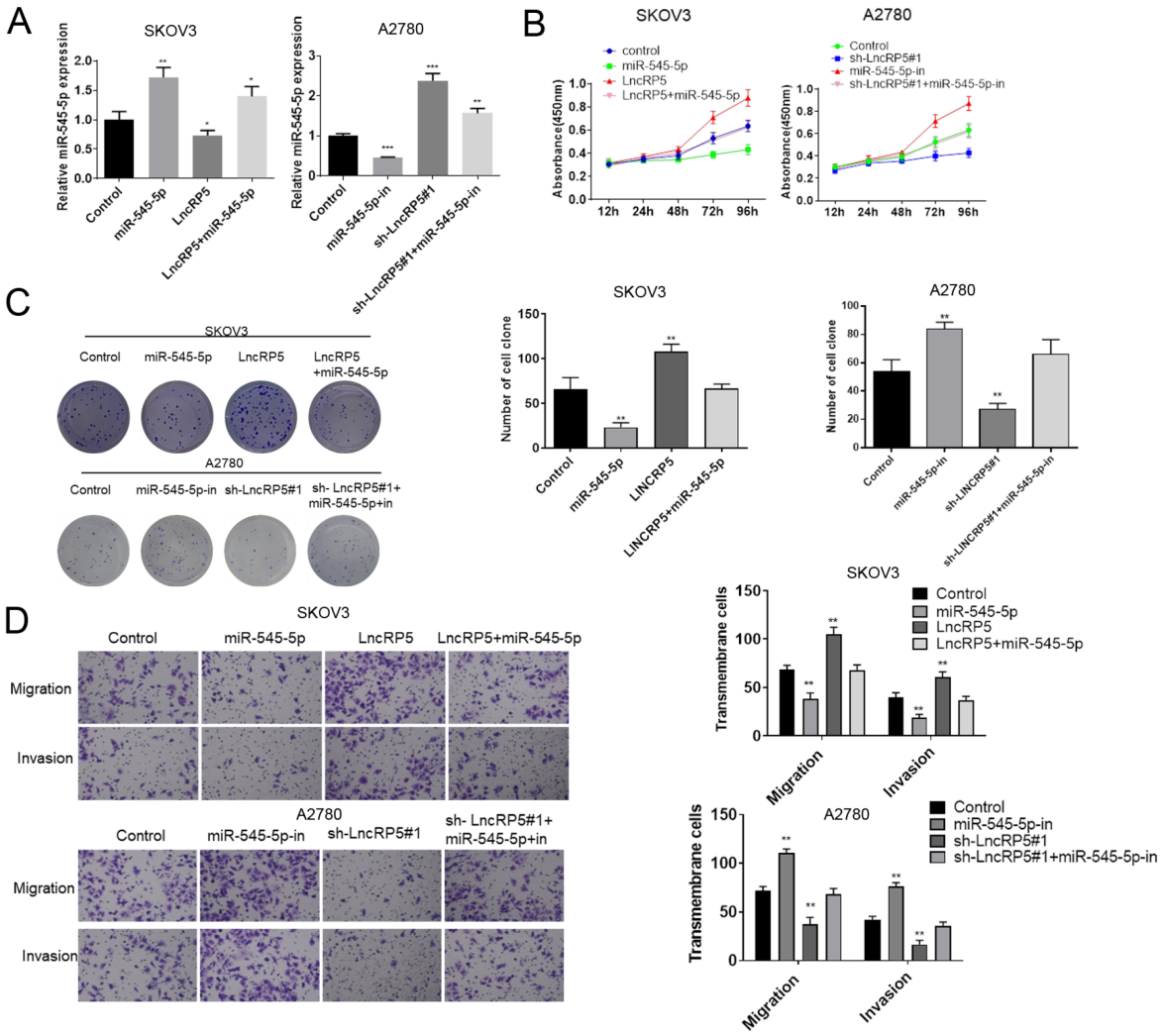

Figure 4. The effects of miR-545-5p on lncRP5-regulated ovarian cancer cell proliferation and metastasis Cancerous cells were transfected with miR-545-5p mimic, miR-545-5p inhibitor, lncPR5, sh-lncPR5 and the related control vectors. (A) The expression of miR-545-5p with different transfections in SK-OV-3 cell line and A2780 cell line. Cancerous cell viability, proliferation, and migration in different cell lines under diverse transfections were detected by (B) CCK-8 assay, (C) colony formation assay, and (D) transwell assay. Each experiment was performed with at least three independent biological replicates. For CCK-8 assay, each point on the line graph is the average value of OD, and the error bar indicates SD. For colony formation assay and transwell assay, representative images are shown. $*P<0.05$, $**P<0.01$, $***P<0.001$ vs the control group.

