A Positive Feedback Regulation Involving PVT1 and HER2/hER3 Promotes Progression of High-grade Serous Ovarian Cancer

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Abstract

Background

In recent years, long noncoding RNAs (lncRNAs) have been reported frequently to play important roles in specific cancers, including high-grade serous ovarian cancer (HGSOC). PVT1 is an important oncogenic lncRNA highly expressed in various cancers. However, little is known about the role of PVT1 in HGSOC and underlying mechanisms.

Methods

The expression levels of PVT1 and HER2/3 in HGSOC tissue and adjacent normal tissue were determined by qRT-PCR. MTT and transwell assays were used to identify the effects of PVT1 cell proliferation and migration respectively. Dual-luciferase reporter assay and RIP experiment were carried out to verify target genes of PVT1. ChIP experiment was used to identify that HER2 was transcription factor of PVT1.

Results

Our results showed that PVT1 expression was up-regulation in human HGSOC specimens and promoted ovarian cancer cell proliferation and migration. We further validated that HER2 was a direct transcription factor for facilitating PVT1 expression. In return, PVT1 enhanced HER2 transcript stability. Moreover, bioinformatics analysis and dual luciferase reporter assay results uncovered that PVT1 functioned as a competing endogenous RNA (ceRNA) for miR-1301-3p to promote cell proliferation and migration by increasing HER3 expression.

Conclusions

Taken together, our results showed that PVT1, controlled by HER2, elevated HER2 and HER3 expression to promote HGSOC progression. Thus, PVT1 can be regarded as a vital diagnostic biomarker for HGSOC and a potential novel therapeutic target.

Background

Epithelial ovarian cancer (EOC) is the most lethal gynecological cancer in women worldwide.[1,2] Most patients relapse and die from this disease despite aggressive frontline treatments with targeted chemotherapy and surgery.[3,4] High-grade serous ovarian carcinoma (HGSOC), which is known for high rate of invasion and metastasis and usually results in mortality, accounting for 60–80% of the women diagnosed with EOC.[5–7] Therefore, it is important to understand the pathophysiological mechanisms of

HGSOC in order to develop new diagnostic techniques and treatment strategies and improve the overall prognosis of patients.

With the advances in genomic analysis technologies, especially next-generation RNA sequencing, a number of new lncRNAs have recently been identified, implying an important role in human diseases, especially cancer.[8–10] PVT1 is an important oncogenic lncRNA highly expressed in cancer cells. Previous studies have reported the carcinogenic effect on PVT1 in various cancers, such as breast cancer,[11] hepatocellular cancer,[12] colon cancer,[13] gallbladder cancer,[14] non-small-cell lung cancer[15] and leukemia.[16] Although many studies have revealed that PVT1 may exhibit malignant biological behaviors in ovarian cancer, the detailed mechanisms and functions of PVT1 in HGSOC have not been clearly explored.

HER2 belongs to epidermal growth factor (EGF) receptor family of receptor tyrosine kinases, is an important oncogenic gene marker in many cancers, especially in HGSOC, HER2 expression is always amplified.[17–20] There are now five approved agents (Trastuzumab, pertuzumab, Lapatinib, Ado-trastuzumab emtansine and Afatanib) that target HER2 in multiple carcinoma. However, the therapeutic effect in HGSOC is barely satisfactory.[21]

In this study, we discovered that PVT1 was overexpressed in HGSOC tissues and positive correlation with HER2 and HER3 expression. Also, PVT1 could promote HGSOC cell proliferation and migration as well as tumor growth in vivo. Functional experiments revealed that PVT1 was modulated by transcription factor HER2, which was stabilized by PVT1 in return. By determining the downstream mechanism of PVT1, we found that PVT1 might increase HER3 expression via sponging miR-1301-3p. These results could provide new insights into the molecular functions of PVT1 and might shed new light on the treatment of HGSOC.

Methods

Clinical Human Samples

A total of 73 pairs of frozen HGSOC tissues were obtained in the Central Hospital of Xinxiang from March 2017 to August 2019. The mean age of these patients was 54.58 ± 11.49 years, and none received radiotherapy or chemotherapy before surgery. After surgical resection of the tumors, all HGSOC tissue samples were determined by histopathological examination. Normal ovarian tissues were collected from cervical cancer surgery patients. Written informed consent obtained from all patients. Tissues were snap frozen in liquid nitrogen and stored at -80℃ for use. The study was approved by the Ethics Committee of the Central Hospital of Xinxiang. All patients provided written informed consent form prior to their inclusion. The written informed consent was obtained from each patient. All the clinical specimens were collected in accordance with the Declaration of Helsinki.

Cell cultures and treatment

FTSEC, OVCAR3, OVCAR4, SNU119, CAOV4 and CAOV3 cell lines were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). They were cultured in 1640 or DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum at 37℃ in tmosphere containing 5% CO_2. As for detecting half-life, cells were treated with 50 μM α-amanitin (Sigma-Aldrich) for 0–24 h in the article.

Lentivirus, siRNA, miR-1301-3p mimic or inhibitor and transfection

The pLV-shPVT1 Lentivirus plasmid was constructed based on pLV-shNC as backbone. siRNAs of PVT1 and miR-1301-3p mimic and inhibitor were purchased from Suzhou Synbio Technologies. The ovarian cell lines were transfected with plasmids or siRNA via into Lipofectamine 2000 transfection reagent (Thermo, Shanghai, China). The sequences were in Table 1.

Western blot and quantitative real-time PCR

Cell sample was lysed with RIPA buffer and then boiled for denaturation. The sample were loading in SDS-PAGE gel, and transferred to PVDF membrane, followed by blocking with 5% BSA for 2 h at room temperature. The primary antibodies for HER2 (ab194976, Abcam), HER3 (ab255607, Abcam) and GAPDH (ab181602, Abcam) for overnight at 4 ℃, the secondary antibodies for 2 h at room temperature.

Total RNA was obtained from cells and tissues using RNAzol (Invitrogen), RNA was reversely transcribed into first-strand cDNAs by using the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa). Real-time qPCR was performed using TaqMan Human microRNA assay (Applied Biosystems) for miRNA analysis and SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) for mRNA analysis.

