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Investigation of the mechanism by which FOXJ2 inhibits proliferation, metastasis and cell cycle progression of ovarian cancer cells through the PI3K/AKT signaling pathway

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Abstract

Background As one member of the Forkhead Box transcription factor, Forkhead Box J2 (FOXJ2) is involved in diverse cancers. At present, the specific role and mechanism of FOXJ2 in ovarian cancer (OC) have not been fully addressed, which allows us to fill the blank.

Materials and methods Accordingly, the expression of FOXJ2 in OC cells and ovarian epithelial cells was quantified via real-time qPCR. Following the transfection, cell counting kit-8, Transwell, wound healing and flow cytometry assays were performed to measure the proliferation, metastasis, apoptosis and cell cycle of OC cells A2780 and HEY. Further, real-time qPCR and Western blotting were both employed for the quantification assays on the expression levels of FOXJ2 as well as phosphoinositide 3-kinase (PI3K) and protein kinase B (AKT) (in both unphosphorylated and phosphorylated forms).

Results Based on the results, FOXJ2 were highly-expressed in OC cells ($P < 0.05$). Silencing of FOXJ2 resulted in attenuated OC cell proliferation, reduced numbers of migrating and invading OC cells, decreased apoptotic capacity, and cell cycle arrest in G1/S phase ($P < 0.05$). In addition, the knockdown of FOXJ2 caused the downward trend on the phosphorylation level of both PI3K and AKT in OC cells ($P < 0.05$).

Conclusion The silencing of FOXJ2 could repress the growth and metastasis potentials and cause the cell cycle G1/S arrest of OC cells in vitro, which was possibly achieved via targeting the PI3K/AKT pathway.

Keywords Cell cycle, Forkhead box J2, Metastasis, Ovarian cancer, PI3K/AKT pathway, Proliferation

Introduction

Ovarian cancer (OC) refers to a group of heterogeneous malignancies involving the ovaries, fallopian tubes and the peritoneal cavity, which is the most prevalent malignancy of the female reproductive system [1–5]. The treatment of OC is based on cytoreductive surgery with the supplementation of chemotherapy, which has indeed made some progresses in the last several decades [6, 7]. However, the therapeutic outcomes of OC patients are mostly thwarted by the development of acquired chemo/radioresistance and the inadequacy of relevant targeted

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therapies [8]. Post-operative chemotherapy in patients with advanced stage of OC, or neoadjuvant chemotherapy followed by the surgery, has achieved an improved progression-free survival and overall survival; however, the increase in recurrence rates has not met the desired expectations [9]. Hence, in an effort to improve the survival of patients and overcome resistance to the therapies, extensive studies have commenced to better understand the precise mechanisms that promote the formation and development of OC [10].

The Forkhead Box (FOX) transcription factors have been considered as potential targets in cancer treatment [11]. As one of the largest transcription factor families, FOX proteins with 44 main family members in humans can be sub-divided into 19 classes [12]. Members of this family has been revealed to have an evolutionarily conserved DNA-binding domain and involve in regulating cancer initiation, progression and chemoresistance [13]. Some existing studies have proven the critical role of FOX transcription factors in OC. For instance, *FOXO1* can increase the transcription and methyltransferase like 14 (METTL14)-mediated N^6-methyladenosine (m^6A) modification of structural maintenance of chromosome 4 (SMC4) [14]. In the meantime, *FOXA1* has been underlined to induce epithelial OC tumorigenesis and progression [15]. Further, *FOXM1* has been established as a multifunctional oncoprotein which can be a therapeutic target in OC as well [16].

Forkhead Box J2 (FOXJ2) is a recently characterized FOX transcription activator that binds to DNA with a dual sequence specificity and expresses at different levels in many types of tissues in adults [17]. Recent studies have additionally demonstrated the participation of *FOXJ2* in diverse cancers. For instance, *FOXJ2* represses the proliferation, migration and epithelial-to-mesenchymal transition (EMT) of prostate carcinoma cells [18]. Also, an integrated analysis has revealed that *FOXJ2* can coordinate with microRNA-20a to mediate the growth and metastasis of colorectal cancer cells [19]. Additionally, *FOXJ2* overexpression is demonstrated to be related to the poor prognosis, development and metastasis in nasopharyngeal carcinoma [20]. Besides, low *FOXJ2* expression in epithelial OC has been shown to be associated with the unfavorable postoperative prognosis [21]. Nonetheless, the specific role of *FOXJ2* in OC has not been fully expounded, which allows us to initiate this study to fill the blank. The current study will accordingly examine the specific effects of *FOXJ2* on the proliferation and metastasis in vitro, and investigate the corresponding pathway(s) accounting for the effects of *FOXJ2* on OC. These investigations, we hope, can be helpful in the identification of possible therapeutic candidates for the intervention of OC.

