RETRACTED ARTICLE: Involvement of NORAD/miR-608/STAT3 axis in carcinostasis effects of physcion 8-O-β-glucopyranoside on ovarian cancer cells

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ABSTRACT
We planned to dig the carcinostasis activity of physcion 8-O-β-glucopyranoside (PG) in ovarian cancer cells and explored whether long non-coding RNA NORAD was the potential cause of the carcinostasis impact of PG. The impacts of PG on the tumour cell behaviours (including cell viability, apoptosis, migration and invasion) of SKOV3 cells were grabbed. The levels of NORAD in cancer tissues and cell lines were determined; afterwards, the impacts of abnormal expression of NORAD on the tumour cell behaviours of SKOV3 cells were assessed. Moreover, we explored whether NORAD modulated the level of STAT3 by competitively sponging miR-608, thus mediating the antineoplastic effects of PG on ovarian cancer cells. PG suppressed cell viability, enhanced apoptosis and lessened migration and invasion of SKOV3 cells. NORAD was upregulated in ovarian cancer tissues and cells. Silencing of NORAD lessened cell viability, migration and invasion, but induced apoptosis of SKOV3 cells, whereas overexpression of NORAD had opposite effects. Moreover, PG decreased the expression of NORAD. Overexpression of NORAD reversed the effects of PG treatment on the cell biological performances of SKOV3 cells, which were further reversed after overexpression of miR-608 simultaneously. Furthermore, STAT3 was tested as a target gene of miR-608, and the impact of NORAD in PG-treated SKOV3 cells were assessed. Overexpression of NORAD reversed the effects of PG treatment on the cell biological performances of SKOV3 cells, whereas overexpression of miR-608 reversed the effects of NORAD. Our findings reveal that NORAD/miR-608/STAT3 axis is pivotal in mediating the antineoplastic impacts of PG on ovarian cancer cells, which may offer a novel explanation in the therapy of ovarian cancer.

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INTRODUCTION

Ovarian cancer turns into one of the most lethal gynaecological tumours [1]. The prognosis of ovarian cancer is poor, which is characterized by only a just 40% of 5-year survival rate [2,3]. Relative absence of early symptoms and the high percentage (>60%) of patients that are diagnosed at an advanced stage result in the high mortality of this disease [4]. Thereby, it is of huge significance to deep grab the underlying biology of ovarian cancer and to explore new therapeutic paradigms.

Long noncoding RNAs (lncRNAs), a group of transcripts without protein-coding potential, have gained massive attention due to their roles in the regulation of diverse physiological and pathological processes [5,6]. In recent years, lncRNAs have been identified to have oncogenic or tumour suppressor roles in tumorigenesis [7]. Plenty of lncRNAs are involved in the process of ovarian cancer, such as CCAT1 [8], GASS [9], HOTAIR [10], tumour protein translationally controlled 1 antisense RNA 1 (TPT1-AS1) [11] and pro-transition associated RNA (PTAR) [12]. Moreover, various studies pointed out that lncRNAs are some new prognostic biomarkers or targets for the detection or treatment of ovarian cancer patients [13]. Therefore, identification of crucial lncRNAs involved in ovarian cancer has great significance for the diagnosis and treatment of this disease.

Recently, the functions of a novel cytoplasmic lncRNA, non-coding RNA Activated by DNA damage (NORAD) have been investigated [14]. NORAD is found to function as an oncogene and is relevant to adverse prognosis in various cancers, such as pancreatic cancer [15] and bladder cancer [16], and oesophageal squamous cell carcinoma [17]. In addition, existing evidence has pointed out that lncRNAs could bind RNA/miRNAs and thereby regulate their translation on the identity of competing for endogenous RNAs (ceRNAs) [18,19]. In patients with pancreatic cancer, NORAD is reported to severe as a ceRNA for miR-125a-3p to regulate the expression of the small GTP binding protein RhoA, thereby promoting epithelial-to-mesenchymal transition to facilitate invasion and metastasis [15]. Also, NORAD is believed to reduce the level of miR-608 as a ceRNA and consequently upregulate FOXO6 expression in gastric cancer cells, thus promoting the tumour growth [20]. However, the potential effects of physcion 8-O-β-glucopyranoside on ovarian cancer have not been explored yet.
impact of NORAD in ovarian cancer remains incomplete investigated, let alone its underlying mechanism.