The primer sequences were as follows:

PVT1 forward primer: 5’- TGGTACCGAGCTCGGATCCTC – 3’,

reverse primer: 5’- CCGCCACTGTGCTGGATGATA-3’;

HER2 forward primer: 5’- TGACTGCCTGGCCTGCCTCCA – 3’,

reverse primer: 5’- GGCAGACGAGGGTGCAGGATC – 3’;

HER3 forward primer: 5’- ACCGAGATGCTGAGATAGTG – 3’,

reverse primer: 5’- GCACACTCATCATGGCAGCA – 3’;

miR-1301-3p:

forward primer: 5’- GCCCGC TTGCAGCTGCCTGGGAG-3’,

reverse primer: 5’- GTGCAGGGTCCGAGGT – 3’,

-actin gene as internal reference, forward primer: 5’- AGGCCAA CCGCGAGAAGATG-3’, reverse primer: 3’-CACACGGAGTACTTG CGCTCAG-5’.

Cell proliferation assays

Cells (1000 per well) were seeded into 96-well plates for proliferation assay. CAOV3 cells were transfected with overexpression plasmid, OVCAR3 cell lines were transfected with siRNAs. Cell viability was measured by using the CCK-8 kit (Dojindo, Japan) according to the manufacturer’s protocol. All experiments were performed for 3 times. Detection of absorbance every 24 hours. The cell proliferation curves were plotted using the absorbance at each time point.

Transwell assay

Cell migration assay was performed using Transwell chamber inserts (8.0 um, Millipore, USA) in a 24-well plate. First, 2 × 104 cells were counted to seed into the upper chamber. Culture medium containing 20% FBS was placed in the bottom chamber. Then cells were incubated at 37 °C for 1–2 day. After incubation, the cells on the upper surface were scraped and washed away gently, then fixed with methanol. Lastly, cells were stained with 0.5% crystal violet. The numbers of sample cells were counted in five randomly selected fields examined by microscopy. The experiments were repeated independently in triplicate.

RNA immunoprecipitation (RIP-MS2) assays

The pcDNA3.1-MS2 or pcDNA3.1-MS2-PVT1 was co-transfected with pMS2-GFP (Add gene) into OVCAR3 cells. After 48 h, cells were used to perform RIP experiments using a GFP antibody (3 μg per reaction; ab290, abcam) and the Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore) according to the manufacturer’s instructions.[22]

As for HER2 mRNA pull down: HER2 mRNA was transcription in vitro and labeled using the Biotin RNA Labeling Mix (Thermo Fisher, USA). Next, the biotinylated RNAs were incubated with cell lysis buffer from the OVCAR3 cells at 4 °C for 4 h, afterwards, add 30 μl streptavidin beads to sample gently and incubated on a rotator overnight according to the manufacturer’s protocol (Thermo Fisher, Waltham, USA). The coprecipitated RNAs were detected by real-time PCR

RBP immunoprecipitation and RIP-qPCR assays

We performed RIP experiments using a Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) according to the manufacturer’s instructions. A anti-HER2 antibody was used for the experiments. The lysates were incubated with the antibody overnight at 4 °C. The coprecipitated RNAs were detected by real-time PCR.

Chromatin Immunoprecipitation

ChIP assays were carried out as described.[23] OVCAR3 cell lines were digested by trypsin. Then cells were harvested after 13000 rpm centrifugation, dissolved in PBS for 4% formaldehyde to fix 10 min at room temperature, added 0.25 M glycine to quench at room temperature for 5 min. The cell pellet through

centrifuging was resuspended in 1 mL lysis buffer followed by sonication to achieve 300–500 bp DNA fragments.The lysates were incubated with 2 mg anti-HER2 antibody and beads overnight at 4℃. The beads were washed with 600 mL wash buffer four times. Then the reverse crosslinking was carried out by adding proteinase K (10 mg/ml) at 55℃ for 2 hr. DNAs were purified by phenol/chloroformextraction extraction and ethanol precipitation to dissolve in 100 mL ddH2O for qRT-PCR. Primers were listed as follows:

D1 (0-500 bp) forward primer: 5’- gccgcagtcgcccaagcc-3’,

reverse primer: 5’- gctctgcccggaggagcgcg-3’;

D2 (500–1000 bp) forward primer: 5’- ctgtaggtcaactctgcc-3’,

reverse primer: 5’- gactgagctccagctccagt-3’;

D3 (1000–1500 bp) forward primer: 5’- gacagagtgaaacttcatct-3’,

reverse primer: 5’- gtggacactggcctcccatgt-3’;

D4 (1500–2000 bp) forward primer: 5’- ttaagcagtgaaaggattaa-3’,

reverse primer: 5’- ggctctgagagggactgggc-3’;

D5 (2000–2500 bp) forward primer: 5’- atcgcttaaacccaggagg-3’,

reverse primer: 5’- tttaatattcctaccatcc-3’;

D6 (2500–3000 bp) forward primer: 5’- aattgcaactatgagacatt-3’,

reverse primer: 5’- tatcagccagaaataattt-3’;

D7 (3000–3500 bp) forward primer: 5’- cccgggagtaagccaggcatg-3’,

reverse primer: 5’- acaggatctcactctgtcat-3’;

Capture of Nascent RNAs

OVCAR3 cells were seeded into 48-well plates and treated with siRNA (Fig. 3B: siNC or siHER2; Fig. 4A: siNC or siPVT1). After 2 days, 0.2 mM EU was incorporated into the cells for 12hr to capture nascent RNAs,. EU-labeled RNAs were biotinylated and captured by using the Click-iT Nascent RNA Capture Kit (Invitrogen) in accordance with the manufacturer’s instructions.

Luciferase reporter assay

PVT1-wt, PVT1-mut, HER3-wt or HER3-mut sequences was subcloned into pGL3 vector. Then the plasmids were co-transfected with miR-1031-3p mimic into OVCAR3 cell lines using Lipofectamine 2000.

After 2 days, luciferase activities were tested using a PmirGLO Dual Luciferase Expression Vector (Promega, Madison) according to the manufacturer's instructions. Luciferase activity in each group was tested and normalized to Renilla.

Animal experiment

The animal studies were approved by the Animal Ethics Committee of the Central Hospital of Xinxiang. Twelve female BALB/C nude mice (5 weeks of age) were randomly divided into two groups. Mice were anesthetized and injected subcutaneously 2*10^6 cells (dissolved in 100 ul PBS) (pLV-NC or pLV-PVT1 stable expression CAOV3 cell lines) into the left or right flanks. Tumor size was monitored and measured once per week via vernier caliper and the tumor volume was calculated as 1/2 LW2, where L and W are the largest and the smallest perpendicular tumor diameter, respectively. Euthanize mice after four weeks. Tumor tissues were snap frozen in liquid nitrogen and stored at -80℃ for use. All experiments were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals of the Central Hospital of Xinxiang and approved by the Animal Ethics Committee of “Animal Ethical and Welfare Committee (AEWC).