Material and methods

Cell culture and transfection

Human ovarian epithelial cells IOSE-80 (BNCC358126) as well as OC cell lines SK-OV-3 (BNCC338639), CoC1 (BNCC101678), A2780 (BNCC351906) and HEY (BNCC353326) were all ordered from BeiNa Culture Collection (Xinyang, China) High-glucose Dulbecco's modified Eagle's medium (11,965,092, Gibco, Grand Island, NY), Roswell Park Memorial Institute-1640 medium (11875101, Gibco) and McCoy's 5A medium (16600082, Gibco) were used in cell culture. 10% fetal bovine serum (A3161001C, Gibco) was supplemented in all the media. Cell incubation was performed using an incubator at 37 ℃ with 5% CO_2 in a humidified atmosphere. Lipofectamine 2000 (11668027) was procured from Invitrogen (Carlsbad, CA) and the relevant small interfering RNAs (siRNAs) targeting *FOXJ2* si-*FOXJ2*#1 (target sequence: 5′-GTGATAACTTCCCCTATTACAAG-3′) and si-*FOXJ2*#2 (target sequence: 5′-GGGTTCCTATTG GACAATTGACA-3′) as well as the control siRNA (si-NC) with the scrambled sequence were all ordered from RiboBio (Guangzhou, China). Then OC cells A2780 and HEY were transfected with the relevant siRNAs with the transfection reagent according to the protocol of the producer. All cells have been STR identified and the mycoplasma detection results are negative.

Cell proliferation assay

The cell counting kit-8 (CCK-8) assay was carried out to test the OC cell proliferation in vitro [22, 23]. Cells following the transfection were seeded at the density of 2×10^3 cells/well into a 96-well plate containing the culture medium. After the incubation for 12, 24, 36 and 48 hours (h), cells were added with 10 μL CCK-8 solution (C0037, Beyotime, Shanghai, China) for additional culture for 4 h. A microplate reader (iMark, Bio-Rad, Hercules, CA) was applied to read the optical density (OD) value at 450 nm.

Cell invasion assays

The invasion capabilities of OC cells were determined in Transwell chamber (pore: 8 μm, 354480, Corning, Inc., Corning, NY) coated without or with the Matrigel (354234, Corning, Inc.) [24]. In detail, the upper chamber was supplemented with OC cells cultured in 200 μL serum-reduced medium, while 600 μL medium containing 10% fetal bovine serum was added to the corresponding lower chamber. Following the culture of 48 h, cells invaded to the lower chamber were visualized in 0.1% crystal violet staining reagent (C0121, Beyotime) and the number of invaded cells was calculated from five different views under the optical microscope (DP27, Olympus, Tokyo, Japan).

Wound healing assay

The migration of OC cells was assessed through wound healing assays [24]. In summary, cells were plated in 6-well plates at a density of 5×10^5 cells per well and cultivated until reaching full confluency. A scratch was created using a 10 mL pipette tip, and the cells were then incubated for 24 h in serum-free medium at 37 °C with 5% CO_2. A light microscope was used to capture images of the migrating cells.

Cell apoptosis assay

Cells of A2780 and HEY were plated in 6-well culture plates and subsequently transfected with either si-NC or si-*FOXJ2*. Following this, the cells were collected, rinsed with PBS, and then resuspended in 195 μL of annexin-V FITC (BD Biosciences, Franklin Lakes, NJ, USA) alongside 5 μL of propidium iodide (PI) in accordance with the manufacturer's instructions. The samples were then incubated in the dark at room temperature for 10 min. Flow cytometry was used for analysis, and the data were processed with Lysis software (EPICS-XL, Ramsey, Minnesota, USA) [25].

Cell cycle analysis

After cell collection, the transfected OC cells were rinsed in phosphate buffer saline (PBS) buffer, followed by being fixed in 70% ethanol (A507050, Sangon Biotech, Shanghai, China) at – 20 ℃ overnight. Then these cells were treated with RNase A (0.1 mg/mL, ST578, Beyotime) and dyed with propidium iodide (PI, ST1569, Beyotime) as per the manuals. The samples were analyzed in independent triplicate with BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ) and cell percentage in each phase was quantified in FlowJo 11 (FlowJo, LLC., Ashland, OR) [26].

RNA isolation and real-time qPCR

Using the TriZol assay kit (15596026, Invitrogen), the total RNA from OC cells and ovarian epithelial cells IOSE80 was extracted to synthesize corresponding complementary DNA (cDNA) with the use of a cDNA synthesis kit (D7178S, Beyotime). To quantify the mRNA level of FOXJ2, the qPCR was then initiated with the help of a qPCR mix (D7260, Beyotime) at the thresholds of at 95 ℃ for 2 min (min) and 40 cycles of at 95 ℃ for 15 s (s) and 60 ℃ for 30 s. Relative FOXJ2 mRNA expression was finally calculated with the $2^{-\Delta\Delta Ct}$ relative quantification method with GAPDH as the normalization control [27]. The primers used are listed as follows: *FOXJ2*-forward: 5′-AACTTTCAGGATCTAAGCTG-3′; reverse: 5′-GTC GTAGACTCTCCATCAGT-3′; GAPDH-forward: 5′-CCTCAACTACATGGTTTACA-3′; reverse: 5′-TGT TGTCATACTTCTCATGG-3′.