*Rumex japonicus* Houtt is a perennial herbal plant widely distributed in China, has been applied in folk medicine because of its antimicrobial, anti-inflammatory and antineoplastic activities [21,22]. Physcion 8-O-β-glucopyranoside (PG) is one of main active ingredients. PG has been pointed out to exert anti-tumour effects in diverse cancers, such as hepatocellular carcinoma [23], melanoma [24], breast cancer [25], oral squamous cell carcinoma [26] and clear-cell renal cell carcinoma [27]. However, the effect of PG on ovarian cancer development has not been clarified. Whether NORAD is a key regulatory principle in mediating the effects of PG on ovarian cancer o remains unclear.

Currently, the impacts of PG on the cell biological performances of ovarian cancer were evaluated. The level of NORAD both in ovarian cancer tissues and in cells was determined, followed by detection of the impacts of abnormal expression of NORAD on the cell biological performances of SKOV3 cells. Moreover, we explored whether NORAD modulated the level of STAT3 by competitively sponging miR-608, thus mediating the anti-tumour effects of PG on ovarian cancer cells. Our findings will offer novel view for the treatment of ovarian cancer.

**Materials and methods**

**Patients**

In total, 56 ovarian cancer patients who underwent surgery in our hospital between April 2012 and April 2018 were enrolled in this study. The mean age of patients was 53.4 ± 12.2 years. The inclusion criteria were as follows: (1) patients' age between 18 and 78 years; (2) histologically confirmed ovarian cancer; (3) patients with adequate cardiac function: all patients underwent an electrocardiogram, and an echocardiogram with a left ventricular ejection fraction ≥ 50% was performed in patients with a history of heart disease or abnormal electrocardiogram; (4) pulmonary function tests displayed forced expiratory volume in 1 s (FEV1) up to 1.2 L, FEV1% higher than 50%, and carbon monoxide diffusing capacity (DICO) more than 50%; and (5) patients with complete liver function (total bilirubin < 1.5 × normal level (ULN); aspartate transaminase (AST) and alanine transaminase (ALT) < 1.5 × ULN). Exclusion criteria were as follows: (1) other malignant diseases metastasized to the ovaries; (2) combined with other diseases, such as serious lung diseases, heart diseases (e.g., symptomatic coronary artery disease or myocardial infarction within 12 months) and significant dysfunction of bone marrow, liver and kidney; (3) pregnant or lactating women; and (4) allergic to any drugs.

All patients did not receive any radiotherapy or chemotherapy before surgery and were pathologically diagnosed after surgery. Histological confirmation revealed 26 (46.42%), 21 (37.5%), 3 (5.36%), 3 (5.36%) and 3 (5.36%) cases were serious carcinoma, endometrioid carcinoma, mucinous carcinoma, clear cell carcinoma and other pathological types of epithelial carcinoma, respectively. In addition, 23 (41.07%), 21 (37.5%) and 12 (21.43%) cases were in G1, G2 and G3 tumor stages, respectively. Based on International Federation of Gynecology and Obstetrics (FIGO) staging, 6 (10.71%), 12 (21.43%), 37 (57.8%) and 5 (1.72%) cases were in G1, G2, G3 and G4 stages, respectively. Furthermore, among these cases, 18 (32.14%) had distant metastasis and the remaining 38 (67.86%) had no metastasis. Ovarian tumour tissues and adjacent non-tumour ovarian tissues taken during an operation were frozen immediately and stored in liquid nitrogen until use. The study gained the approval from the ethics committee of our hospital, and each patient was informed consent before research.