Statistical Analysis

Statistical analysis was performed using Graphpad Prism software (La Jolla, CA, USA). The measurement data were expressed by the mean ± SD. The differences between groups were analyzed by t-test. A P value < 0.05 indicated a significant difference. When representative figures are shown, these are representative of three independent repeats.

Results

PVT1 was amplified in HGSOC and correlated with HER2 expression

To determine the expression pattern of PVT1 in HGSOC, qRT-PCR was conducted in clinical specimens. We observed a significantly high expression in HGSOC compared to adjacent para-cancerous tissues (Fig. 1A). Similarly, HER2 mRNA expression was remarkable increase compared with corresponding noncancerous tissue in the majority of HGSOC specimens (Fig. 1B). Then, HGSOC patients were divided into high- and low- expression group based on HER2 median expression value, we found that PVT1 expression was higher levels in HER2 high group than low group (Fig. 1C). In addition, the expression of PVT1 and HER2 mRNA were strongly positive correlation in HGSOC patients (p□0.001) (Fig. 1D). Then, we further investigated the correlations between PVT1 expression and clinicopathological features in HGSOC tissues. Patients were divided into high and low expression groups according to the median level of PVT1 expression in HGSOC tissues. The results illustrated that PVT1 upregulation was correlated with FIGO stage, ascites and residual leisions, but not associated with patient age (Table 2).

PVT1 promoted ovarian cell proliferation and migration

Firstly, we examined the expression level of PVT1 in five HGSOC cell lines[24] and normal ovarian epithelial cell line. The result showed that PVT1 expression was obviously higher in HGSOC cell lines (Fig. 2A). To investigate the biological function of PVT1 in HGSOC progression, PVT1 was knocked down or over-expressed to detect the phenotype of cell proliferation and migration. The data showed that the capacity of cell proliferation and migration were reduced when PVT1 was knocked down (Fig. 2B, 2D). Conversely, overexpression of PVT1 accelerated ovarian cell proliferation and migration (Fig. 2C, 2E). Furthermore, Lentivirus-mediated knock-down of PVT1 with shRNA in OVCAR3 cell line was used to examine the effect on tumorigenesis in vivo. The results showed that stable knock-down of PVT1 could inhibit tumor weights and volumes markedly compared with control group (Fig. 2F, 2G, 2H). Therefore, our data suggested that PVT1 promotes ovarian carcinoma progression.

PVT1 formed a positive feedback loop with HER2

It has been reported that HER2 was involved in transcriptional activation of many genes serving as a transcription factor.[25,26] To address whether HER2 plays a role in PVT1 transcription in vivo, we performed chromatin immunoprecipitation (ChIP) and q-PCR assay, found that HER2 bound to the region of PVT1 DNA promoter (Fig. 3A). To further elucidate whether HER2 occupies PVT1 DNA promoter, dual-luciferase report system was constructed that contain PVT1 promoter and showed significant reduction of fluorescence signal in HER2 knockdown group (Fig. 3B). In addition, the expression of newly synthesized nascent PVT1 RNA was detected via incorporating 5-ethynyluridine (EU) into cells. As expected, the captured nascent PVT1 RNA was obviously decreased in HER2 knockdown cells (Fig. 3C). Consistently, the mature PVT1 RNA expression was reduced via silencing HER2 expression (Fig. 3D). Furthermore, OVCAR3 cells were treated with a-amanitin to block new RNA synthesis and then the degradation of PVT1 RNA was measured. The results showed that HER2 did not influence the half-life of PVT1 RNA (Fig. 3E). Together the data suggested that HER2 increased PVT1 RNA transcription, not degradation, as a transcription factor.

Then we asked whether PVT1 played an important role in HER2 transcription and translation progression. The captured nascent HER2 RNA level was unchanged (Fig. 4A), but the degradation rate was accelerated in PVT1 knockdown group (Fig. 4B). Also, HER2 mRNA and protein expression levels were decreased in PVT1 knockdown group (Fig. 4C, D). Based on the above results, we speculated that PVT1 could bind with HER2 transcript. PVT1-MS2-RIP experiments confirmed above statement (Fig. 4E). Then we further validated that HER2 pulled down endogenous PVT1 transcript using in vitro transcribed biotin-labelled HER2 (Fig. 4F). HER2-RIP assay revealed that combining capacity of PVT1 transcript and HER2 protein was lacking (Fig. 4G). These results suggested PVT1 elevated HER2 expression through combining with HER2 mRNA, not HER2 protein, eventually, developing a positive feedback loop involving PVT1 and HER2.

PVT1 directly bound to miR1301-3p

To explore the molecular mechanism of PVT1 in HGSOC, StarBase V3.0 (http://starbase.sysu.edu.cn/) was used to predict the potential miRNAs bound to of PVT1. The analysis revealed that miR1301-3p

contained a binding site for PVT1 (Fig. 5A). Then we performed dual luciferase reporter assay and found that the luciferase report activity of PVT1-wt group was significantly lower than PVT1-mut group (Fig. 5B). Furthermore, the RIP experiment was used to validate the direct interaction between PVT1 and miR1301-3p. As shown in Fig. 5C, miR1301-3p-wt group displayed a remarkable enrichment of PVT1 than NC group and miR1301-3p-mut group. Second, we measured the miR1301-3p expression level in HGSOC and adjacent para-cancerous tissues. RT-qPCR showed that miR1301-3p expression was frequently declined and inversely correlated with the expression of PVT1 in HGSOC (Fig. 5D, E). Meanwhile, overexpression of PVT1 robustly decreased miR1301-3p expression and silencing PVT1 significantly increased the expression level of miR1301-3p (Fig. 5F, G).

PVT1 increased HER3 expression via sponging miR-1301-3p to promote HGSOC progression

It has been reported that miR1301-3p is a tumor suppressor and plays critical roles in multiple human cancers. However, the effect of miR-1301-3p on HGSOC is still unclear. Then, bioinformatics prediction (StarBase V3.0) was used to explore the underlying mechanism and found HER3 was a potential target gene for miR1301-3p (Fig. 6A). Dual luciferase reporter assay was performed and the result showed the luciferase activity of HER3-wt group was obviously decreased than HER3-mut group (Fig. 6B). Subsequently, RT-qPCR showed that miR1301-3p could significantly inhibit expression level of HER3 (Fig. 6C). These data suggested that miR1301-3p could combine and influence HER3 expression.

