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Role of INPP4B in the proliferation, migration, invasion, and survival of human endometrial cancer cells

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Summary. Background. Inositol polyphosphate 4-phosphatase type II (INPP4B) has been identified as a tumor repressor in several human cancers while its role in endometrial cancer has not been investigated yet. Therefore, the current study was designed to determine whether INPP4B participates in the progression of endometrial cancer by utilizing clinical data and experimental determination.

Materials and methods. We first include six chemotherapy-treated patients with recurrent and metastatic endometrioid carcinoma to determine the relationship between *INPP4B* mutation and relative tumor burden. By using siRNA-mediated gene silencing and vector-mediated gene overexpression, we further determined the effect of manipulating INPP4B expression on the proliferation, invasion, and survival of endometrial cancer cells. Furthermore, the repressing effect of INPP4B together with its role in chemotherapy was further validated by xenograft tumor-bearing mice models. Western blot analysis was used to explore further downstream signaling modulated by INPP4B expression manipulation.

Results. Two of the patients were found to have *INPP4B* mutations and the mutation frequency of *INPP4B* increased during the progression of chemotherapy resistance. Endometrial cancer cells with silenced INPP4B expression were found to have promoted tumor cell proliferation, invasion, and survival. Endometrial cancer cells overexpressing INPP4B were found to have decreased tumor cell proliferation, invasion, and survival. An *in vivo* study using six xenograft tumor-bearing mice in each group revealed that INPP4B overexpression could suppress tumor progression and enhance chemosensitivity. Furthermore, INPP4B overexpression was found to modulate the activation of Wnt3a signaling.

Conclusion. The current study suggested that INPP4B could be a suppressor in endometrial cancer progression and might be a target for endometrial cancer treatment. Also, INPP4B might serve as a predictor of chemosensitivity determination.

Key words: INPP4B, Endometrial cancer, Wnt3a, Chemosensitivity

Introduction

Endometrial cancer is one of the most common malignant tumors in the female reproductive system in developed countries and China. The global incidence of endometrial cancer has been on the rise in recent years, with an estimated 61,880 new cases and 12,160 deaths from endometrial cancer in 2019 (Siegel et al., 2019). According to the statistics of the National Cancer Center in 2015, the incidence rate of endometrial cancer in China is 63.4/100,000, and the mortality rate is 21.8 per 100,000 (Chen et al., 2016b). Particularly, the mortality rate of endometrial cancer in China has increased faster than the incidence rate. The increased mortality may be associated with an increase in advanced cases, high-risk pathologic types (e.g., plasma mammary carcinoma), and advanced age at diagnosis. Additionally, a study from the Gynecologic Oncology Group (GOG) has shown that the effective rate of a single drug including chemotherapy and endocrine therapy is 0~31%, and six-month progression-free survival (PFS) is 0~43% (Charo and Plaxe, 2019). Therefore, it is of great urgency to further explore the pathogenesis of endometrial cancer and to develop novel therapeutic strategies for endometrial cancer treatment.

Molecular typing has been widely adopted in cancer diagnosis and along with the development of targeted therapy for many cancers. In endometrial cancer, molecular characterization was proven to provide better risk stratification and treatment recommendations

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(Zighelboim et al., 2007; Mjos et al., 2017). Additionally, several targeted therapies for endometrial cancer treatment were under testing based on their molecular typing. Phosphoinositide signaling was proven to play critical roles in cell proliferation, survival, and other critical cellular physiological processes. Inositol polyphosphate 4-phosphatase type II (INPP4B), an important enzyme modulating phosphoinositide signaling and encoded by the *INPP4B* gene, was shown to be associated with the progression of breast, ovarian, prostate, lung, and melanocytic cancers (Hodgson et al., 2011; Perez-Lorenzo et al., 2014; Chen et al., 2017; Tang et al., 2019; Liu et al., 2020; Wang et al., 2022), while its role in endometrial cancer has not been studied.

Therefore, the current study was designed to determine the role of INPP4B in endometrial cancer progression. We have found that the frequency of *INPP4B* mutation increased during the progression of chemotherapy resistance in patients. *In vitro* and *in vivo* studies have revealed that overexpression of INPP4B could significantly suppress tumor progression and promote the therapeutic effect of chemotherapy. Our study suggested that INPP4B might be a promising target for endometrial cancer treatment.

Materials and methods

Patients and DNA extraction, sequencing, and processing

The experimental procedures performed in the current study were approved by the Ethics Committee of the Capital Medical University Cancer Center/Beijing Shijitan Hospital, Beijing, China, and followed the guidelines of the Declaration of Helsinki. A total of six paclitaxel-treated patients with recurrent endometrial carcinoma at our hospital were enrolled in the current study. Informed consent was obtained from all patients involved.

Peripheral blood (8 ml) was collected from each patient before and after paclitaxel treatment. The plasma was separated by centrifuging the blood sample at 1,600g at 4°C for 10 min, the harvested plasma was then retained and stored at -80°C before extraction of cell-free DNA (cfDNA). Next-generation sequencing (NGS) was conducted by Gene+Technology Co., Ltd.

Cell culture

Ishikawa cells (humanendometrial adenocarcinoma cell line, purchased from PUMCH) and RL95-2 cells (human endometrial adenocarcinoma cell line, purchased from Wuhan Punosai Life Technology Co., LTD) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen)supplemented with 10% fetal bovine serum (FBS, HyClone), 50 Unit/ml penicillin, and 50 μg/ml streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2.

Reverse transcription and quantitative polymerase chain reaction (RT-Q-PCR)

Total cellular RNA was extracted using Trizol according to the manufacturer's instructions. One μg of total RNA was reverse transcribed using the SuperScript III Reverse Transcriptase kit (ABI and Invitrogen) with random primers in a 20 μl reaction system. The following primers were used: β-Actin, 5'-3', TCCTCCTGAGCGCAAGTACTCC; 3'-5', CATAC TCCTGCTTGCTGATCCAC. INPP4B, 5'-3', TTCC GATTTAAAGAAAGTTGCAT; 3'-5', ACCACGTA ATGTTCTCGTC. The Roche 480 sequence detection system was used with the following conditions: 5 min at 95°C, 40 cycles at 95°C for 10 sec, and 1 min at 60°C. Relative INPP4B mRNA levels were calculated as follows: Δ*C*T (sample) =*C*T (INPP4B) - *C*T (actin); Relative expression $=2^{-\Delta\Delta CT}$.