Western blotting analysis

The cell samples were lysed in RIPA lysis buffer (P0013B, Beyotime) to extract the protein sample. Then around 50 mg protein samples were separated in SDSPAGE separation gel and moved onto the polyvinylidene fluoride membrane. Hereafter, the non-specific binding in the membrane was clocked by 5% non-fat milk and the membrane was incubated overnight with the relevant primary antibodies against FOXJ2 (#8186, 1:1000, Cell Signaling Technology, Danvers, MA), phosphoinositide 3-kinase (PI3K, #4292, 1:1000, Cell Signaling Technology), phosphorylatedPI3K (#4228, 1:1000, Cell Signaling Technology), protein kinase B (AKT, ab8805, 1:500, Abcam, Cambridge, UK), phosphorylated-AKT (ab38449, 1:1000, Abcam) and housekeeping control GAPDH (ab181602, 1:10000, Abcam) at 4 ℃ overnight. Further, TBST was washed with the membrane three times for 10 min and probed with the horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (A0208, 1:1000, Beyotime) at room temperature for 1 h. Following another rinse in TBST, the membranes were developed with an ECL visualization reagent (P0018S, Beyotime) and the band density was quantified by the densitometry using Quantity One 4.6.6 (Bio-Rad).

Statistical analysis

All quantified data represented an average of three independent samples and shown as mean ± SD. GraphPad Prism 10.4.1 was applied for statistical analysis. Significant differences were determined via one/two way ANOVA test, followed by Dunnett's/Turkey's multiple comparisons test or Student's t test. The values of $P<0.05$ were deemed to indicate the statistically significant results in all cases. In the figures, ns denotes P-value > 0.05, ** denotes P-value < 0.01, *** denotes P-value < 0.001 and **** denotes P-value < 0.0001.

Results

Upregulated expression of FOXJ2 in OC cells

The qPCR and western blotting assays were carried out to quantify the mRNA and protein level of *FOXJ2* in OC cells and ovarian epithelial cells IOSE-80, so as to investigate the role of *FOXJ2* in OC. According to the relevant results, the mRNA and protein expression of *FOXJ2* were markedly higher in OC cells than that in ovarian epithelial cells IOSE-80 (Fig. 1A–C, P < 0.001). Considering the relatively high expression in A2780 and HEY cells, these two cells were applied in subsequent assays.

Repressive effects of FOXJ2 knockdown on the proliferation and metastasis of OC cells *in vitro*

According to the above findings, the relevant *FOXJ2*-specific siRNAs were customized and applied in the

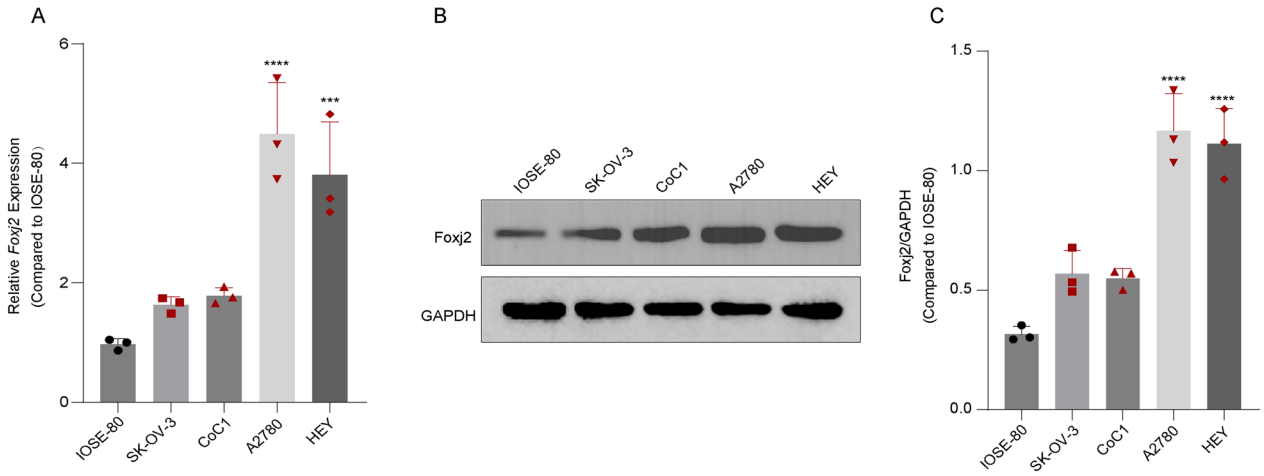

Fig. 1 FOXJ2 was highly expressed in ovarian cancer cells. **A**–**C** Relative mRNA and protein expression of FOXJ2 in ovarian cancer cells (SK-OV-3, CoC1, A2780 and HEY) and ovarian epithelial cells IOSE-80 were calculated using qPCR (**A**) and western blotting (**B**, **C**) with GAPDH as the normalization control. Experimental data (n = 3) were expressed as mean ± SD. ***denotes P-value < 0.001, ****denotes *P*-value < 0.0001

subsequent knockdown assays. The transfection efficiency was firstly tested, and the evidently low expression of *FOXJ2* in A2780 and HEY cells has suggested the successful transfection (Fig. 2A, B, P < 0.001).