Next we explored the relationship between PVT1 and HER3. We found that mRNA expression pattern of PVT1 was positive correlated with HER3 through spearman correlation analysis (p□0.001)(Fig. 6D). Rescue experiment showed that PVT1 significantly promoted HER3 expression and miR1301-3p could reverse the effect (Fig. 6E, F). CCK-8 assay and transwell assay were performed and exhibited that PVT1 promoted HGSOC cell proliferation and migration through sponging miR1301-3p to elevate HER3 expression (Fig. 6G, H). In brief, these results demonstrated that PVT1 promoted the HGSOC progression via miR1301-3p/HER3 axis.

Discussion

With the recent development of high-throughput techniques, a number of systematic cancer genomics projects have been performed to investigate different molecular pathways, and identify epigenomic, genomic and transcriptomic alterations in cancers. These projects have focused on protein-coding genes and long noncoding transcripts, which were showed to be involved in the regulation of many biological processes.

This study focused on PVT1, a plasmacytoma variant translocation1 lncRNA, has been shown as an important regulator of cancer progression.[27,28] For example, Zeng et al. showed that PVT1 suppressed CRC progression by sponging miR-216a to modulate YBX1 expression.[29] In esophageal carcinoma, PVT1 promoted cancer progression by regulating miR-145/VEGF pathway.[30] Moreover, PVT1 was reported to have clinical significance and found to be associated with malignant progression in ovarian cancer.[31–33]

However, further investigation for elucidating its detailed involvement in HGSOC tumorigenesis was required.

In our study, we found PVT1 expression was significantly higher in HGSOC than adjacent para-cancerous tissues. In addition, cell proliferation, migration and tumor formation of ovarian cancer cells were restrained in PVT1 knockdown group. These results implied that PVT1 may be a functional oncogenic lncRNA in HGSOC. Then we searched out the underlying mechanisms to decipher malignant phenotypes of PVT1 inducing. HER2 was an important oncogenic gene marker in ovarian cancer,[19,20] we found the expression of PVT1 and HER2 mRNA were strongly positive correlation in HGSOC patients. Also, in HER2-high HGSOC tissues, PVT1 expression was obviously increased. Recent reports have shown that HER2 functions as a transcription factor to regulate transcriptional activation of many genes.[25,26] Chromatin immunoprecipitation (ChIP) and dual-luciferase report system uncovered HER2 bound to the region of PVT1 DNA promoter. In return, PVT1 interacted with HER2 transcript to enhance its stability. It suggested PVT1 and HER2 could form a positive feedback loop to promote HGSOC tumorigenesis.

Conclusions

In conclusion, in this study, bioinformatics prediction uncovered that miR1301-3p was a potential target miRNA for PVT1. Furthermore, we found increase HER3 expression via sponging miR-1301-3p to promote HGSOC progression. Our results showed that PVT1, controlled by HER2, elevated HER2 and HER3 expression to promote HGSOC progression. Thus, PVT1 can be regarded as a vital diagnostic biomarker for HGSOC and a potential novel therapeutic target.

Abbreviations

lncRNAs
long noncoding RNAs; HGSOC:high-grade serous ovarian cancer; EGF:epidermal growth factor; cDNA:complementary DNA; RIPA:radio immune precipitation assay; SDS:sodium dodecyl sulfate; PAGE:polyacrylamide gel electrophoresis

Declarations

Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Central Hospital of Xinxiang. All patients and healthy volunteers provided written informed consent prior to their inclusion. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki.

Consent for publication

All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare that they have no competing interests.

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Authors' contributions

WW, WF and YG wrote the main manuscript text, YL and LL collected the data, and LY and QH prepared all figures. CC designed the experiments. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: The siRNA or shRNA sequences

siRNA	Sense 5'-3'
shPVT1-1	GCCAUCAUGAUGGUACUUUAA
siPVT1-2	GCCAGGACACUGAGAUUUGGA
shNC	ACGUGACACGUUCGGAGAAA
siNC	ACGUGACACGUUCGGAGAA
siPVT1-1	GCUAGUGGACAUGAGAAGG
siPVT1-2	CUCAAGAUGGCUGUGCCUG
siHER2-1	caacacagacacgUUUgag
siHER2-2	UcaagUggaUggcgcUgga
miR-1301-3p mimic	CUUCAGUGAGGGUCCGUCGACGUU
miR-1301-3p inhibitor	AACGUCGACCCGACACUGAAG
miR-NC	UUGUACUACACAAAAGUACUG

Table 2: Clinicopathological features of HGSOC tissues with regard to the relative expression of PVT1

Patients	Total	PVT1		χ2	P
	n	low expression	high expression		
	73	(n=36, 67.6%)	(n=37, 43.3%)		
Age(Y)					
≤50	32	17(47.2%)	15(52.8%)	0.006	0.938
>50	41	19(46.3%)	22(53.7%)		
FIGO stage					
I-II	19	15(78.9%)	4(21.1%)	9.023	0.003
III-IV	54	21(38.9%)	33(61.1%)		
Ascites					
positive	57	24(42.1%)	33(57.9%)	5.408	0.02
negative	16	12(75%)	4(25%)		
Residual lesions					
<1 cm	52	31(59.6%)	21(40.4%)	7.673	0.006
≥1 cm	21	5(23.8%)	16(76.2%)		