INPP4B knockdown and overexpression.

The specific siRNA sequences for INPP4B were designed and listed as follows: hINPP4Bsi-1-sense, CAGAAUGUUUGAGUCACUAdTdT; hINPP4Bsi-1-antisense, UAGUGACUCAAACAUUCUGdTdT; hINPP4Bsi-2-sense, CCAGGAGGCAUUCUUAA GAdTdT; hINPP4Bsi-2-antisense, UCUUAAGA AUGCCUCCUGGdTdT; hINPP4Bsi-3-sense, CGAU GUCAGUGACACUUGAdTdT; hINPP4Bsi-3-antisense, UCAAGUGUCACUGACAUCGdTdT. d-NC, dNCFAM, hACTB, and pCDNA3.1-EGFP-T2A-puro-INPP4B were synthesized. The siRNA and over-expression sequences (pCDNA3.1-EGFP-T2A-puro-INPP4B) were transiently transfected into the Ishikawa and RL95-2 cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Western blot assay

The total proteins of cultured cells were exacted and an equal amount of total proteins (40 μg) were subjected to electrophoresis. Proteins were then transferred to nitrocellulose membranes for further blocking (1% BSA). The blocked membranes were then incubated with primary antibodies (anti-INPP4B antibody, Cell signaling; anti-PTEN antibody, anti-CTNNB1 antibody, anti-P53 antibody, and anti-Wnt antibody, all from Abcam) overnight at 4°C. The corresponding HRP-labeled secondary antibodies were then added and protein expression was visualized by chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The relative protein expression was calculated by normalizing it to the expression of β-actin; the protein expression was determined using more than three independent experiments.

Cell survival and proliferation assay

The effect of manipulating the expression of

INPP4B on the survival of Ishikawa and RL95-2 cells was determined using the CCK8 kit according to the manufacturer's instructions. Briefly, cells from different treatments were seeded on 96-well plates at a density of 5,000 cells/well with the CCK8 kit incorporated in the medium. The absorbance was determined at different time intervals (0h, 24h, 48h, 72h, 96h, and 120h) using a Multiskan plate reader at 450nm.

Cell migration and invasion assay

Cell migration and invasion abilities were assessed by scratch wound-healing and Transwell assays. Briefly, for the scratch wound-healing assay, different treated Ishikawa and RL95-2 cells were seeded in six-well plates (approximately 5×10^5 cells/well). The next day, after the cells had adhered to the bottom, linear wounds were created by scratching the center of the cell monolayer. At different time points, wound images were obtained using a Celigo instrument. For the Transwell assay, Matrigel was diluted to the final concentration of 1mg/ml by precooling serum-free medium. Differently treated Ishikawa and RL95-2 cells (approximately 2×10^5 cells/well) in serum-free medium were added to the upper chamber of 24-well plates, and DMEM containing 10% FBS was added to the lower chamber. The plate was incubated for 48h after which non-invading cells were removed with cotton swabs; the remaining cells were fixed in 4% paraformaldehyde for 30 min, stained with 0.5% crystal violet, and counted.

Apoptosis detection

The effect of different treatments on the apoptosis of Ishikawa and RL95-2cells was determined by the TUNEL assay according to the manufacturer's instructions. Briefly, differently treated Ishikawa and RL95-2 cells were seeded in 96-well plates (approximately 5×10^4 cells/well). The next day, cells were fixed with 4% formaldehyde for 20 to 30 minutes at room temperature and then subjected to the TUNEL staining protocol. At the end of TUNEL staining, DAPI was added for nuclear staining. Then the cells were washed with PBS and their fluorescence captured by a fluorescence microscope. The fluorescence intensity was further determined using ImageJ software.

Xenograft tumor growth assay

All procedures involving mice were approved by the Institutional Animal Care and Use Committee. Each group was randomly assigned six mice. A total of 1×10^7 cells were suspended in 200 μL phosphate-buffered saline and injected subcutaneously into the armpit of these seven- to eight-week-old female BALB/c nu/nu mice (SPF Biotechnology Co., Ltd. Beijing). When the tumor grew to 1 cm^3 in size, different treatments were given (paclitaxel was given at a dose of 150 nM/kg). The size of the tumors was measured twice a week for four weeks. Tumor volumes were calculated as $V\ (mm^3) = 0.5\times ab^2$. At the end of the experiment, mice were sacrificed and tumors were excised and weighed (Pentobarbital (0.3%) intraperitoneal injection anesthesia (0.1 mL/10 g); Cervical dislocation execution).

Statistical analysis

Enumeration data in the current study were presented as percentages and numerical data were presented as mean ±SD. Student's t-test was used to determine the difference between two independent groups, and one-way ANOVA was used to calculate the difference among groups using LSD for intergroup comparison. A P value less than 0.05 was considered statistically different.

Results

INPP4B gene mutation occurred in refractory endometrioid carcinoma patients

Six chemotherapy-treated (paclitaxel combined with

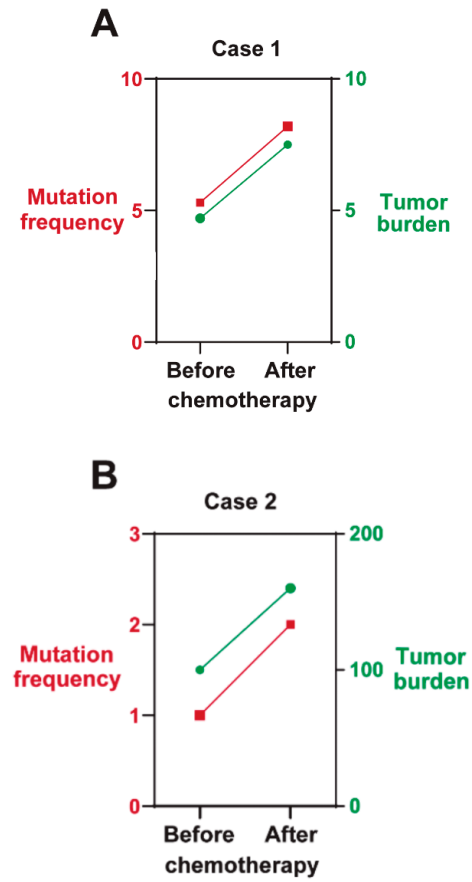

Fig. 1. *INPP4B* gene mutation and the progression of chemotherapy resistance. Tumor burden was determined in two *INPP4B*-mutated patients (Case 1, **A**; Case 2, **B**) before and after chemotherapy.