The proliferation and metastasis of OC cells in vitro were evaluated by the CCK-8 and Transwell assays. Accordingly, the silencing of *FOXJ2* led to the diminished OD value in both A2780 and HEY cells at 12, 24, 36 and 48 h (Fig. 3A–B, P < 0.0001) and the reduced number of invaded cells based on the Transwell assay, which was applied to quantify the number of invaded

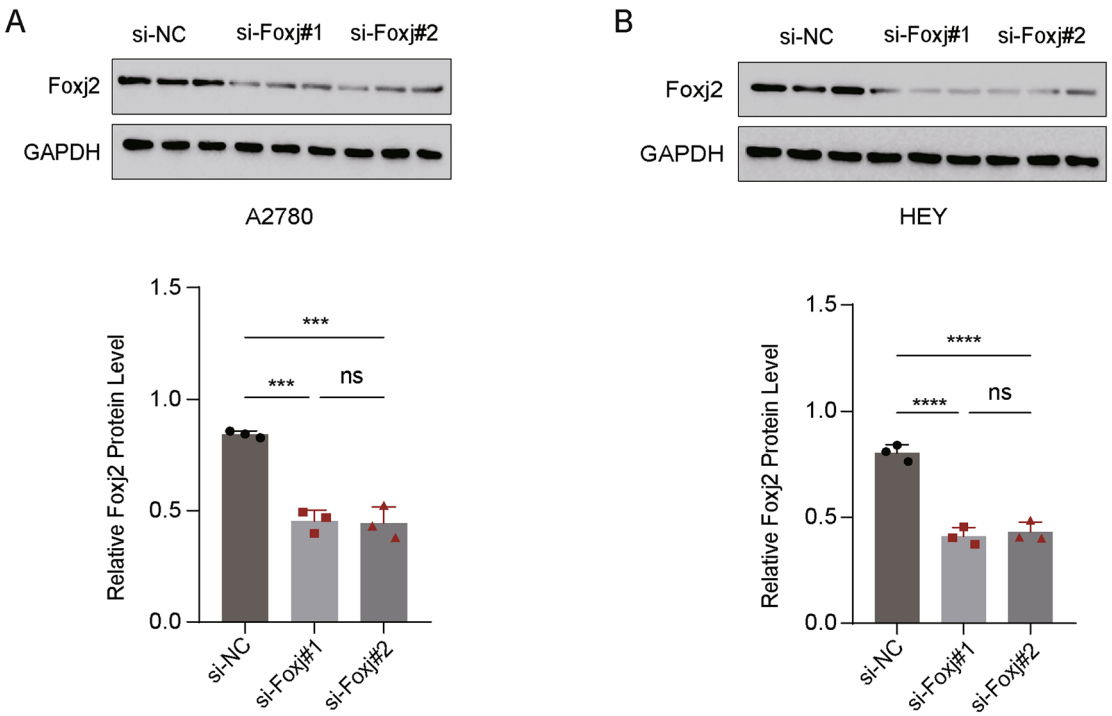

Fig. 2 Validation on the transfection efficiency. **A**, **B** The knockdown efficiency of FOXJ2-specific siRNAs on ovarian cancer cells A2780 (**A**) and HEY (**B**), as tested in western blotting assay. Experimental data (n = 3) were expressed as mean ± SD. ns denotes *P*-value > 0.05, ***denotes *P*-value < 0.001 and ****denotes *P*-value < 0.0001