Figures

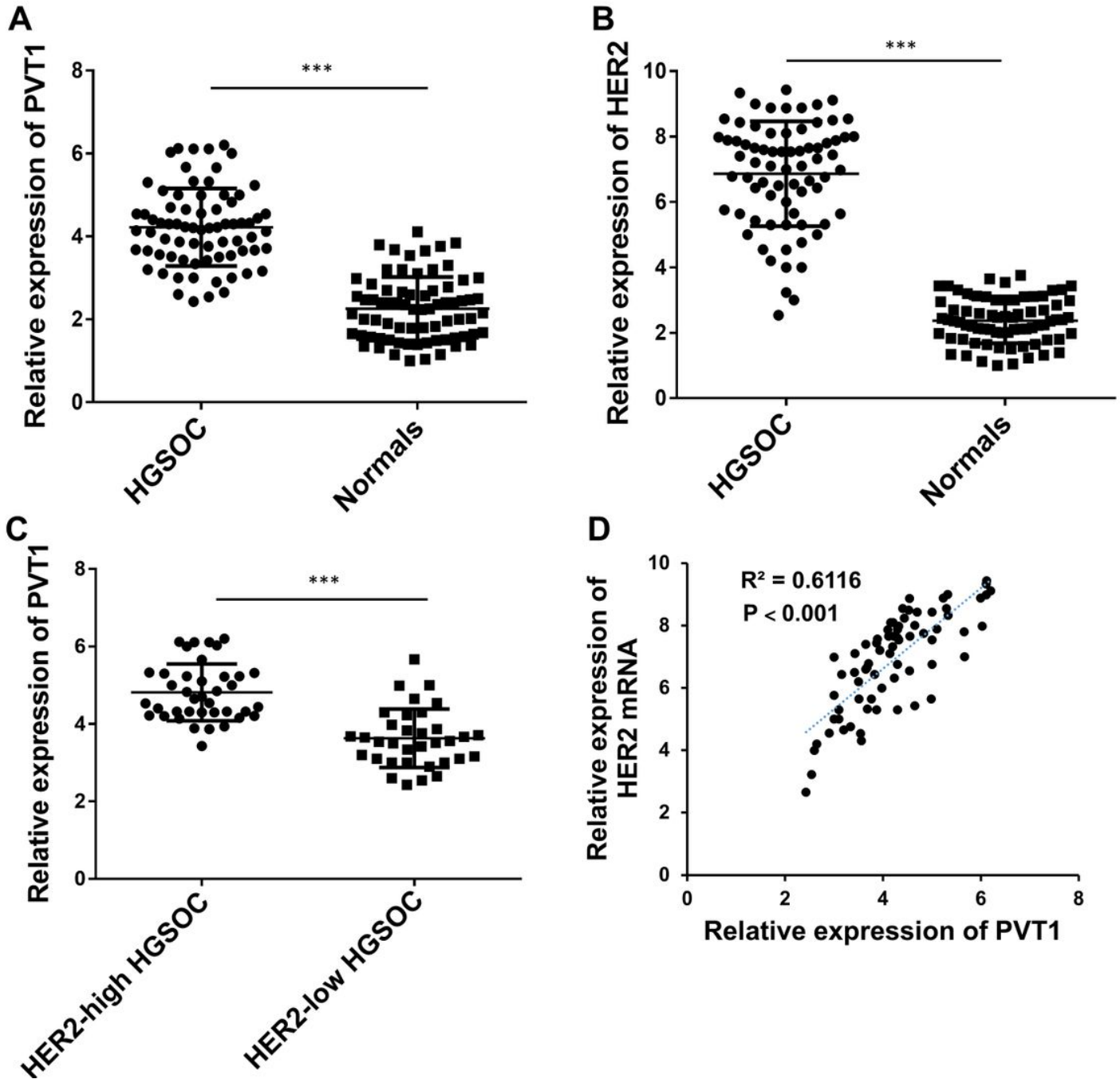

Figure 1

PVT1 was amplified in HGSOC and correlated with HER2 expression. (A) PVT1 expression was significantly higher in HGSOC tissues than normal tissues. (B) HER2 expression was significantly higher in HGSOC tissues than normal tissues. (C) PVT1 expression was higher levels in HER2 high HGSOC group. (D) The correlation was detected between PVT1 and HER2 expression in HGSOC carcinoma tissue. Data are reported as means ± SD. ***P<0.001.