**Cell culture**

A human ovarian epithelial cell line HOEpiC and five human ovarian cancer cell lines SKOV3, Caov3, A2780, HO-8910 and OVCAR3 were purchased from Chinese Type Culture Collection, Chinese Academy of Sciences. These cells were then grown in RPMI 1640 medium (TaKaRa, Japan) containing 100 mg/ml streptomycin sulfate, 100 mg/ml streptomycin sulfate and 10% fetal bovine serum (TaKaRa) and cultured in a 37 °C humidified air atmosphere with 5% CO₂.

**Cell treatment and transfection**

PG was gained from Chengdu Xunchen Biological Technology Co., Ltd. (Chengdu, China). For detecting the effect of PG on ovarian cancer cells, SKOV3 cells were disposed with various concentrations of PG (0, 20, 50 and 100 µM) for 24 h, 48 h and 72 h, respectively, according to the study of Xie et al. [28].

For investigation of the impact of NORAD in ovarian cancer, the sequence of NORAD was cloned into a pcDNA3.1 (+) vector (Thermo Fisher Scientific, Inc.), named pc-NORAD. The pcDNA3.1 (+) vector was chosen as negative control (NC). The sh-NORAD, sh-NC, miR-608 mimic, mimic NC, pEX-STAT3 and pEX-2 were designed by GenePharma (Shanghai, China). For cell transfection, SKOV3 cells were cultured until reaching 50–70% confluence, these vectors or oligonucleotides were then transfected into SKOV3 cells by means of Lipofectamine 2000 reagents (Invitrogen). Afterwards, cells were harvested after 48 h.

**MTT assay**

SKOV3 cells were seeded into each well of 96-well plate with the density of 2 × 10³ cells each well. Following different treatments at the indicated time, MTT solution (5 g/L, 20 µL/well) was added to each well and used to incubate the cells at 37 °C for another 4 h. DMSO (150 µL/well) was then mixed with cells for dissolving crystals for 10 min. Absorbance values at 492 nm were then measured for assessing cell viability using the microplate reader (BioTek, VT, United States).

**Flow cytometric analysis**

After different treatments, SKOV3 cells were harvested and washed with ice-cold PBS. Cell apoptosis was then analyzed.
by annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (Sangon Biotech, Shanghai, China) and detected on a FACSscan instrument (Becton Dickinson, Mountain View, CA, USA).

**Transwell assays**

After different treatments, SKOV3 cells (5 × 10⁴ cells) were harvested and then placed into the top chamber of Transwell assay inserts (8-mm pore size; Millipore, Billerica, MA, USA) in 200 μL of serum-free RPMI 1640 medium. Unlike migration assay, the top chamber of an insert was precoated with Matrigel for the invasion assay. RPMI 1640 containing 10% FBS was then added to the bottom chamber as a chemo-attractant. Afterwards, any remaining cells on the top layer of the inserts were swept by a sterile cotton swab. The migrated or invaded cells through the membrane were fixed with methanol, stained 0.1% crystal violet, and counted and imaged using digital microscopy (Olympus, Tokyo, Japan).

**Quantitative PCR (qPCR) test**

We isolated the total RNA from cells using Trizol kit (TaKaRa, Japan); afterwards, the isolated and purified RNA was chosen for synthesizing cDNA using an M-MLV Reverse Transcriptase kit (TaKaRa). The expression levels of target genes were then detected by qPCR using the SYBR® Green kit (Takara) on an ABI StepOne PCR instrument. With β-actin as an internal control for RNA and U6 as a reference for miRNA, comparative Ct (2⁻ΔΔCT) method was used for relative quantification of target genes.