carboplatin) patients with recurrent and metastatic endometrioid carcinoma were included in the current study. Circulating Tumor DNA (CtDNA) was detected before and after two cycles of chemotherapy. Two chemotherapy-resistant patients were found to have gene mutations (mutations including *FGFR2*, *TP53*, *TSC1*, *ARID1A*, *PIK3CA*, *DNMT3A*, *INPP4B*, *PPM1D*, *OR4C6*, *B2M*, and *INPP4B*). As presented in Figure 1, the frequency of INPP4B mutation increased during the progression of chemotherapy resistance.

INPP4B silencing promotes the proliferation, migration, invasion, and survival of endometrial cancer cells

To determine the effect of decreased expression of INPP4B on endometrial cancer cells, we first generated three siRNAs for silencing the expression of INPP4B. As presented in Figure 2A, the expression of INPP4B was mostly decreased by si-INPP4B-2, we therefore took this siRNA for further study. By using the CCK8 kit for cellular proliferation ability determination, we have found that silencing INPP4B could significantly promote the proliferation of endometrial cancer cells (Figs. 2B, 3A). Additionally, silencing INPP4B could significantly promote the migration and invasion of endometrial cancer cells as determined by the wound healing and Transwell assays, respectively (Figs. 2C,D, 3B,C). TUNEL staining together with flow cytometry analysis revealed that silencing INPP4B could significantly enhance the survival of endometrial cancer cells (Fig. 2E,F). These results suggested that silencing INPP4B might promote the progression and metastasis of endometrial cancer.

Overexpression of INPP4B inhibits the proliferation, migration, invasion, and survival of endometrial cancer cells

As silencing INPP4B could promote the proliferation, migration, invasion, and survival of endometrial cancer cells, we then decided to determine whether overexpression of INPP4B could suppress the cellular behavior of endometrial cancer cells. We first generated an INPP4B overexpression vector and transinfected it into endometrial cancer cells. RT-QPCR and western blot analysis revealed that the overexpression vector could significantly promote the expression of INPP4B mRNA levels (Fig. 4A). CCK8 assay revealed that overexpression of INPP4B could significantly inhibit the proliferation of endometrial cancer cells (Figs. 4B, 5A). Also, the wound healing and Transwell assays revealed that overexpression of INPP4B significantly suppressed themigration and invasion of endometrial cancer cells (Figs. 4C,D, 5B,C). TUNEL staining together with flow cytometry analysis revealed that overexpression of INPP4B could significantly suppress the survival of endometrial cancer cells (Figs. 4E,F, 5D,E). Therefore, these results suggested that overexpression of INPP4B might inhibit the progression and metastasis of endometrial cancer.

INPP4B overexpression inhibits tumor growth and could cooperate with paclitaxel in tumor treatment

To determine whether INPP4B overexpression could inhibit tumor growth *in vivo*, we applied the overexpression vector to endometrial cancer-bearing mice using the xenograft tumor growth animal model. As presented in Figure 6, the size and weight of the tumor were significantly lower in INPP4B over-expression-treated mice than in control mice. More interestingly, INPP4B overexpression combined with paclitaxel can significantly inhibit tumor growth than single-treated mice, suggesting that INPP4B could cooperate with paclitaxel in tumor treatment.

The INPP4B overexpression inhibition of tumor growth might partly be through modulating the Wnt3a signaling pathway.

As Wnt signaling critically participates in the progression of endometrial cancer, we further determined whether the therapeutic effect of INPP4B overexpression was achieved by modulating Wnt signaling. Via western blot, we found that compare with control group, overexpression of INPP4B could significantly promotes the protein expression of P53, PTEN, and Wnt3a ($P<0.01$), while the expression of CTNNB1 was significant decreased when INPP4B was overexpressed ($P<0.01$) (Fig. 7), suggesting that the endometrial cancer-suppressing property of INPP4B overexpression is closely related to its ability to modulate the Wnt3a signaling pathway.

Discussion

Endometrial cancer is known for being very aggressive at its advanced stages;the efficacy rate of current chemotherapy regimens is relatively low. One of the main reasons for this low success rate is the acquired chemoresistance of these cancers during their progression due to multiple mechanisms, like abnormalities in DNA repair, survival-related pathways (PI3K/AKT, MAPK), and other pathways (Brasseur et al., 2017).

In 2013, The Cancer Genome Map (TCGA) research network reported a large-scale and comprehensive genome analysis from 373 cases of endometrial cancer. Common mutations in the microsatellite unstable subgroup include mutations in *PTEN*, *ARID5B*, *PIK3CA*, and *PIK3R1*. These molecular analyses provided novel insights into the molecular determinants of endometrial cancer subtyping (Yen et al., 2020). In the transgenic endometrial cancer mouse model, biallelic *PTEN* deletion led to the occurrence of complex atypical hyperplasia, and *PIK3CA* mutation led to the development of atypical hyperplasia to endometrial cancer (Yen et al., 2020). *PTEN* deletion, *CTNNB1*