sponging miR-545-5p.

lncRP5 regulates PTP4A1 expression via miR-545-5p

To identify the genes involved in this lncRP5/miR-545-5p pathway, we predicted the target genes for miR-545-5p using miRwalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) and TargetScan 7.2 (http://www.targetscan.org/vert_72/) and obtained 203 overlapping candidates (Figure 5A and Supplemental Table S1). Among these listed candidates, we selected PTP4A1 for further study, not only because lncRP5 is the antisense RNA of PTP4A1 (Figure 5B), but also because bioinformatics analysis suggests that it is closely related to lncRP5 (Figure 5C). Luciferase reporter plasmid harboring WT or MUT 3′-UTR binding site of PTP4A1 was transfected into HEK293T cells and luciferase reporter assay was performed. miR-545-5p overexpression caused a significant reduction in the luciferase activity of PTP4A1-WT cells. Nevertheless, transfection with miR-545-5p inhibitor notably enhanced the relative luciferase activity in PTP4A1-WT cells. But no significant change was observed in the luciferase activity in the PTP4A1 MUT group (Figure 5D), indicating an interaction between miR-545-5p and PTP4A1. In SK-OV-3 cells, transfection with miR-545-5p inhibited the expression of PTP4A1. lncRP5 significantly increased the protein level of PTP4A1, whereas transfection with miR-545-5p reversed the effect of lncRP5. Consistently, in A2780 cells, the expression of PTP4A1 was decreased by sh-lncRP5, but miR-545-5p inhibitor restored the expression of PTP4A1 (Figure 5E). Furthermore, the expression of PTP4A1 was negatively correlated with miR-545-5p (Figure 5F) and positively correlated with lncRP5 (Figure 5G). These data demonstrated the lncRP5/miR-545-5p/PTP4A1 regulatory axis in OC.

Overexpression of PTP4A1 reverses the inhibitory effects of miR-545-5p on OC

To confirm the role of PTP4A1 in OC cells, cellular functions were examined after A2780 cells were transfected with PTP4A1 overexpression plasmid or/and miR-545-5p mimic. It turned out that