**Western blot test**

After different treatments, SKOV3 cells were lysed using the mammalian protein extraction reagent RIPA (Beyotime, Beijing, China). After quantitation, protein extraction (50 μg per lane) was divided by 10% SDS-PAGE and then the divided proteins were transferred to PVDF membrane (Sigma, USA). The membranes were then incubated with specific primary antibodies (1:1000) at 4 °C overnight. After incubation with the recommended secondary antibodies (1:5000) at 37 °C for 2 h, the reacted protein signals were revealed using ECL detection kit (Pierce, Rockford, IL, USA). Primary antibodies to STAT3, Bcl-2, Bax, pro-caspase-3, cleaved caspase-3, pro-caspase-9, cleaved caspase-9 and β-actin were all gained from Abcam (Cambridge, UK). β-actin was chosen as the control.

**Dual-luciferase reporter test**

The pMIR-REPORT-STAT3-wt/mut (Sangon Biotech, Shanghai, China) were produced and then were transfected into SKOV3 cells, together with miR-608 mimic or mimic NC. Dual-Luciferase Reporter Assay System (E1910, Promega, WI, USA) was chosen for the evaluation of luciferase activity of reporter vectors after 48 h of transfection.

**Statistical analysis**

We carried out all experiments independently with three times repeats. The SPSS 16.0 software (SPSS, Chicago, IL) was chosen for statistical analysis. The obtained data were displayed as the mean ± standard error (SD). The statistical differences were calculated using a Student t-test for two groups or one-way analysis of variance (ANOVA) for more than two groups. p < .05 was chosen as statistically significant.

**Results**

**PG inhibits cell viability, migration and invasion but promotes apoptosis of SKOV3 cells**

To grab the impact of PG in ovarian cancer development, we used different concentrations of PG to treat SKOV3 cells for different times and then detect the effects of PG on cell behaviours. As shown in Figure 1(A), MTT test uncovered that PG treatment significantly depressed SKOV3 cell viability in a dose- and time-dependent way (p < .05). According to the cell viability, 48 h of PG treatment was chosen for the following experiments. Flow cytometry test uncovered that 48 h of PG treatment dramatically induced SKOV3 cell apoptosis in a dose-dependent way (p < .05); meanwhile, western blot revealed that 48 h of PG treatment remarkably decreased the level of Bcl-2 but accelerated the levels of Bax, cleaved-caspase-3 and cleaved-caspase-9, confirming that PG promoted SKOV3 cell apoptosis (Figure 1(B)). Moreover, the results of Transwell assays displayed that 48 h of PG treatment prominently suppressed the migration (Figure 1(C)) and invasion (Figure 1(D)) of SKOV3 cells in a dose-dependent way (p < .05). These findings proved to us that PG might exhibit an anti-tumour effect on ovarian cancer.