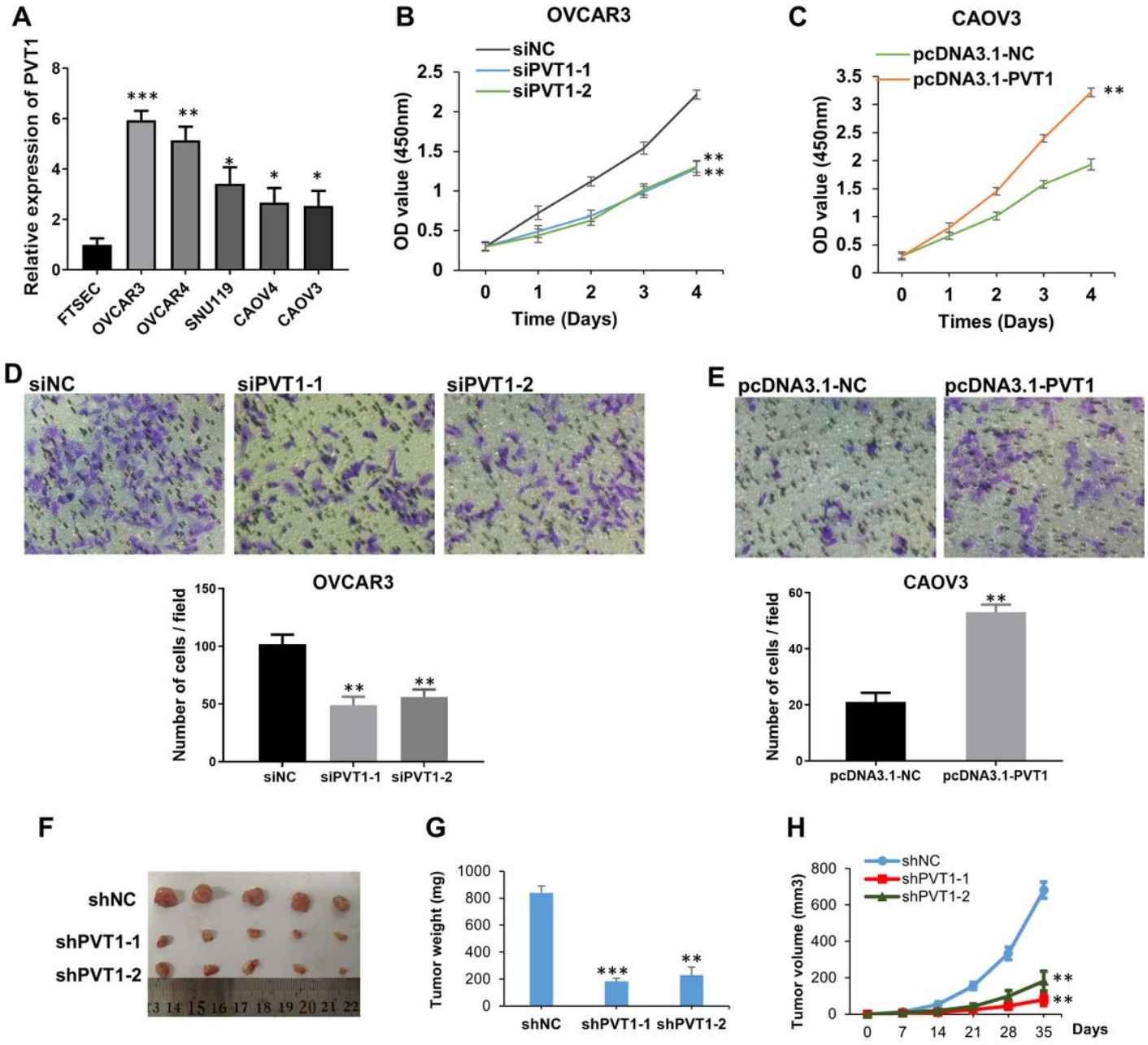

Figure 2

PVT1 promoted ovarian cell proliferation and migration. (A) PVT1 expression was examined in HGSOC cell lines and normal ovarian epithelial cell line. (B, C) CCK-8 assays showed that the effect of PVT1 on ovarian cell (OVCAR3 and CAOV3) proliferation ability. (D, E) Transwell assays showed that PVT1 promoted ovarian cell migration capacity. (F) Mice tumors was represented and PVT1 increased tumor volumes. (G, H) The weights and volumes of the xenograft tumors derived from OVCAR3 cells with stable knock-down PVT1 or the negative control cells were examined. Data are reported as means ± SD. *P<0.05; **P<0.01; ***P<0.001.

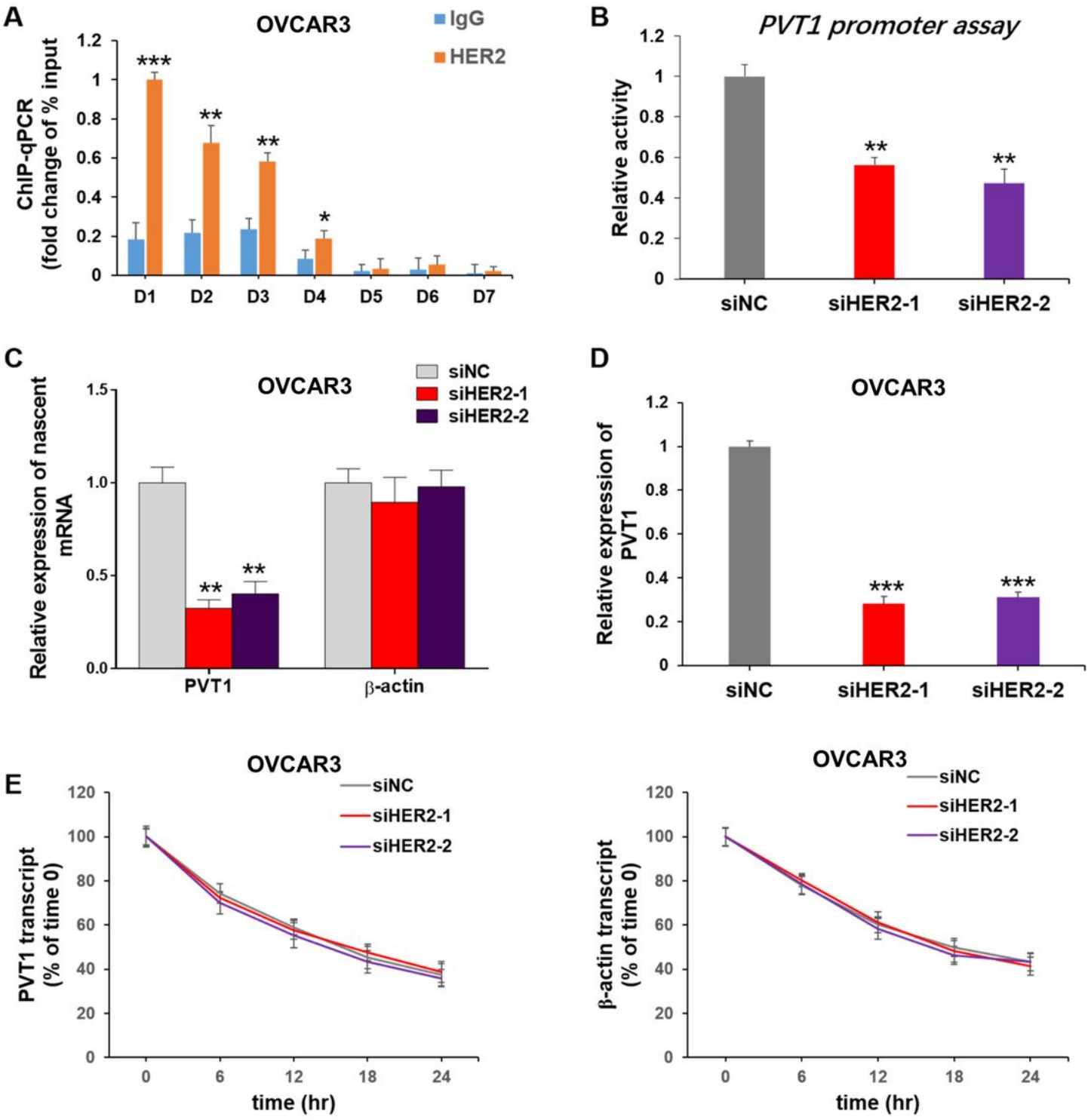

Figure 3

HER2 increased PVT1 RNA transcription, not degradation, as a transcription factor. (A) HER2 bound to the region of PVT1 DNA promoter. “D’s” represent distances from the transcription start site to specific primer sets (B) Dual-luciferase reporter assay was carried out and found that the luciferase activity of siHER2 group was lower in OVCAR3 cell lines. (C) The captured nascent PVT1 RNA was obviously decreased in

HER2 knockdown cells. (D) Mature PVT1 RNA expression was detected by qPCR. (E) The half-life of PVT1 RNA was examined when cell treated with 50μM α-amanitin. *P<0.05; **P<0.01; ***P<0.001.