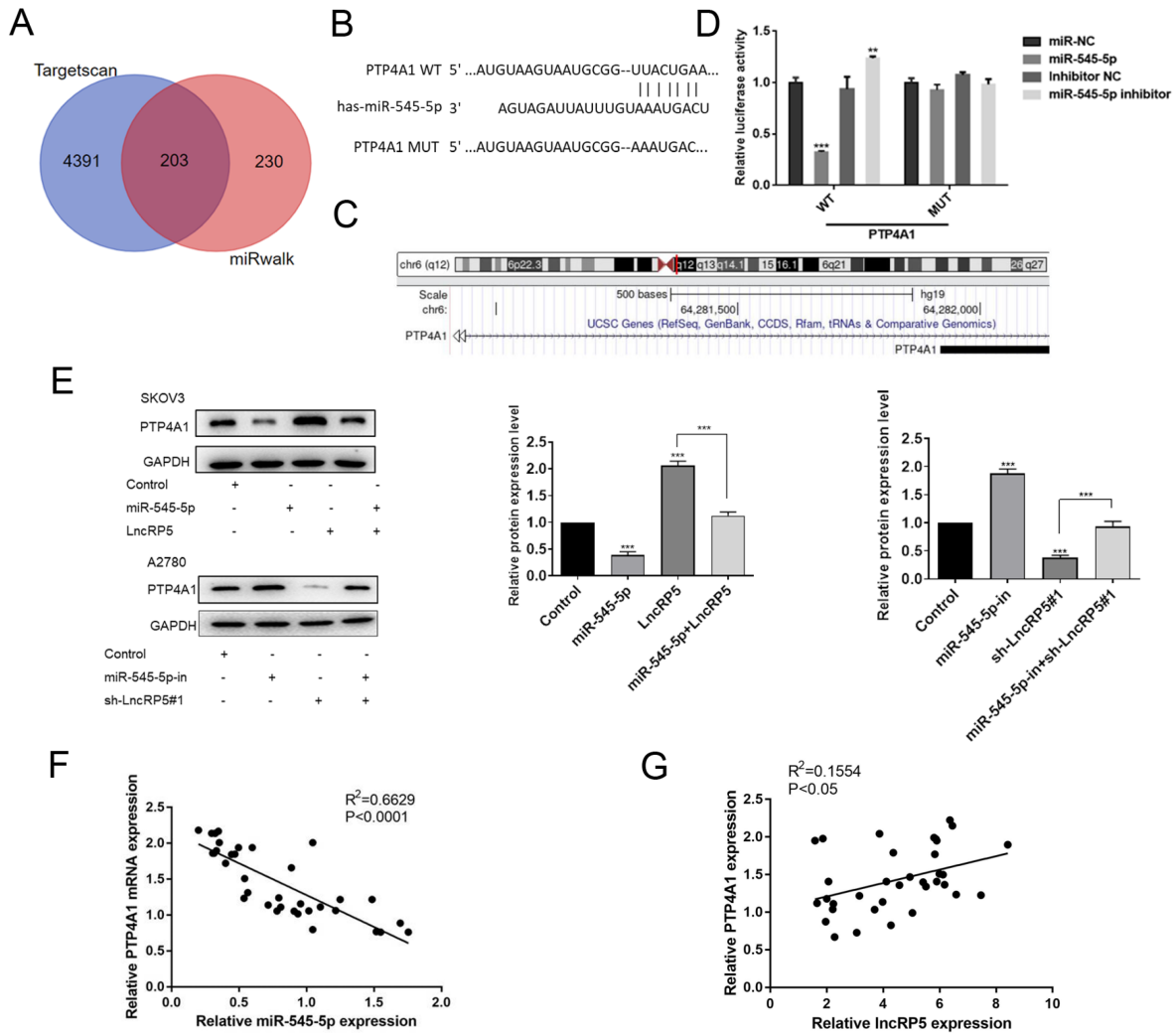

Figure 5. lncRP5 regulated PTP4A1 expression via miR-545-5p (A) Screening of target genes for miR-545-5p. The potential target genes for miR-545-5p were predicted using miRwalk 2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) and TargetScan 7.2 (http://www.targetscan.org/vert_72/) and shown in Venn graph. (B) PTP4A1 and miR-545-5p binding site. (C) The UCSC database shows that PTP4A1 gene is closely related to lncRP5. (D) Dual luciferase reporter gene assay for PTP4A1 and miR-545-5p. Luciferase reporters containing PTP4A1-WT/MUT 3′-UTR miR-545-5p binding sites were co-transfected into HEK293T cells with miR-545-5p mimic, miR-545-5p inhibitor or respective NCs. Data are presented as the mean ± SD, $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs miR-NC group. (E) Western blot analysis of PTP4A1 in SK-OV-3 cell line and A2780 cell line with different transfections. SK-OV-3 cell line was transfected with/without miR-545-5p mimic or lncRP5. A2780 cell line was transfected with miR-545-5p inhibitor or sh-lncRP5. Total proteins were extracted and PTP4A1 were detected using anti-PTP4A1 antibody with GAPDH as the loading control. The gray values were analyzed using Image J and shown in bar graph. The correlation between PTP4A1 and (F) miR-545-5p or (G) PTP4A1 and lncRP5 were calculated using SPSS.

miR-545-5p abrogated the promotive effects on both mRNA and protein expression of PTP4A1 (Figure 6A,B). Moreover, transfection of PTP4A1 overexpression plasmid enhanced cell viability, proliferation, migration, and invasion in OC cells, which were reduced by miR-545-5p (Figure 6C–E), collectively verifying the inhibitory effects of miR-545-5p on PTP4A1 in OC cells. These data indicated that miR-545-5p functionally inhibits PTP4A1.

lncRP5 regulates ovarian tumor progression *in vivo*

To verify our finding that lncRP5 is involved in OC progression via miR-545-5p/PTP4A1 axis, xenograft mouse model was constructed. It was found that in comparison with the control group, silencing of lncRP5 showed adverse influence on tumor growth (Figure 7A). Tumor growth rate in the sh-lncRP5 mice was markedly decreased (Figure 7B). Similarly, sh-lncRP5 also led to repressed tumor weight (Figure 7C). In addition, sh-lncRP5 increased the expression of miR-545-5p, and decreased lncRP5 level and PTP4A1 mRNA and protein levels, compared with the control group (Figure 7D–G). These data indicated that lncRP5 is a potential therapeutic target for OC.