**NORAD is upregulated in ovarian cancer and PG decreases the expression of NORAD**

There were studies reporting the role of NORAD in several cancers [15–17,28], but the impact of NORAD in ovarian cancer remains incomplete clarified. To grab if NORAD played a significant role in ovarian cancer, we assessed the levels of NORAD both in ovarian cancer tissues and in cells. The data uncovered that NORAD was markedly higher expressed in ovarian tumour tissues than that in non-tumour tissues (p < .01, Figure 2(A)). Moreover, NORAD was also remarkably up-expressed in five ovarian cancer cell lines, including A2780, Caov3, HO-8910, SKOV3 and OVCAR3, compared to that in normal ovarian epithelial HOEpiC cells (p < .05, Figure 2(B)). SKOV3 cells were selected for subsequent experiments due to the highest expression levels of NORAD. Subsequently, when transfecting with pc-NORAD and sh-NORAD, NORAD was dramatically up-expressed or depressed in SKOV3 cells, respectively (p < .001, Figure 2(C)), implying a high transfection efficiency. Moreover, silencing of NORAD by transfection with sh-NORAD significantly inhibited cell viability (p < .05, Figure 2(D)), induced apoptosis (p < 0.001, Figure 2(E)) and suppressed migration (p < .05, Figure 2(F))
Physcion 8-O-β-glucopyranoside (PG) inhibited cell viability, promoted apoptosis and suppressed migration and invasion of SKOV3 cells in a dose-dependent manner. (A) MTT showed the SKOV3 cell viability after treatment with different concentrations of PG at different times. (B) Flow cytometry showed SKOV3 cell apoptosis and western blot revealed the expression of apoptosis-related proteins after treatment with different concentrations of PG for 48 h. (C,D) Transwell assays showed SKOV3 cell migration and invasion after treatment with different concentrations of PG for 48 h. All experiments were repeated three times. Data were expressed as the mean ± standard error (SD). *, p < .05; **, p < .01 and ***, p < .001.
Figure 2. NORAD was upregulated in ovarian cancer and PG decreased the expression of NORAD. (A) NORAD expression in ovarian tumour tissues and non-tumour tissues. (B) NORAD expression in ovarian cancer cells and normal ovarian epithelial cells. (C) NORAD expression in SKOV3 cells after transfection with pc-NORAD, sh-NORAD and their controls. (D–G) After transfection with pc-NORAD, sh-NORAD and their controls SKOV3 cell viability, apoptosis and the expression of apoptosis-related proteins, migration and invasion were detected, respectively. (F) NORAD expression in SKOV3 cells after treatment with different concentrations of PG for 48 h. All experiments were repeated three times. Data were expressed as the mean ± standard error (SD). *, p < .05; **, p < .01 and ***, p < .001.
and invasion ($p < .05$, Figure 2(G)) of SKOV3 cells. Opposite effects on SKOV3 cell viability (Figure 2D), migration (Figure 2(F)) and invasion (Figure 2(G)) were observed after silencing of NORAD by transfection with sh-NORAD compared to transfection with sh-NC (all $p < .05$), but apoptosis did not exhibit significant change (Figure 2(E)). These data indicated that NORAD might play a tumour-promotion function in ovarian cancer. To further investigate whether PG exhibited anti-tumour effects on ovarian cancer by regulating NORAD, we determined the NORAD expression in PG-treated SKOV3 cells. The results showed that 48 h of PG treatment remarkably decreased NORAD expression in a dose-dependent manner (Figure 2(G)). Based on this result, 100 μg/ml of PG was selected in the following experiments.

**PG inhibits the cell biological performances of SKOV3 cells through decreasing NORAD expression**

To explore whether the effects of PG on ovarian cancer via inhibiting NORAD expression, SKOV3 cells were transfected with pc-NORAD after treatment of 100 μg/ml PG for 48 h. We found that compared to PG + pc-NORAD transfection, overexpression of NORAD by transfection with pc-NORAD significantly increased the cell viability ($p < .05$, Figure 3(A)), migration ($p < .05$, Figure 3(C)) and invasion ($p < .05$, Figure 3(D)) but inhibited apoptosis ($p < .01$, Figure 3(B)) of PG-treated SKOV3 cells, indicating that overexpression of NORAD reversed the impacts of PG treatment on the cell biological performances of SKOV3 cells.

**NORAD promotes the growth of PG-treated SKOV3 cells by sponging miR-608**

A recent study has confirmed that NORAD accelerates the growth of gastric cancer cells through sponging miR-608 [20]. We thus hypothesized that whether this kind of correlation between NORAD and miR-608 existed in ovarian cancer. As presented in Figure 4(A), miR-608 was dramatically enhanced after transfection with miR-608 mimic relative to transfection with mimic NC ($p < .001$), suggesting that miR-608 was successfully overexpressed. In comparison to PG + pc-NORAD + mimic NC group, miR-608 mimic concurrently in PG + pc-NORAD + miR-608 mimic group significantly changeover the impacts of NORAD high level on PG-treated SKOV3 cells by decreasing cell viability ($p < .05$, Figure 4(B)), migration ($p < .05$, Figure 4(D)) and invasion ($p < .05$, Figure 4(E)) but promoting apoptosis ($p < .01$, Figure 4(C)). We thereby deduced that NORAD might promote the growth of PG-treated SKOV3 cells by sponging miR-608.