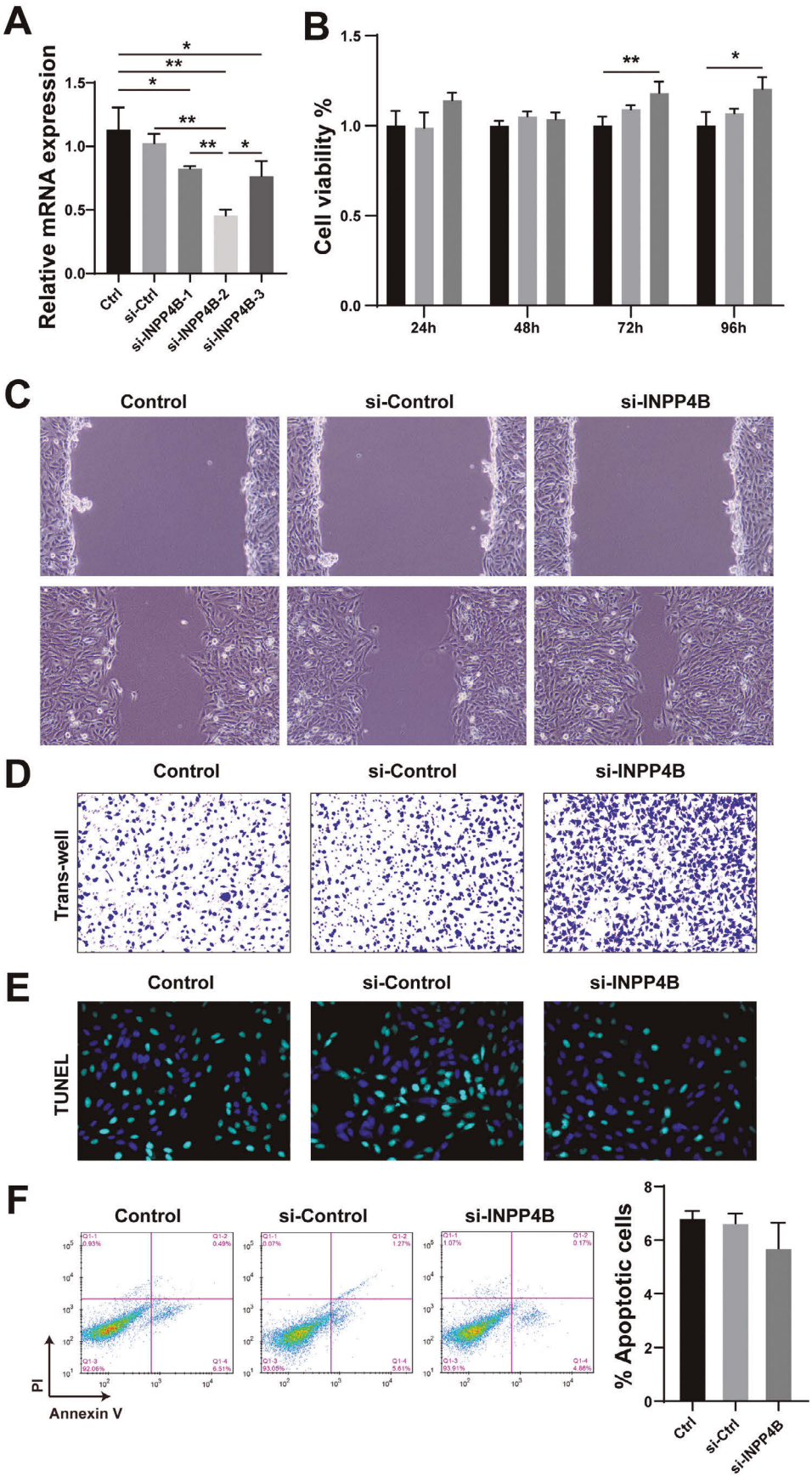

Fig. 2. INPP4B silencing promotes the proliferation, migration, invasion, and survival of endometrial cancer cells in Ishikawa cells. **A.** mRNA expression of *INPP4B* in cells treated with different INPP4B siRNAs. **B.** Viability of endometrial cancer cells under different treatments at indicated time points as determined using the CCK8 assay. **C.** Representative images of cell migration capacity determination using the wound healing assay. **D.** Representative images of cell invasion ability determination using the Transwell assay. **E.** Apoptotic cell determination using TUNEL staining. Representative images are shown and the green-labeled cells were regarded as apoptotic cells. **F.** Representative flow cytometry diagram of double-staining with Annexin V-FITC/PI. The percentage of cells in each quadrant is indicated. Data were presented as mean ±SD. **P*<0.05, ***P*<0.01.