Discussion

OC is a common gynecological malignant tumor. Since OC is often diagnosed at the advanced stages, it is featured with low cure rate, high recurrence rate, and low five-year survival rate [24]. When diagnosed with OC, patients usually undergo standard surgery and chemotherapy. Although the treatments are effective initially, most patients harbor a high risk of recurrence. Patients with recurrent OC are sometimes incurable due to chemoresistance [25]. Therefore,

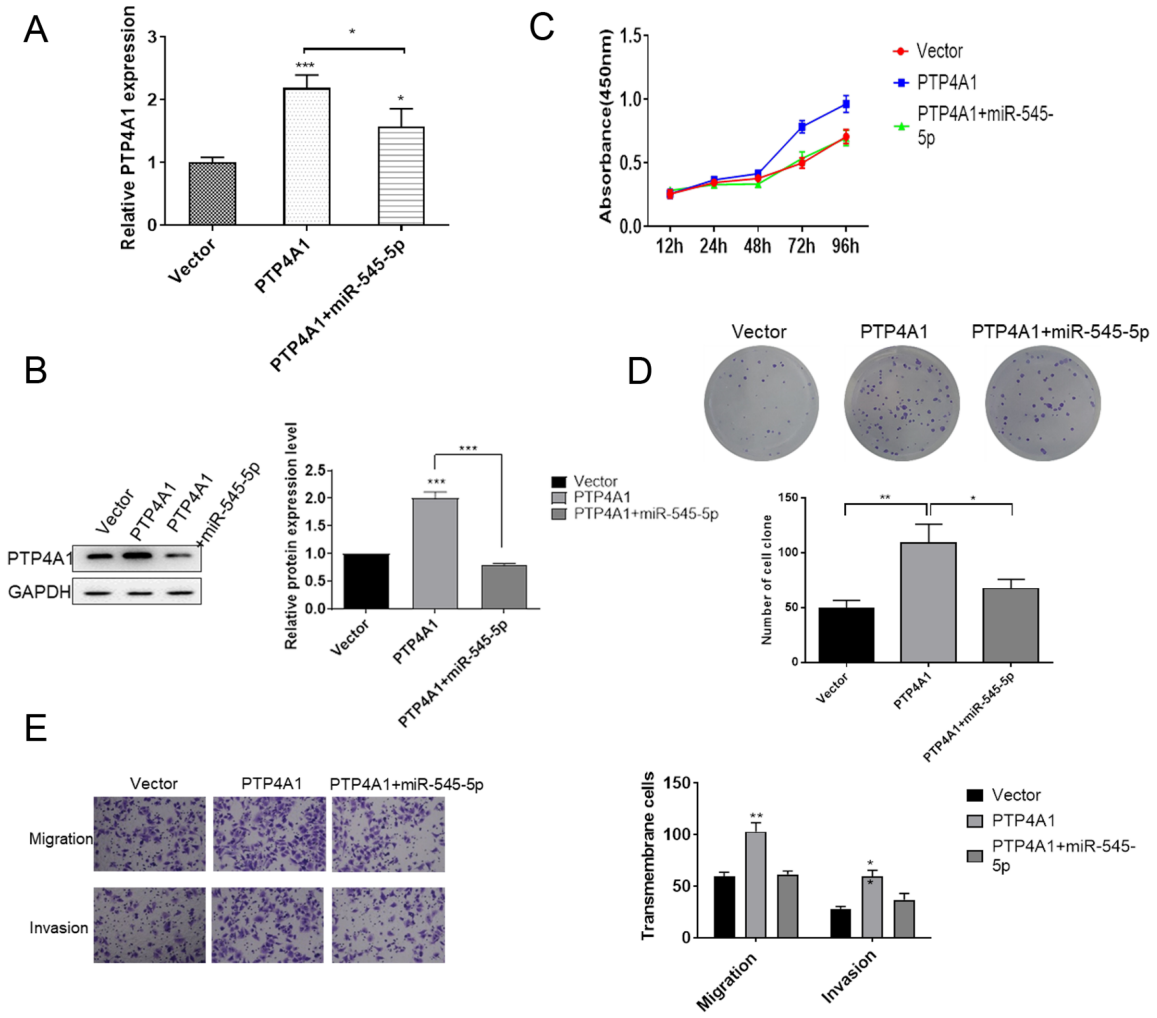

Figure 6. miR-545-5p inhibited ovarian cancerous cell growth and migration by downregulating PTP4A1 in A2780 cell line (A) qRP-PCR analysis of PTP4A1 mRNA level in cells under different transfections. (B) Western blot analysis of PTP4A1 protein in cells with different transfections. Total proteins were extracted and PTP4A1 were detected using anti-PTP4A1 antibody with GAPDH as the control. The gray values were analyzed using Image J and shown in bar graph. Cancerous cell viability, proliferation, and migration in cells under diverse transfections were detected by (C) CCK-8 assay, (D) colony formation assay, and (E) transwell assay. Each experiment was performed with at least three independent biological replicates. For CCK-8 assay, each point on the line graph is the average value of OD, and the error bar indicates SD. For colony formation assay and transwell assay, representative images are shown. $*P<0.5$, $**P<0.01$, $***P<0.001$ vs the control group.

more effective treatment strategies are required for OC patients, where targeting therapy for OC is the most essential approach.