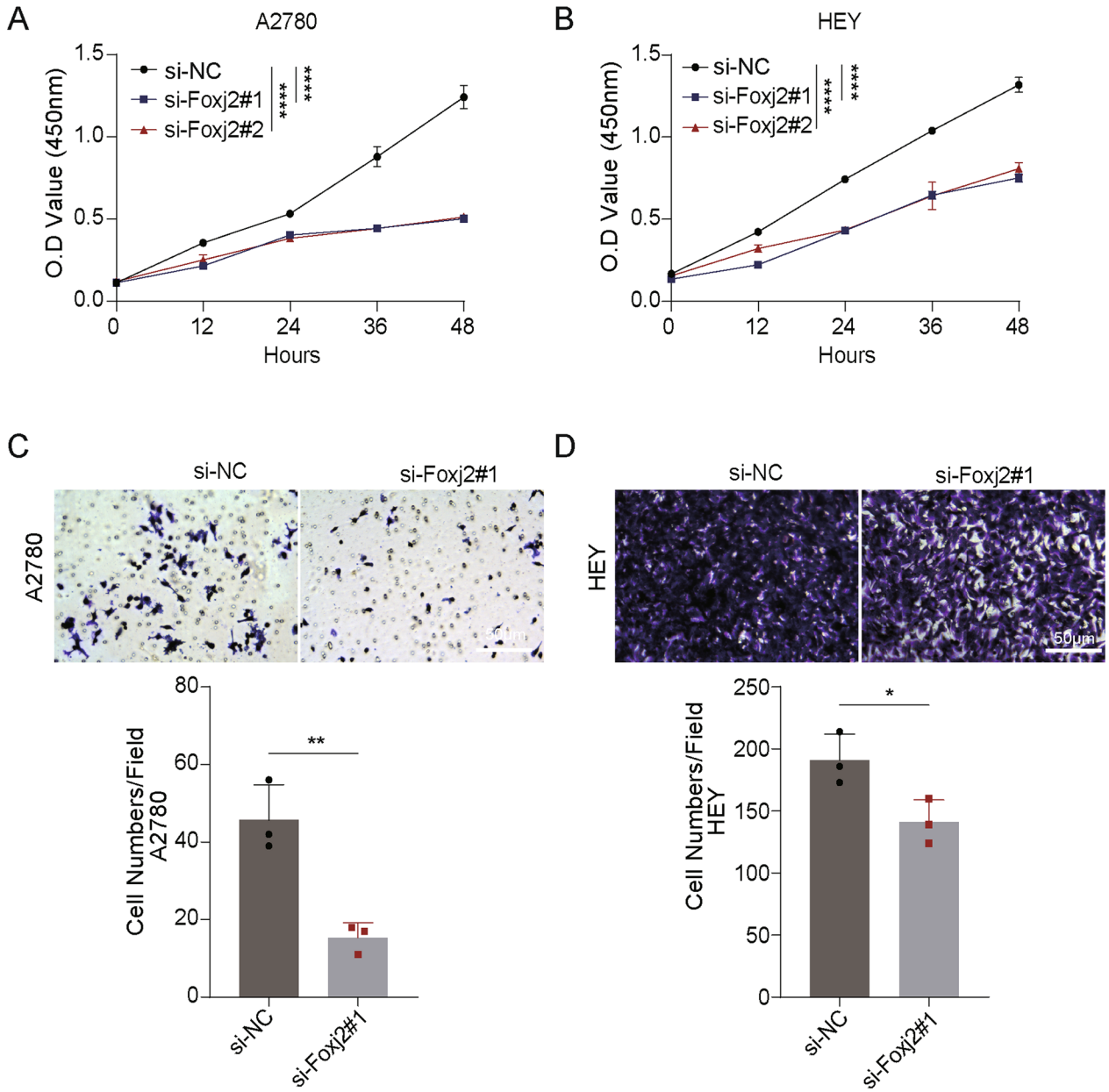

Fig. 3 Repressive effects of FOXJ2 knockdown on the proliferation and metastasis of ovarian cancer cells in vitro. **A**, **B** CCK-8 assay results on ovarian cancer cells A2780 (**A**) and HEY (**B**), which was applied to evaluate the proliferation. **C**, **D** Number of migrated ovarian cancer cells A2780 based on the observation from Transwell assay. Experimental data (n = 3) were expressed as mean ± SD. *denotes *P*-value < 0.05, **denotes *P*-value < 0.01 and ****denotes *P*-value < 0.0001

A2780 cells (Fig. 3C, P < 0.01) and invaded HEY cells (Fig. 3D, P < 0.05).

Effect of FOXJ2 silencing on apoptosis and migration capacity of OC cells

To further investigate whether *FOXJ2* expression could affect the apoptotic capacity of OC cells, to this end, we observed that *FOXJ2* silencing significantly increased the apoptotic rate of A2780 and HEY cells (Fig. 4A, B, P < 0.01). Subsequently, wound healing assays also demonstrated that the migration ability of cells in the si-*FOXJ2* group was significantly diminished relative to the control group (Fig. 4C, D, P < 0.05). These results further support the possibility of *FOXJ2* as a therapeutic target for OC, especially its potential value in

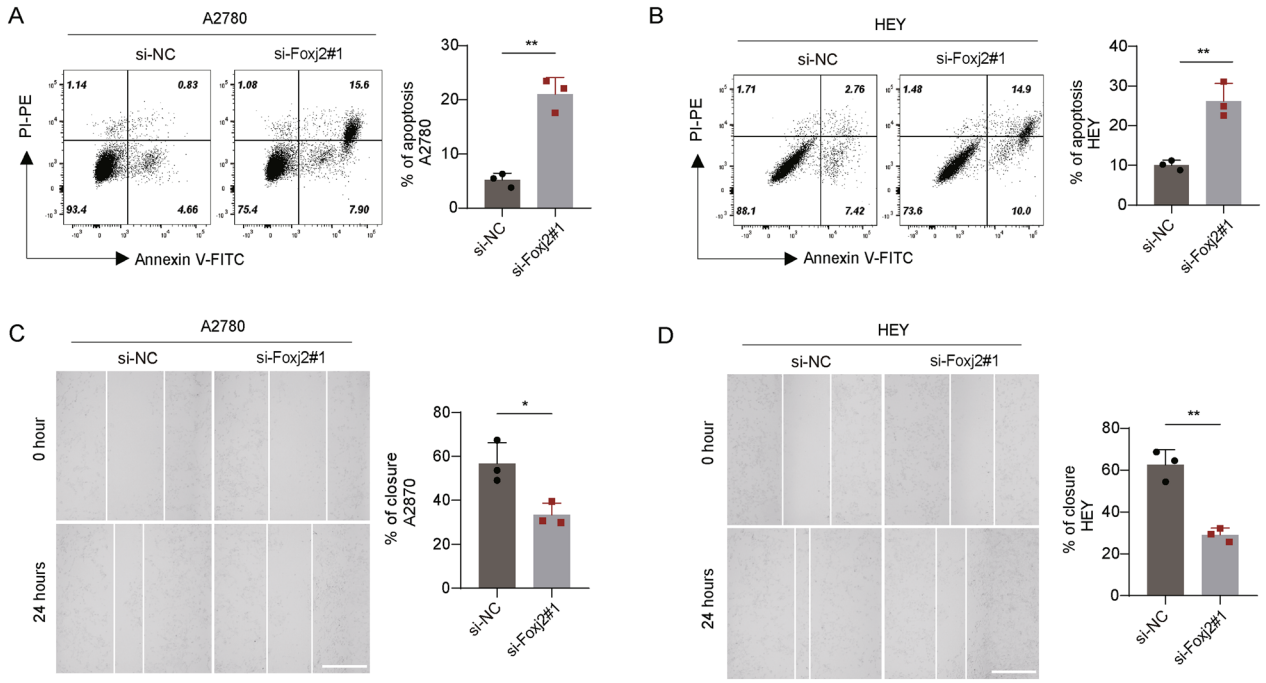

Fig. 4 Effect of FOXJ2 silencing on the apoptotic and migratory capacity of ovarian cancer cells in vitro. **A**, **B** The effects on the apoptotic ability of A2780 (**A**) and HEY (**B**) cells after silencing by FOXJ2 were detected based on flow cytometry, respectively. **C**, **D** The effects of silencing by FOXJ2 on the migratory ability of A2780 (**C**) and HEY (**D**) cells were examined separately based on wound healing assays. Experimental data (n = 3) were expressed as mean ± SD. *denotes P-value < 0.05 and **denotes P-value < 0.01

therapeutic strategies to inhibit tumor metastasis and induce apoptosis in tumor cells.