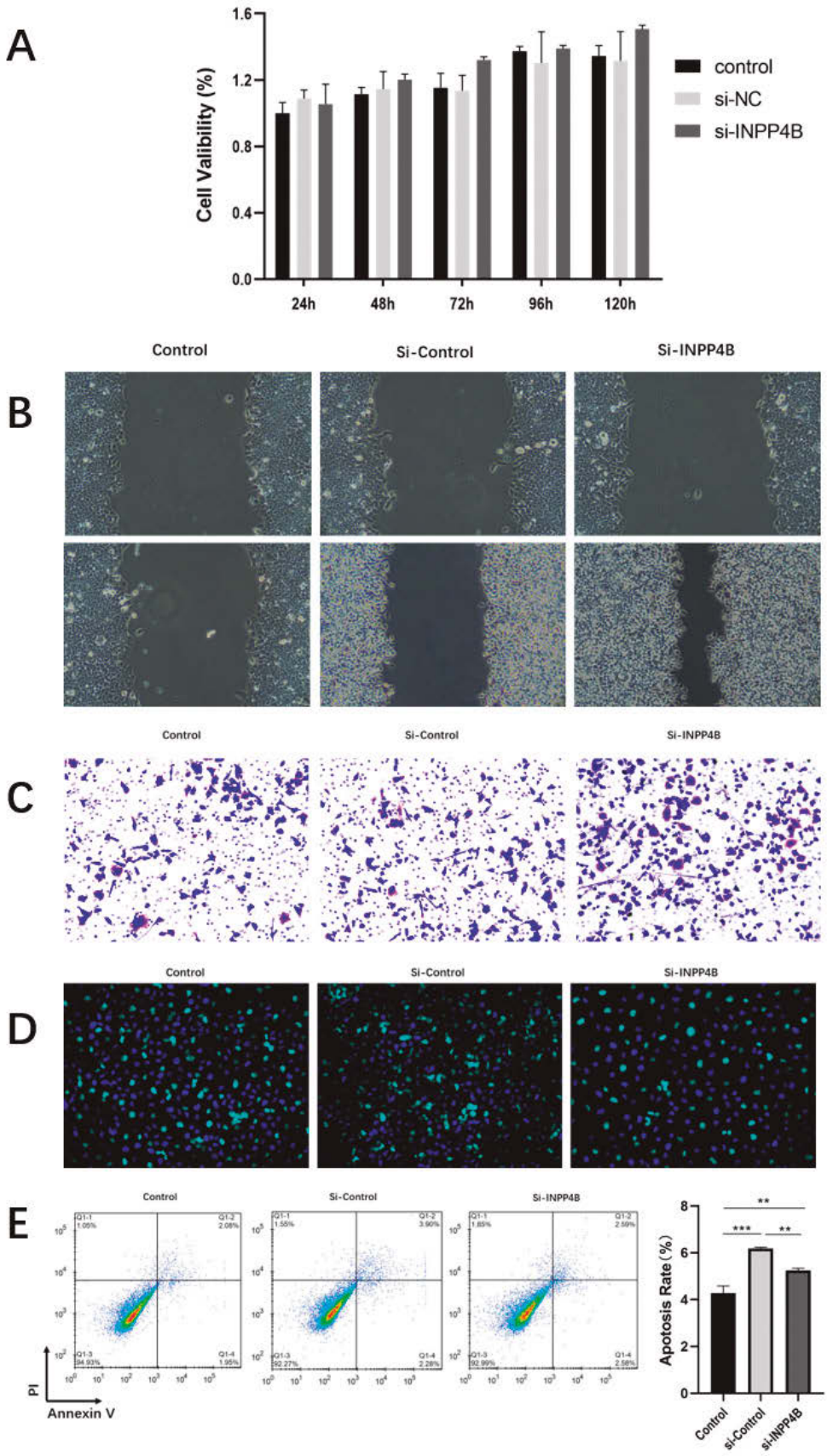

Fig. 3. INPP4B silencing promotes the proliferation, migration, invasion, and survival of endometrial cancer cells in RL95-2 cells. **A.** Viability of endometrial cancer cells under different treatments at indicated time points as determined using the CCK8 assay. **B.** Representative images of cell migration capacity determination using the wound healing assay. **C.** Representative images of cell invasion ability determination using the Transwell assay. **D.** Apoptotic cell determination using TUNEL staining. Representative images are shown and the green-labeled cells were regarded as apoptotic cells. **E.** Representative flow cytometry diagram of double-staining with Annexin V-FITC/PI. The percentage of cells in each quadrant is indicated. Data were presented as mean ±SD. ** P<0.01, *** P<0.001.

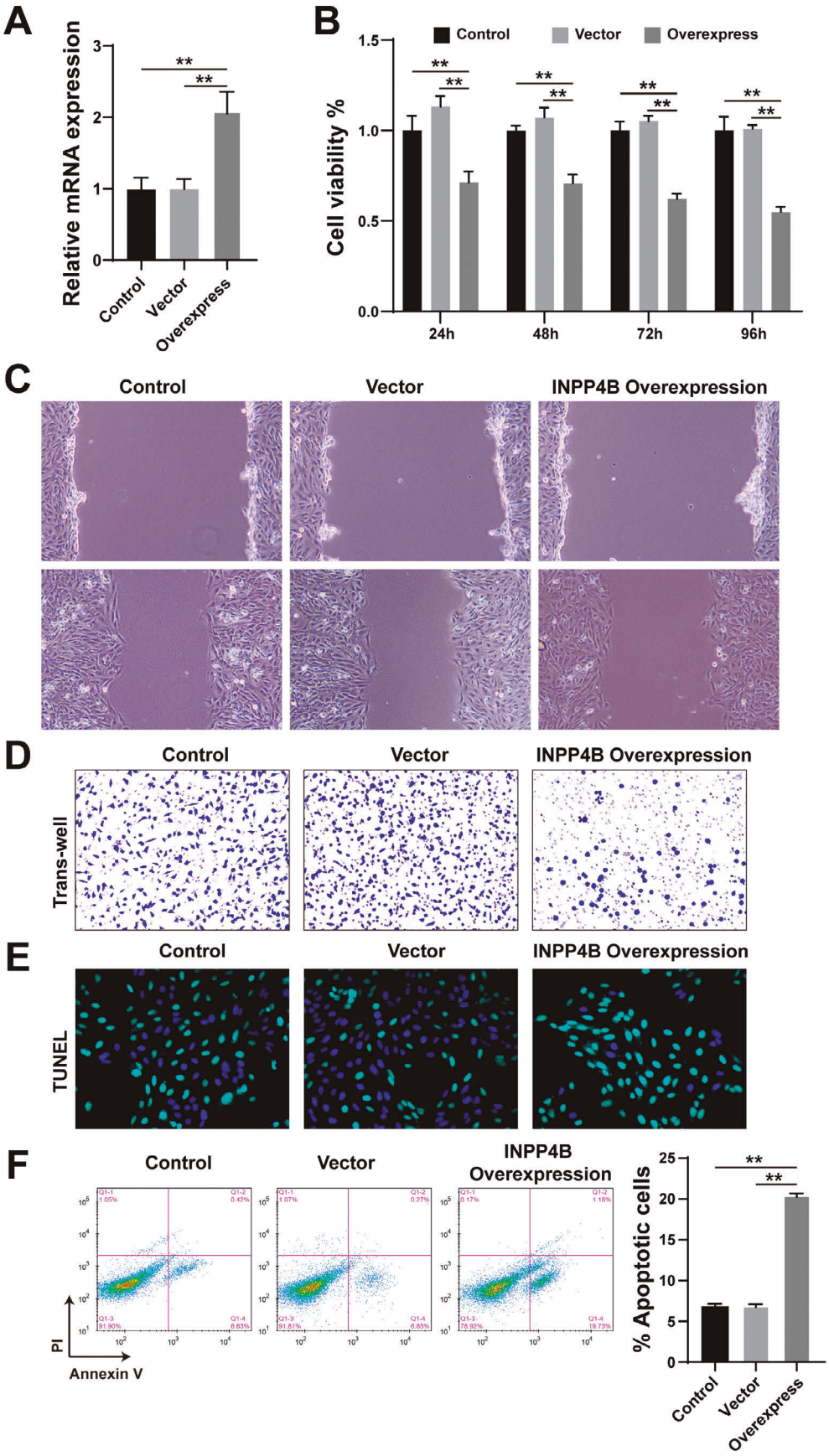

Fig. 4. Overexpression of INPP4B inhibits the proliferation, migration, invasion, and survival of endometrial cancer cells in Ishikawa cells. **A.** mRNA expression of INPP4B in differently treated endometrial cancer cells. **B.** Viability of endometrial cancer cells under different treatments at indicated time points as determined using the CCK8 assay. **C.** Representative images of cell migration capacity determination using the wound healing assay. **D.** Representative images of cell invasion ability determination using the Transwell assay. **E.** Apoptotic cell determination using TUNEL staining. Representative images are shown and the green-labeled cells were regarded as apoptotic cells. **F.** Representative flow cytometry diagram of double-staining with Annexin V-FITC/PI. The percentage of cells in each quadrant is indicated. Data were presented as mean ±SD. **P*<0.05, ***P*<0.01.