**STAT3 is tested as a target of miR-608, and the impacts of NORAD in PG-treated SKOV3 cells are through miR-608-mediated STAT3**

We identified the target genes of miR-608 in ovarian cancer cells. Using Targetscan online tool, we found that STAT3 was targeted by miR-608 (http://www.targetscan.org/cgi-bin/targetscan/vert_71/view). Subsequently, luciferase reporter test was carried out and the data displayed that STAT3-wt group obtains a very low luciferase activity after co-transfection with miR-608 mimic ($p < .05$), but the luciferase activity of STAT3-mut did not exhibit obvious change (Figure 5(A)). Meanwhile, the levels of STAT3 were conspicuously underexpressed in miR-608 mimic group relative to mimic NC group ($p < .01$, Figure 5(B)). These data confirmed the target relationship between miR-608 and STAT3. Furthermore, we further overexpressed STAT3 to verify whether the role of NORAD in PG-treated SKOV3 cells was through regulation of miR-608-mediated STAT3. The results showed that STAT3 was successfully overexpressed by transfection of pEX-STAT3 ($p < .001$, Figure 5(C)). Our results further showed that, the impacts of pc-NORAD and miR-608 synchronously on PG-treated SKOV3 cells were dramatically changeover after pc-NORAD transfection simultaneously, which were testified by the increased cell viability ($p < .015$, Figure 5(D)), decreased apoptosis ($p < .01$, Figure 5(E)) and the enhanced abilities of migration ($p < .05$, Figure 5(F)) and invasion ($p < .05$, Figure 5(G)) in PG + pc-NORAD + miR-608 mimic + pEX-STAT3 group compared to PG + pc-NORAD + miR-608 mimic + pEX group. We thereby deducted that the role of NORAD in PG-treated SKOV3 cells might be regulated by miR-608-mediated STAT3.

**Discussion**

Mounting evidence has revealed that deregulation of IncRNAs is involved in the development of human malignancies [29], providing insights into novel strategies for cancer treatment. We grabbed the antineoplastic activity of PG in ovarian cancer cells and explored whether NORAD was responsible for the antineoplastic impact of PG. And, we discovered that PG depressed cell viability, migration and invasion but enhanced apoptosis of SKOV3 cells, suggesting the potential of PG as a chemotherapeutic agent in the progress of ovarian cancer.

One of other important findings of our study was that NORAD was upregulated in ovarian cancer tissues and cells, and silencing of NORAD resulted in depressions on cell viability, migration and invasion, but an enhancement on apoptosis of SKOV3 cells. Consistent with previous findings in other cancers [30–32], our results confirmed that NORAD may also play a tumour-promotion impact in ovarian cancer. However, we did not investigate the association between NORAD expression and overall patients, further studies are still required to evaluate the correlation between NORAD and patients’ prognosis. Moreover, overexpression of NORAD reversed the impacts of PG treatment on the cell biological performances of SKOV3 cells; we thus speculate the PG may exert anti-tumour impact on ovarian cancer via targeting NORAD.