Effects of FOXJ2 silencing on the cell cycle of OC cells *in vitro*

Subsequently, flow cytometry assay was adopted to determine the cell cycle of OC cells following the silencing of *FOXJ2*. As shown in Fig. 5A, B, the proportion of G1 phase was significantly higher and the proportion of S phase was significantly lower in the si-*FOXJ2* group relative to the si-NC group ($P < 0.0001$). These results reflect that *FOXJ2* silencing resulted in cell cycle block in G1/S phase, which may inhibit cell proliferation and DNA synthesis.

Inactivation of PI3K/AKT pathway in FOXJ2-silencing OC cells

Finally, we aimed to investigate the downstream pathway underlying the repressive effects of *FOXJ2* on OC cells. The levels of PI3K/AKT pathway-related proteins in OC cells were quantified to confirm whether *FOXJ2* could act on PI3K/AKT pathway so as to exert its effects on OC. Following the silencing of *FOXJ2*, we observed a sharp reduction in the phosphorylated levels of both PI3K and AKT in A2780 (Fig. 6A–C, P < 0.01) and HEY cells (Fig. 6D–F, P < 0.01).

Discussion

OC is one of the most frequently diagnosed gynecological cancers worldwide [28]. The discovery on reliable biomarkers for OC plays a critical role in the management of disease and has a strong impact on both the prognosis and survival of patients with OC [29]. Recently, with the deepening of the research, existing studies have paid great attention on relevant gene research [30]. Transcription factors represent those proteins controlling gene expression via binding to specific DNA sequences, thereby manipulating gene transcription through up- or down-regulation of RNA polymerase or other regulatory proteins [31]. *FOXJ2* is a fork head transcriptional activator that expresses early in embryonic developmental stage and is widely distributed in adults [32]. Here, we provided novel evidence on the involvement of *FOXJ2* in OC. Our current study, based on the *in-vitro* cultured OC cells A2780 and HEY, has proven that *FOXJ2* is high-expressed in OC cells and the siRNA-mediated knockdown of *FOXJ2* could evidently repress the proliferation and metastasis and promote cell cycle G1/S arrest. Further, the silencing of *FOXJ2* could target PI3K/AKT signaling pathway in OC cells. These evidences, collectively, provided solid evidence on the involvement of *FOXJ2* in OC.