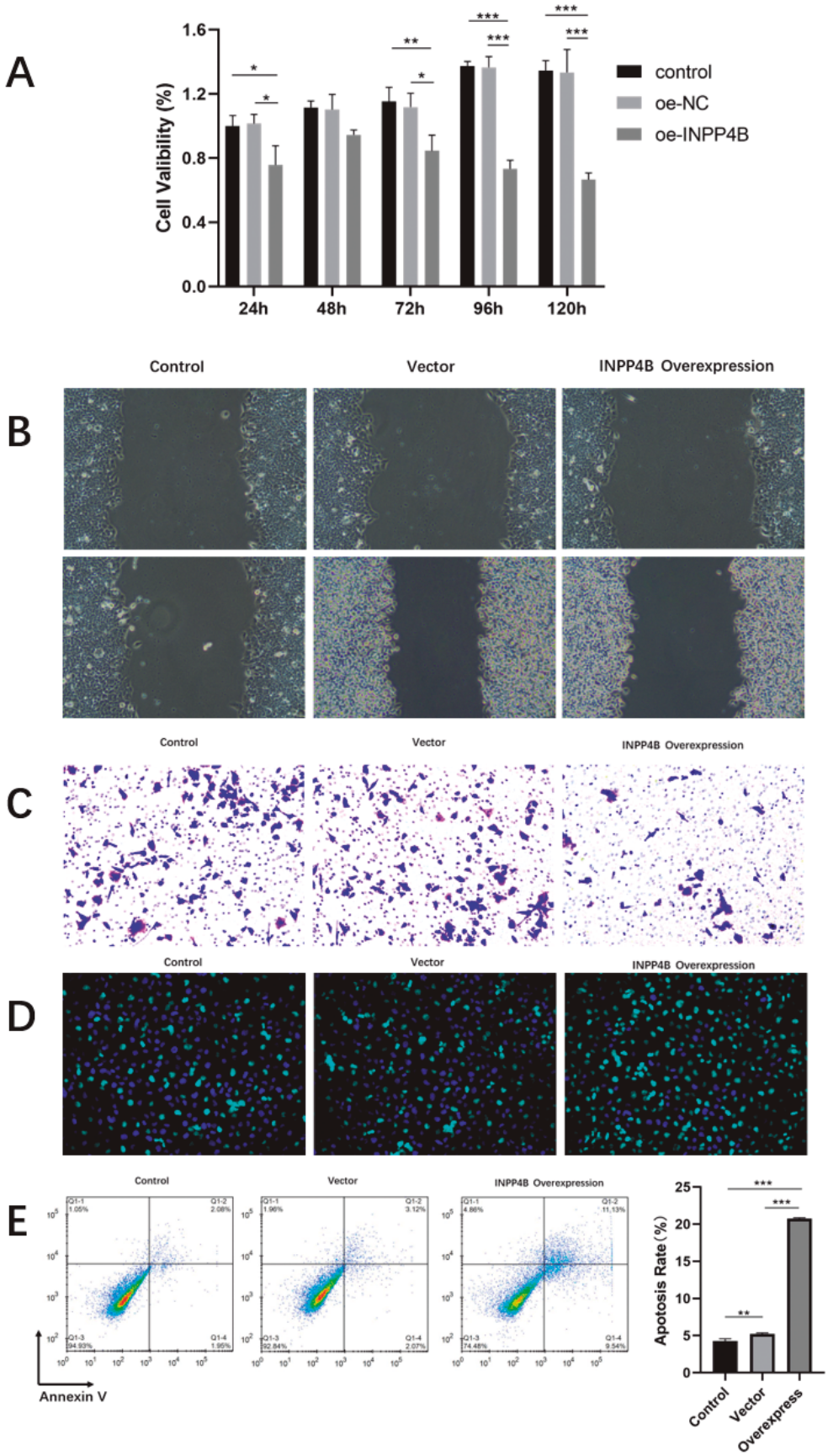

Fig. 5. Overexpression of INPP4B inhibits the proliferation, migration, invasion, and survival of endometrial cancer cells in RL95-2 cells. **A.** Viability of endometrial cancer cells under different treatments at indicated time points as determined using the CCK8 assay. **B.** Representative images of cell migration capacity determination using the wound healing assay. **C.** Representative images of cell invasion ability determination using the Transwell assay. **D.** Apoptotic cell determination using TUNEL staining. Representative images are shown and the green-labeled cells were regarded as apoptotic cells. **E.** Representative flow cytometry diagram of double-staining with Annexin V-FITC/PI. The percentage of cells in each quadrant is indicated. Data were presented as mean ±SD. ***P*<0.01, ****P*<0.001.