Accumulating evidence together pointed out that IncRNAs could bind miRNAs/RNAs and thereby running their regulator functions on the identity of ceRNAs in many diseases [33,34]. Strikingly, our results found that the role of NORAD in PG-treated SKOV3 cells were across the miR-608-mediated STAT3. There is a study that has confirmed the regulatory
Figure 3. PG inhibited the malignant behaviours of SKOV3 cells through decreasing NORAD expression. SKOV3 cells were transfected with pc-NORAD and pcDNA3.1 in presence of PG treatment. (A–D) SKOV3 cell viability, apoptosis and the expression of apoptosis-related proteins, migration and invasion were detected, respectively. All experiments were repeated three times. Data were expressed as the mean ± standard error (SD). *, p < .05, **, p < .01 and ***, p < .001.
Figure 4. NORAD promoted the growth of PG-treated SKOV3 cells by sponging miR-608. (A) miR-608 expression in SKOV3 cells after transfection with miR-608 mimic and mimic NC. (B–E) SKOV3 cells were cotransfected with pc-NORAD and miR-608 mimic in presence of PG treatment. Then, SKOV3 cell viability, apoptosis and the expression of apoptosis-related proteins, migration and invasion were detected, respectively. All experiments were repeated three times. Data were expressed as the mean ± standard error (SD). *, p < .05; **, p < .01 and ***, p < .001.
Figure 5. STAT3 was identified as a target gene of miR-608, and the role of NORAD in PG-treated SKOV3 cells were through regulation of miR-608-mediated STAT3. (A) Luciferase reporter assay showed the target relationship between miR-608 and STAT3. (B) STAT3 expressions in SKOV3 cells after transfection with miR-608 mimic and mimic NC. (C) STAT3 expression in SKOV3 cells after transfection with pEX-STAT3 and pEX-2. (D–G) SKOV3 cells were cotransfected with pc-NORAD, miR-608 mimic and pEX-STAT3 in presence of PG treatment. Then, SKOV3 cell viability, apoptosis and the expression of apoptosis-related proteins, migration and invasion were detected, respectively. (H) The technical chart of this study. All experiments were repeated three times. Data were expressed as the mean ± standard error (SD). *, p < .05, **, p < .01 and ***, p < .001.
relationship between NORAD and miR-608 [20], implying the reliability of our results. Moreover, miR-608 is shown to serve as a tumour suppressor in lung adenocarcinoma [35] and is associated with the prognosis of lung adenocarcinoma treated with tyrosine kinase inhibitors targeting EGFR [36].

Expect in lung adenocarcinoma, previous studies also confirmed the tumour suppressive role in some other types of human tumours, including glioma [37], colon cancer [38] and bladder cancer [39]. Notably, HOXD cluster antisense RNA 1 (HOXD-AS1), a cancer-related IncRNA, has been shown to promote the malignant behaviours of ovarian cancer cells through miR-608 [40]. Given the tumour suppressive role of miR-608 in various cancers, we deduced that NORAD promotes the progression of ovarian cancer via regulating miR-608.

Moreover, STAT3 was tested as a target gene of miR-608 in ovarian cancer cells. It is shown that STAT3 plays a leading role in several processes such as inflammation and immunity in tumours by regulating numerous oncogenic signalling pathways, such as nuclear factor-κB and Janus kinase pathways [41]. In ovarian cancer ascites, elevated STAT3 expression is shown to promote tumour invasion and metastasis [42]. Moreover, STAT3 is abnormally highly expressed and is shown to be relevant to the poor outcome in patients with ovarian cancer [43]. Furthermore, scholars pointed out that STAT3 exhibits utility in both monotherapy and combination therapy and thereby may be a promising target in ovarian cancer models [44,45]. In this study, we found that the impacts of pc-NORAD and miR-608 mimic synergistically on PG-treated SKOV3 cells were dramatically changeover after overexpression of STAT3 synchronously. We thus deduced that NORAD could regulate STAT3 through mediating miR-608, thus regulating the antineoplastic activity of PG in ovarian cancer cells.

To sum up, our findings confirm the antineoplastic activity of PG in ovarian cancer cells. Moreover, NORAD/miR-608/STAT3 axis is an important regulatory chain in mediating the antineoplastic impacts of PG in ovarian cancer cells. Our discoveries may offer a novel experimental basis for the explanation of the progression of ovarian cancer. Further in vivo tests including animal or human clinical trials are to carry out to verify our conclusion.

Disclosure statement
Authors declare that there is no conflict of interests.

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