In the human genome, more than 98% of RNAs are incapable of encoding proteins, namely non-coding RNAs (ncRNAs) [26]. Based on the number of nucleotides, ncRNAs are classified into small ncRNAs and lncRNAs [27]. miRNAs are typical small ncRNAs and have been evidenced to exert multiple functions on cancer progression [28]. In OC, some miRNAs were reported to act as cancer promoters [29], whereas others were considered as suppressors [30]. lncRNAs, another type of ncRNAs, have also been proved to be aberrantly expressed or mutated in diverse cancerous tissues [31] and exhibit anti-oncogenic or oncogenic effects on OC [32]. One typical regulatory mechanism of lncRNA in mediating the progression of OC is to sponge the miRNAs and further regulates the target mRNA translations [33]. For example, lncRNA TUG1 is promoted in OC, and it exacerbates OC through the miR-186-5p/ZEB1 axis [34]. lncRNA SNHG12 was upregulated in OC patients and cell lines, and the regulatory axis SNHG12/miR-129/SOX4 was further proved in the OC cells [35]. In this work, we confirmed that lncRP5 was upregulated in OC and it positively regulated cell growth and metastasis of the OC cells. miR-545 was confirmed to be the target of lncRP5 and was downregulated in the OC cells [36]; however, miR-545 mimics suppressed cell viability and migration, acting as an anti-oncogenic regulator in OC, which was consistent with the findings of the previous studies [37].

The PRL family members are protein tyrosine phosphatases, including PRL-1, PRL-2 and PRL-3, and they are highly homologous. Phospho-relay signaling systems tend to participate in tumor cell proliferation and metastasis. Therefore, the function of PRL-1/PTP4A1 has been intensively explored in oncology. High levels of PTP4A1 were observed in various cancerous cells, such as pancreatic cancer [38], breast cancer [39], and cervical cancer [40]. Zeng *et al.* [41] reported that elevated PTP4A1 is responsible for increased cell clonogenicity and migration in OC cells. In the present