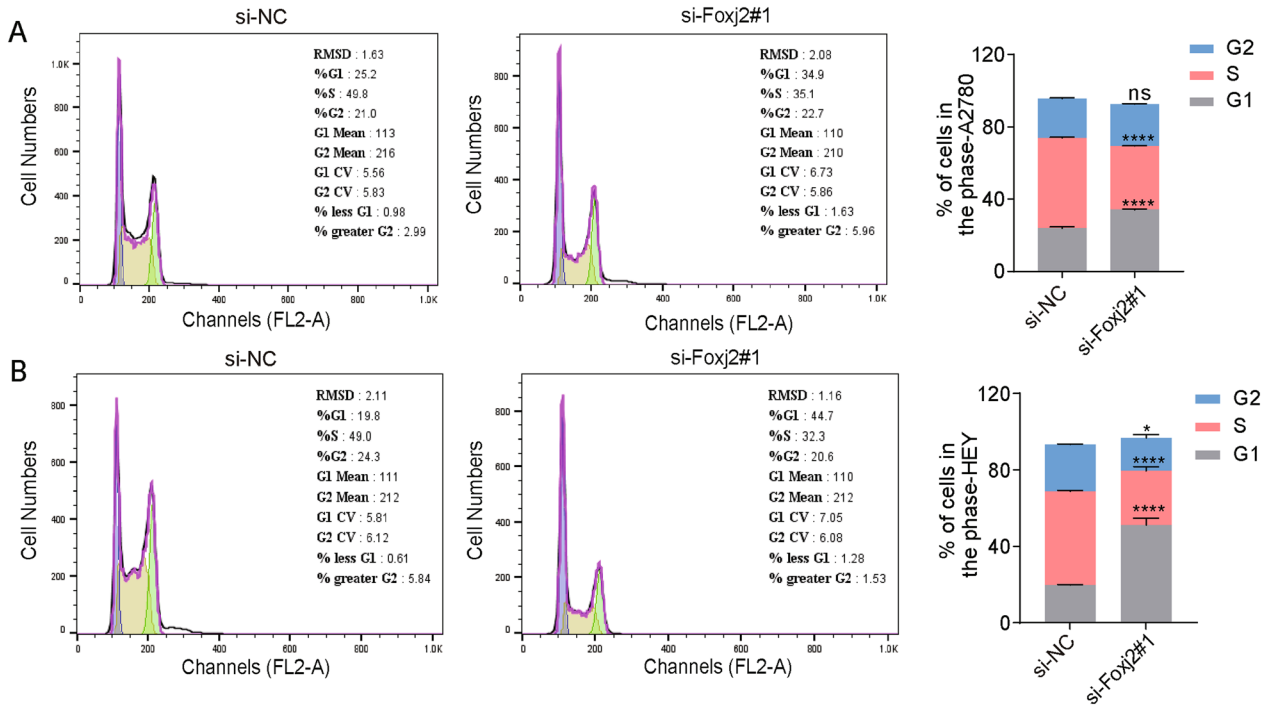

Fig. 5 Effects of FOXJ2 silencing on the cell cycle of ovarian cancer cells in vitro. **A** Number of cells at different phases of cell cycle in ovarian cancer cells A2780 in vitro, based on flow cytometry assay. **B** Number of cells at different phases of cell cycle in ovarian cancer cells HEY in vitro, based on flow cytometry assay. Experimental data (n = 3) were expressed as mean ± SD. *denotes *P*-value < 0.05, and ****denotes *P*-value < 0.0001

Proliferation, characterized by the altered expression and/or activity of cell cycle related proteins, is one manifestation of cancer development and progression, with the other one being metastasis [33, 34]. Existing studies have widely explored the involvement of *FOXJ2* in the proliferation and metastasis of tumors, as *FOXJ2* is implicated in cell cycle regulation and promoting tumorigenesis [35]. Like we mentioned earlier, *FOXJ2* can mediate the growth and metastasis of colorectal cancer cells and dramatically repress microRNA-20a-enhanced proliferation, migration, invasion in vitro and xenografts in vivo, while inducing cell cycle G1 arrest [19]. In another study of glioma, *FOXJ2* was seen to be positively correlated with E-cadherin and overexpressed *FOXJ2* suppressed the migration of glioma cells via increasing E-cadherin expression [36]. Besides, another study on nasopharyngeal carcinoma has suggested that overexpression of *FOXJ2* is related to the development and metastasis, as *FOXJ2* silencing via RNA interfering (RNAi) represses the cell proliferation and metastasis [20]. While trying to interpret the specific effects of *FOXJ2* on OC, a series of assays including CCK-8 and Transwell were applied to evaluate the proliferation and metastasis of OC cells A2780 and HEY in vitro. According to the relevant results, it was evident that *FOXJ2* silencing could evidently reduce the proliferation and metastasis of OC cells A2780 and HEY in vitro. Nonetheless, the specific molecular mechanisms accounting for such repressive effects of *FOXJ2* silencing on OC have not been fully explored, rendering a limitation of this study.

Then we sought to explore the relevant downstream signaling pathway underlying the effects of *FOXJ2*. A prior study has demonstrated that *FOXJ2* could repress the transforming growth factor-β1-induced EMT on non-small lung cancer via Notch signaling [37]. This evidence has therefore built up our confidence that there's at least 1 pathway which could be targeted by *FOXJ2*. Accordingly, we shifted our attention to the specific pathways which could be modulated by FOX transcription family members in cancers, and PI3K/AKT pathway has caught our attention in that PI3K/AKT pathway has been reported as the frequently altered pathway in OC [38]. Moreover, copy number changes in genes which encode both the PI3KCA (p110α) and PI3KCB (p110β) subunits of PI3K are related to a worse prognostic outcome in OC [39]. Further, the expression of PI3KCA and phosphorylated AKT in OC were analyzed, revealing their association with the poor survival [40]. The overall targeting of PI3KCA caused the decrease in the proliferation of OC, as seen in the increased level of P27 [41]. As G1 cell cycle progression is modulated by P27, such inhibition of PI3KCA seems