mutation, or *MIH-1* inactivation jointly promote the occurrence of endometrial cancer (Cheung et al., 2011; Urick et al., 2011; Byron et al., 2012; Joshi et al., 2015). As a newly discovered tumor suppressor, INPP4B has a similar effect to PTEN. It blocks the signal transduction pathway of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) by inhibiting the signal of phosphatidylinositol kinase (PIK), thereby weakening the growth and proliferation capacity of tumor cells, inducing tumor apoptosis, and playing the role of a tumor suppressor gene (Gewinner et al., 2009). It has been confirmed that INPP4B can inhibit tumor growth in thyroid cancer, melanoma, prostate cancer, liver cancer, breast cancer, and multiple myeloma (Hodgson et al., 2011; Perez-Lorenzo et al., 2014; Chen et al., 2017; Tang et al., 2019; Liu et al., 2020; Wang et al., 2022). The decreased INPP4B levels can enhance cell proliferation and migration through AKT inactivation, indicating that INPP4B has an antitumoral effect in these cancer cells. However, in contrast to some research, INPP4B can promote tumor growth as a carcinogen in colon cancer (Yang et al., 2020), acute myeloid leukemia (Jin et al., 2018), and gallbladder cancer (Fedele et al., 2010; Rijal et al., 2015; Guo et al., 2016;Wu et al., 2021). The increased expression of INPP4B is related to the poor clinical prognosis of pancreatic cancer (Dzneladze et al., 2018). Compared with normal tissues, the expression of INPP4B is up-regulated in pancreatic cancer tissues. *INPP4B* gene knockout inhibits the proliferation of pancreatic cancer cells and promotes apoptosis (Dzneladze et al., 2018; Chi et al., 2015).

Patients with strong INPP4B expression in human primary oral squamous cell carcinoma tissues had statistically significantly poorer overall survival than patients with weak expression of INPP4B (Yang et al., 2020b). Interestingly, the expression of INPP4B in gastric cancer is lower than that in normal tissues; INPP4B may play dual roles as an oncogene and tumor suppressor gene in different tissues and clinical stages. Therefore, the role of INPP4B in tumorigenesis and development is still controversial. Recent studies have shown that INPP4B is related to docetaxel drug

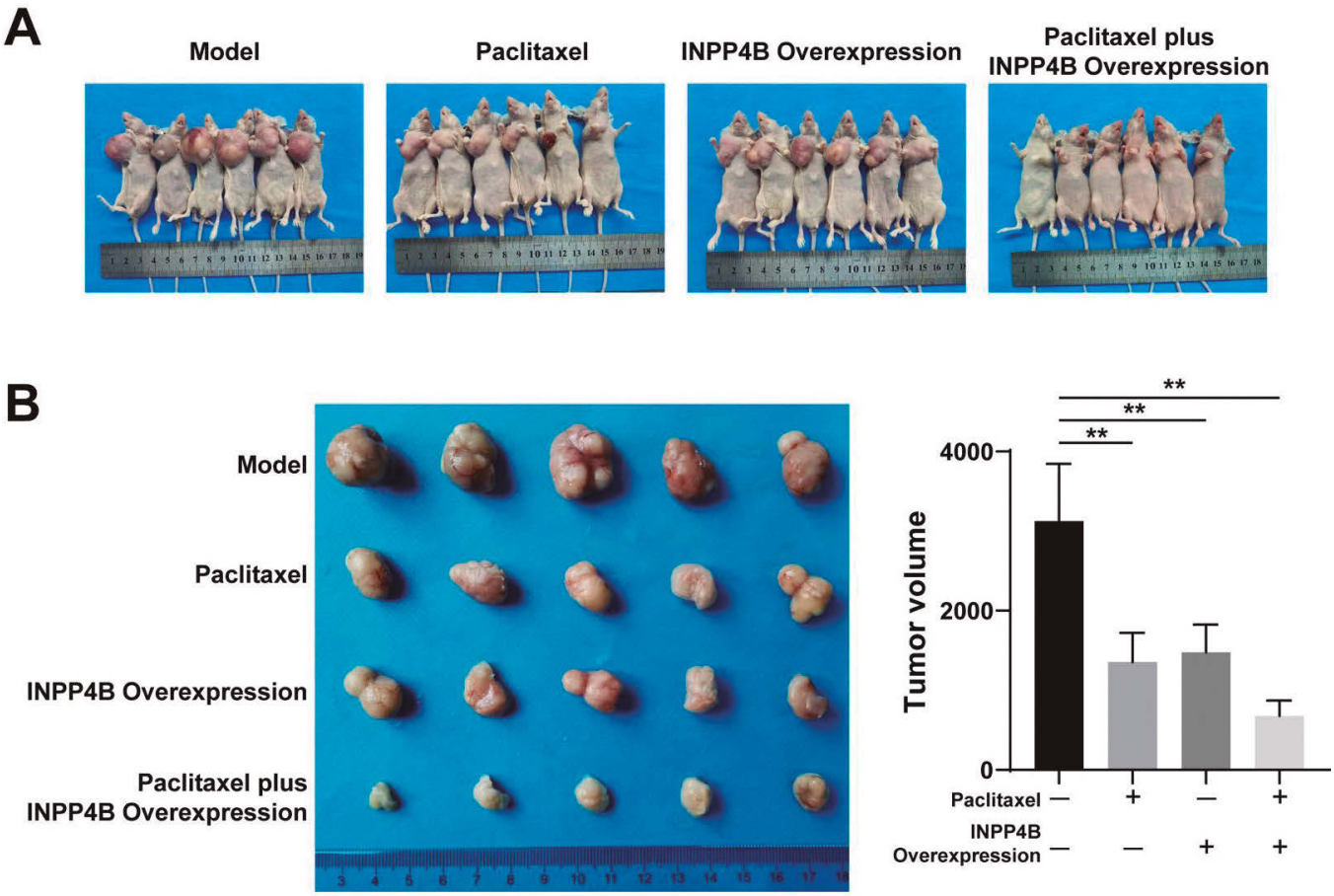

Fig. 6. INPP4B overexpression inhibits tumor growth and could cooperate with paclitaxel in tumor treatment. **A.** Representative images of tumor-bearing mice treated before sacrificed. **B.** The tumors from differently treated mice were harvested and the volume was calculated. Data were presented as mean ±SD. ** $P<0.01$.

resistance in acute myeloid leukemia, laryngeal cancer cells, and prostate cancer (Min et al., 2013; Rijal et al., 2015; Chen et al., 2016a), suggesting that INPP4B has a potential role as a new tumor drug resistance-related gene. However, the expression of this gene in endometrial carcinoma is still unclear.