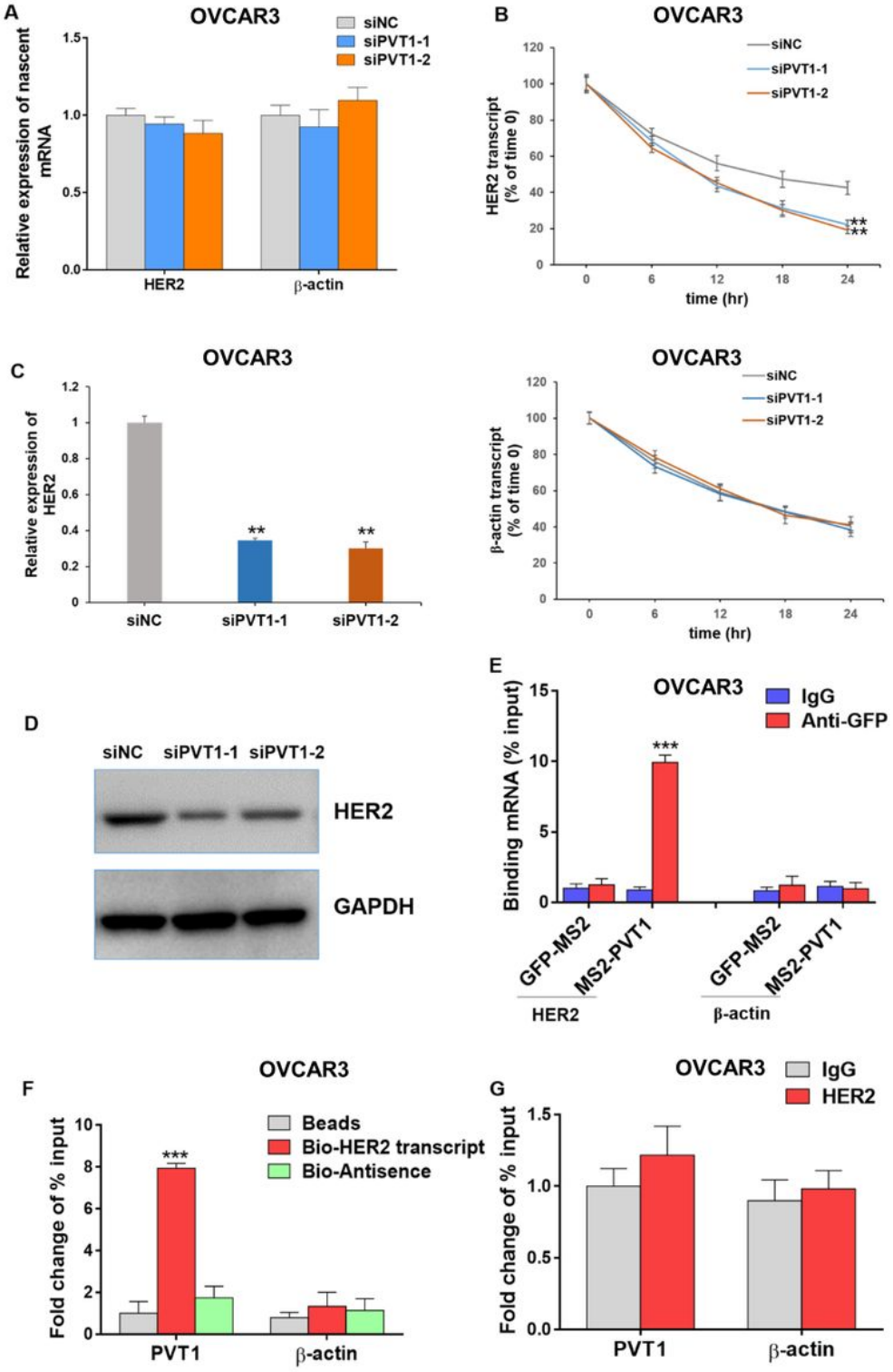

Figure 4

PVT1 elevated HER2 expression through combining with HER2 mRNA. (A) The captured nascent HER2 RNA was detected in PVT1 knockdown cells. (B) The half-life of HER2 RNA was examined when cell treated with 50μM α-amanitin. (C, D) mRNA and protein expression of HER2 were detected by qPCR and

western blot. (E) MS2-RIP followed by mRNA qRT-PCR to detect mRNAs endogenously associated with PVT1. (F) mRNA was extracted and assessed by qRT-PCR after pull-down with biotin-labeled HER2. (G) The combining capacity of PVT1 transcript and HER2 protein was examined by HER2-RIP assay. **P<0.01; ***P<0.001.