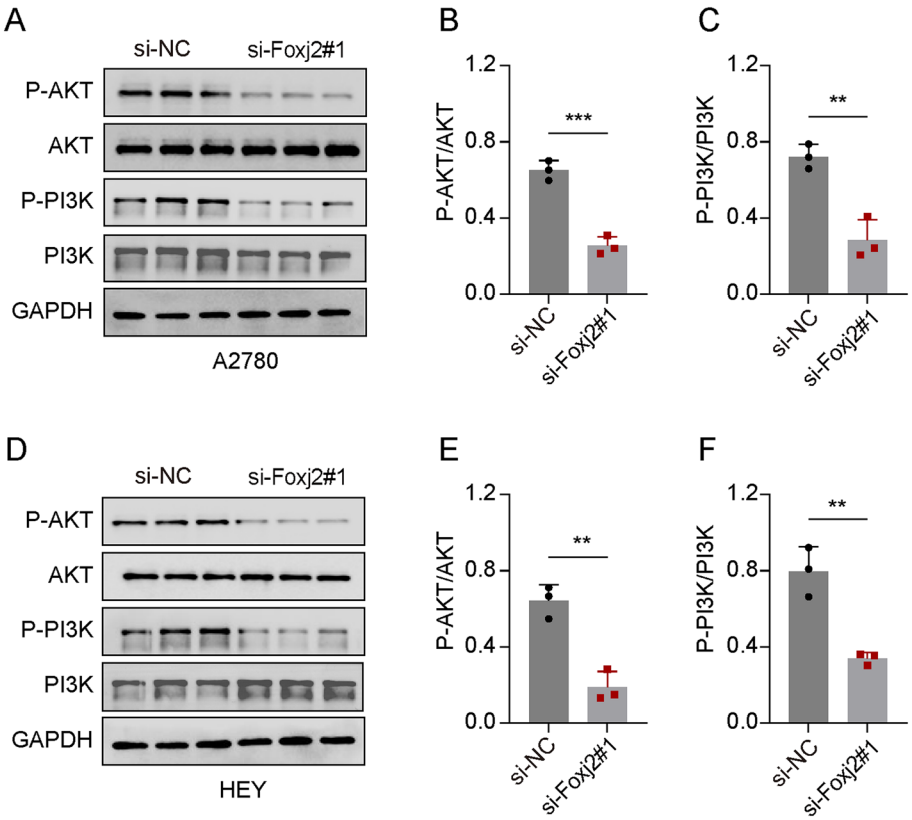

Fig. 6 Inactivation of PI3K/AKT pathway in FOXJ2-silencing ovarian cancer cells. **A**–**C** Phosphorylation levels of PI3K and AKT in ovarian cancer cells A2780, as quantified via western blotting assay. **D**–**F** Phosphorylation levels of PI3K and AKT in ovarian cancer cells HEY, as quantified via western blotting assay. Experimental data (n = 3) were expressed as mean ± SD. **denotes *P*-value < 0.01 and ***denotes *P*-value < 0.001

to be responsible for the decrease in cell proliferation [42]. In addition, the direct targeting of AKT can also affect the proliferation and invasion of OC. The siRNA against AKT1 isoform diminished the proliferation of OC cells OVCAR-3, although to a lesser degree than the inhibition on PI3KCA [42]. Also, targeting the AKT2 isoform is unveiled to enhance the activation of apoptosis [43]. These evidences collectively demonstrated the potentials on targeting PI3K/AKT pathway on the malignant behaviors of OC, which made us wonder whether *FOXJ2* could indeed target PI3K/AKT pathway in OC. In our current study, the relationship between *FOXJ2* and PI3K/AKT pathway was illustrated in OC firstly, as seen in the results where *FOXJ2* silencing led to the diminished phosphorylated levels of PI3K and AKT in OC cells. Some relevant rescue assays using PI3K/AKT agonists or antagonists should be incorporated in the future to additionally confirm the targeting relationship between *FOXJ2* and PI3K/AKT pathway. Also, we can not rule out the possibility that there may be other pathways that may be at play here, like MAPK, JAK/STAT and NF-κB [44–46]. Whether *FOXJ2* can act on these pathways to exert its effects on OC will become the goal of our future studies so as to expand the discovery on the involvement of *FOXJ2* on OC.

However, there are certain research limitations of our study. First, the experimental results of this study were based on in vitro cell models, and future studies could construct OC mouse xenograft models to further validate the effects of *FOXJ2* knockdown on tumor proliferation, migration, and PI3K/AKT signaling activity through in vivo experiments. In addition, although it was experimentally verified that *FOXJ2* knockdown significantly reduced the phosphorylation levels of PI3K and AKT, the regulatory mechanisms were not further clarified. For this reason, subsequent studies could explore the direct role of *FOXJ2* in relation to genes or proteins related to the PI3K/AKT pathway by protein immunoprecipitation (Co-IP) or chromatin immunoprecipitation (ChIP) combined with sequencing. Finally, the study focused only on the PI3K/AKT pathway and did not consider the possible effects of *FOXJ2* on OC through other signaling pathways. Therefore, we will further explore other potential regulatory pathways by high-throughput sequencing (screening for

differentially expressed genes or proteins after *FOXJ2* knockdown.

Conclusion

In conclusion, we, so far as we are concerned, provide novel evidence that the silencing of *FOXJ2* could lead to the suppression on the growth and metastasis and the cell cycle arrest at G1/S phase. These results highlighted *FOXJ2* as a potential therapeutic target in OC, but detailed investigation is still required to further analyze the complex molecular mechanism of *FOXJ2* in promoting OC development.

Data availability statement

The data is available upon reasonable request from the corresponding authors Zengrong Tu and Rong Huang.

Abbreviations

OC	Ovarian cancer
FOX	Forkhead box
FOXJ2	Forkhead box J2
METTL14	Methyltransferase like 14
m6A	N6-methyladenosine
SMC4	Structural maintenance of chromosome 4
siRNAs	Small interfering RNAs
CCK-8	Cell counting kit-8
h	Hours
OD	Optical density
PBS	Phosphate buffer saline
PI	Propidium iodide
cDNA	Complementary DNA
min	Minutes
s	Seconds
PI3K	Phosphoinositide 3-kinase
AKT	Protein kinase B
RNAi	RNA interference

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Author contributions

All authors contributed to this present work: [LYW], [HH] and [RH] designed the research; [RFZ], [RFG] and [MS] performed interpreted the results; [RYD], [ZRT] and [RH] performed the experiments and analyzed the results; [LYW], [RFZ], [MS], and [ZRT] drafted the manuscript; [HH], [RFG], [RYD] and [RH] revised the manuscript and gave the final approval of the version to be published. All authors read and approved the final manuscript.

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Data availability

The data is available upon reasonable request from the corresponding authors Zengrong Tu and Rong Huang.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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