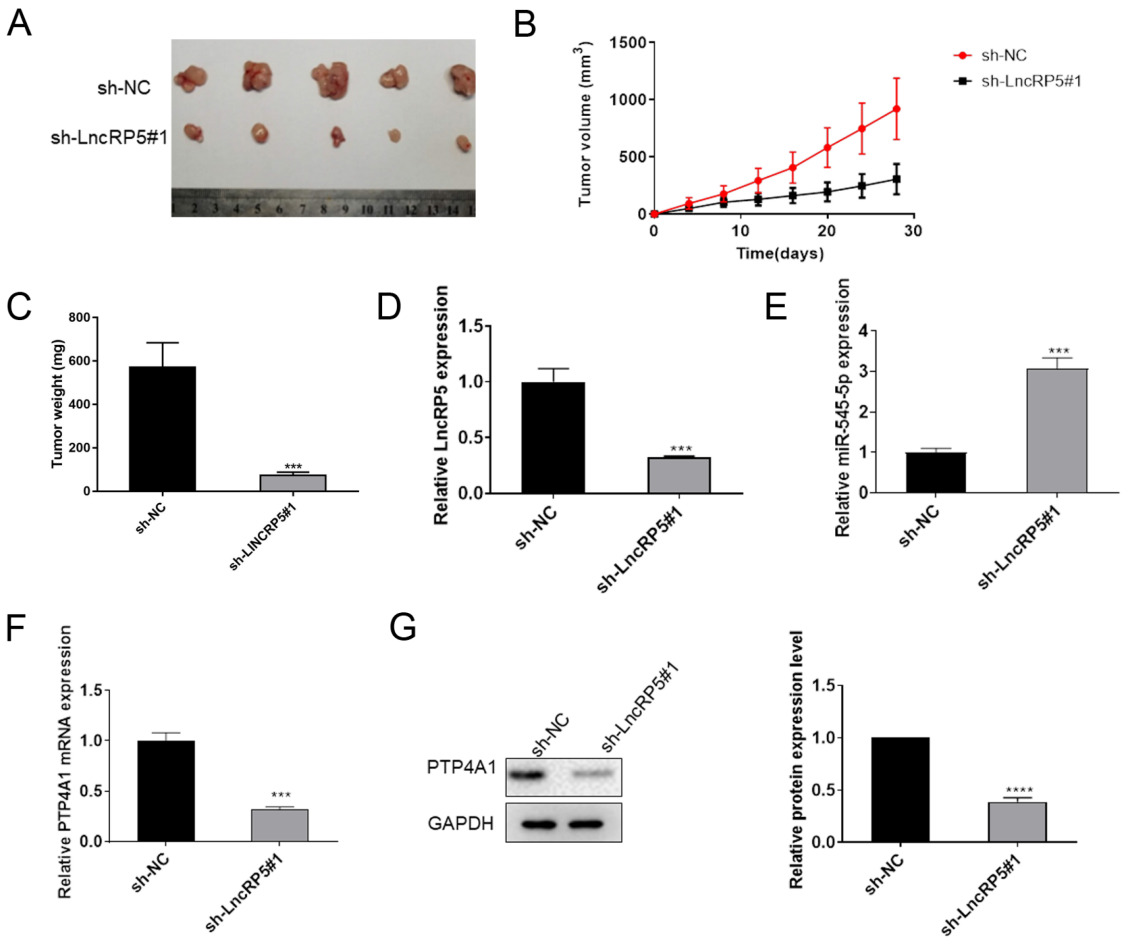

Figure 7. The role of lncRP5 in ovarian tumor formation in xenograft model mice (A) Representative images of tumor tissues from mice. (B) Tumor volume and (C) weight of different groups were measured. The expression level of (D) lncRP5, (E) miR-545-5p, and (F) PTP4A1 were detected by qRT-PCR. (G) Western blot analysis of PTP4A1 in cells with different transfections. Total proteins were extracted and PTP4A1 were detected using anti-PTP4A1 antibody with GAPDH as the control. The gray values were analyzed using Image J and shown in bar graph. Data are presented as the mean ± SD, $n = 5$. ***$P < 0.001$, ****$P < 0.0001$ vs the control group.

work, we verified that upregulation of PTP4A1 conferred increased cell viability, migration, and invasion in OC cells. Mechanically, lncRP5 was proved to upregulate the expression of PTP4A1 via sponging miR-545-5p.

In summary, our work revealed the oncogenic role of lncRP5 in OC. It promotes cell viability, migration, and invasion of the cancerous cells by sponging miR-545-5p, which is a negative regulator for PTP4A1. This study offered a novel regulatory mechanism under the progression of OC, potentially contributing to cancer diagnosis or therapy in the future.

Supplementary Data

Supplementary Data is available at *Acta Biochimica et Biphysica Sinica* online.

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Conflict of Interest

All the authors have declared no competing financial interests.

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