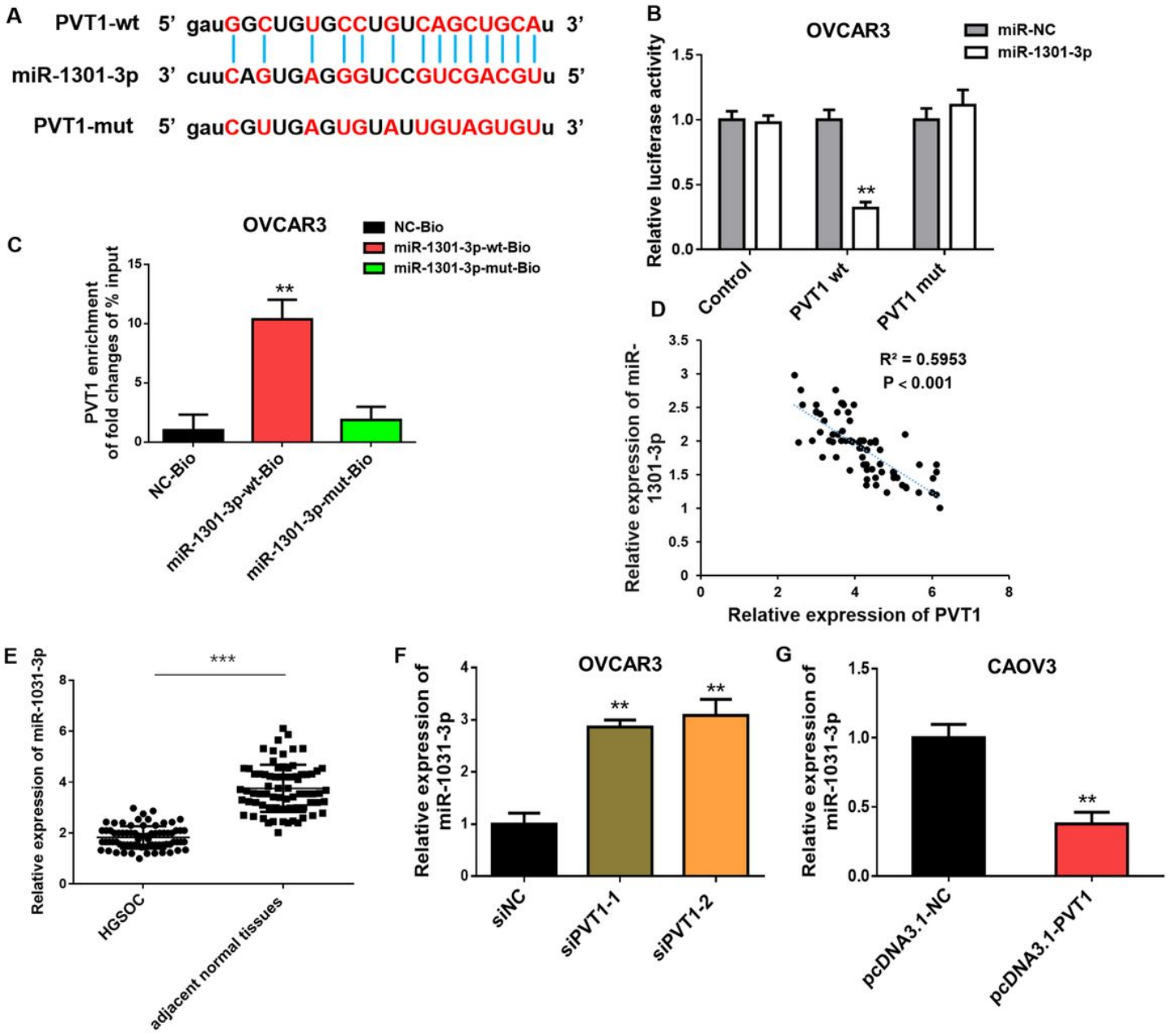

Figure 5

PVT1 directly bound to miR 1301-3p (A) A schematic outline of sequence sites of miR-1301-3p targeted to PVT1 predicted by bioinformatic analysis. (B) Dual-luciferase reporter assay was carried out to examine the luciferase activity of PVT1-wt group and PVT1-mut group. (C) RNA pull-down assay was used to detect the direct interaction between miR-1301-3p and PVT1. (D) The relationship was examined between miR-1301-3p and PVT1 in HGSOC tissues. (E) miR-1301-3p expression was significantly lower in

HGSOC tissues than normal tissues. (F, G) The expression level of miR-1301-3p was detected via qRT-PCR. Data are reported as means ± SD. **P<0.01; ***P<0.001.

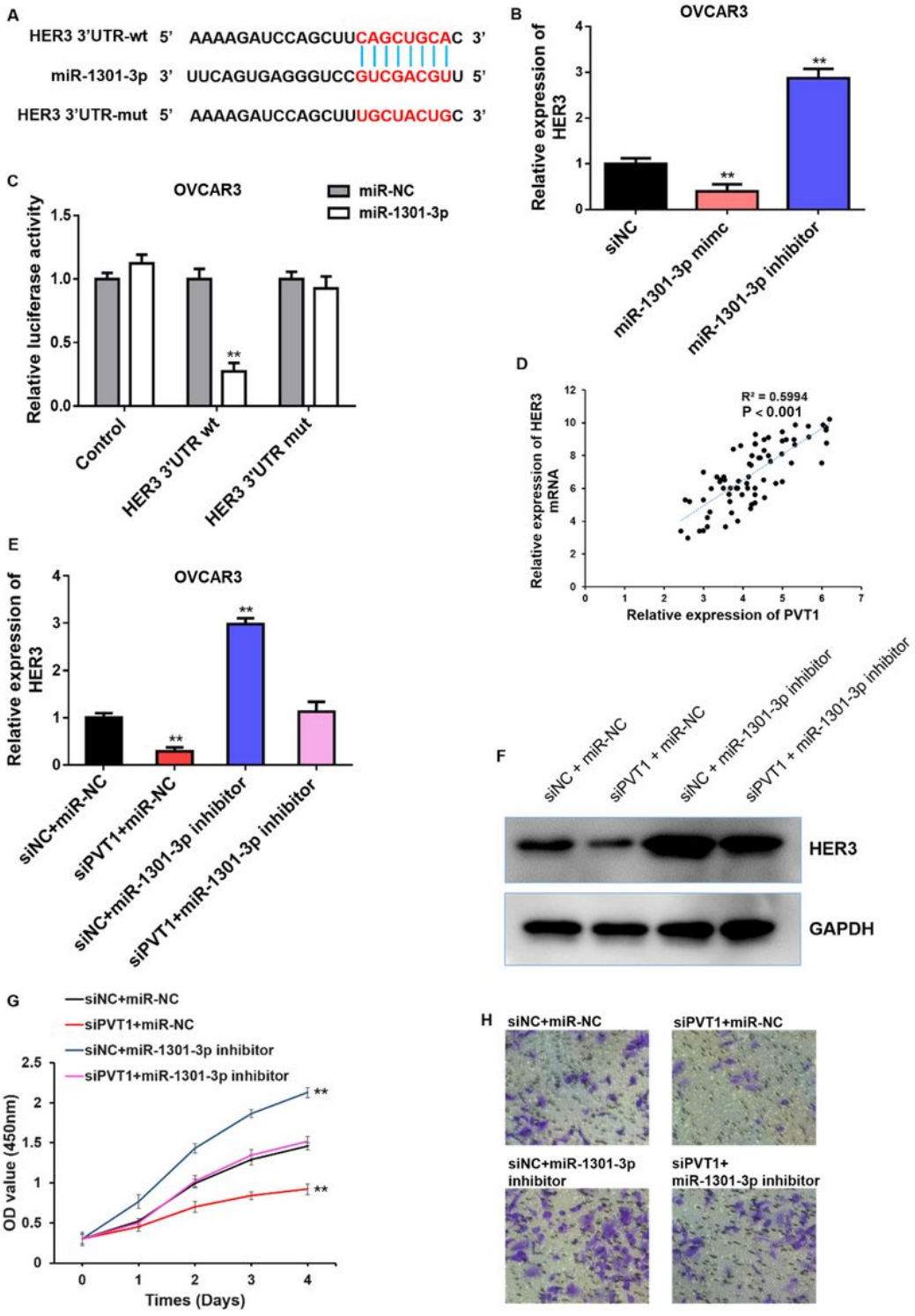

Figure 6

PVT1 increased HER3 expression via sponging miR-1301-3p to promote HGSOC progression. (A) A schematic outline of sequence sites of miR-1301-3p targeted to HER3 predicted by bioinformatic analysis. (B) The expression of HER3 was detected in miR-1301-3p knockdown cells by qRT-PCR. (C)

Dual-luciferase reporter assay was carried out to examine the luciferase activity of HER3 3'UTR-wt group and HER3 3'UTR -mut group. (D) The relationship between mRNA expressions of miR-1301-3p and HER3 in HGSOC tissues was detected. (E, F) qRT-PCR and Western blot validated that miR-1301-3p inhibitor reversed the PVT1 knockdown mediated expression level of HER3 in OVCAR3 cell line. (G, H) miR-1301-3p inhibitor elevated the cell proliferation and migration capability after PVT1 knockdown via CCK-8 and transwell assays in OVCAR3 cell line. Data are reported as means ± SD. **P<0.01.