In this study, we first found *INPP4B* gene mutations in peripheral blood ctDNA of patients with refractory and recurrent endometrial cancer, and the frequency of *INPP4B* mutations in patients with chemotherapy resistance increased, suggesting that *INPP4B* gene mutations may be related to the recurrence and drug resistance of endometrial cancer. The *in vitro* and *in vivo* studies further showed that the overexpression of INPP4B could inhibit tumor growth, indicating that *INPP4B* may act as a tumor suppressor gene in intrauterine carcinoma.

Wnt/β-catenin signaling has been found to play an essential role in many oncogenic processes in gynecologic malignancies, including tumorigenesis, metastasis, recurrence, and chemotherapy resistance (McMellen et al., 2020). It was reported that INPP4B commonly suppresses PI3K/AKT signaling by converting $PI(3,4)P_2$ to PI(3)P and INPP4B inactivation is common in triple-negative breast cancer. INPP4B promotes PI3Kα-dependent late endosome formation and Wnt/β-catenin signaling in breast cancer. Wnt inhibition or depletion of the PI(3)P-effector, Hrs, reduced INPP4B-mediated cell proliferation and tumor growth (Rodgers et al., 2021). In this study, after the upregulation of INPP4B expression, the expression of catenin and PTEN are upregulated, indicating that INPP4B played an antitumoral role in endometrial carcinoma by modulating the Wnt/β-Catenin signaling pathway. At the same time, this study found that the increased level of INPP4B could increase the chemosensitivity of paclitaxel in endometrial cancer cells and tissues, which may also be associated with the Wnt/β-Catenin signaling pathway; this specific mechanism needs further experimental verification.

On the other hand, the paper also has some limitations. First of all, due to the limitation of clinical data, the sample size of our study was small. Second, this paper lacks the bioinformatics analysis of relevant gene and protein expression. If these data can be supplemented, the content and depth of this paper will be further enriched and improved. In the next study, we will expand the sample size and improve the above defects to make the study more rigorous and perfect.

Conclusion

This study shows that INPP4B may play a role as a tumor suppressor gene in endometrial cancer, and

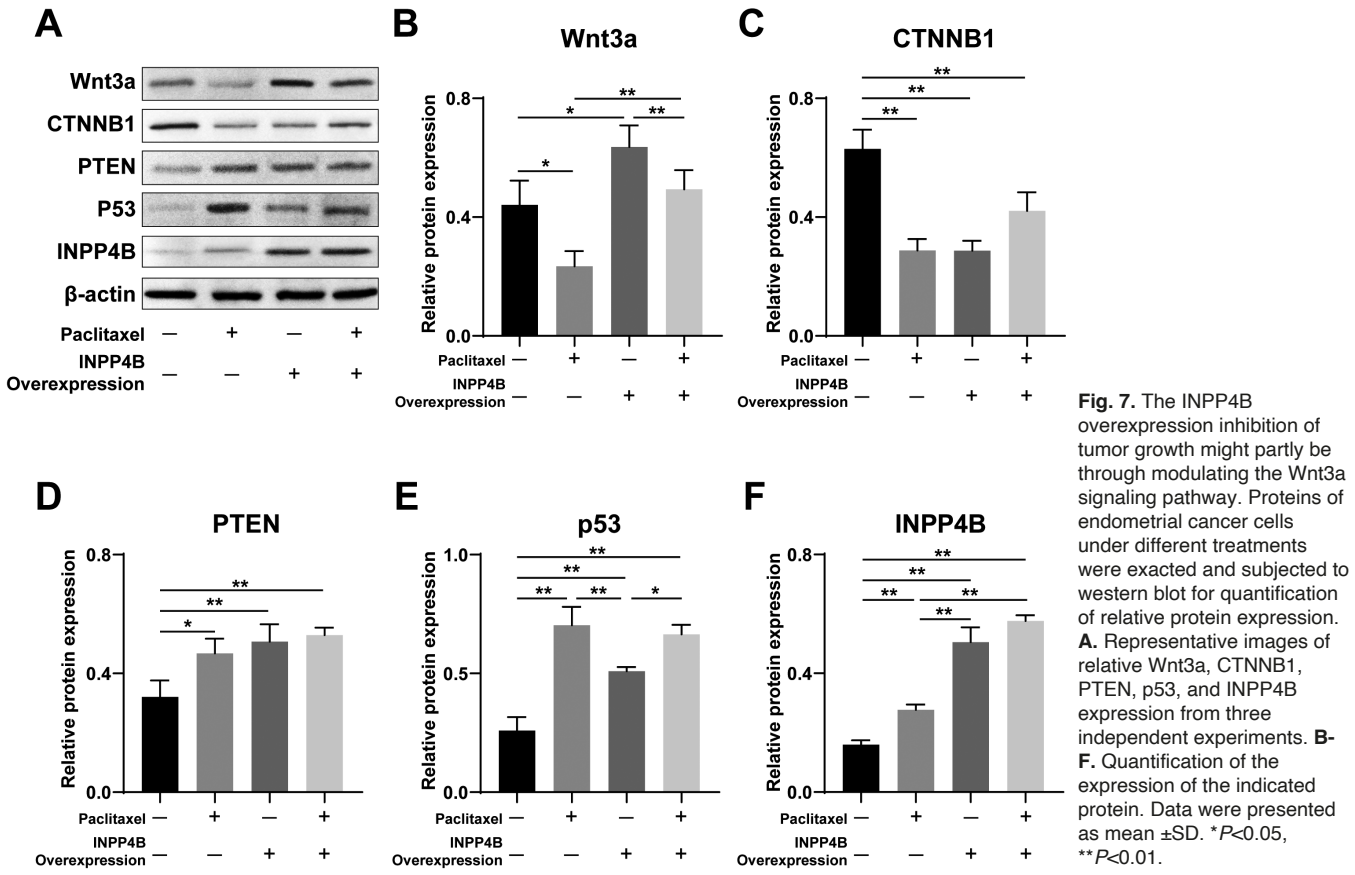

Fig. 7. The INPP4B overexpression inhibition of tumor growth might partly be through modulating the Wnt3a signaling pathway. Proteins of endometrial cancer cells under different treatments were exacted and subjected to western blot for quantification of relative protein expression. **A.** Representative images of relative Wnt3a, CTNNB1, PTEN, p53, and INPP4B expression from three independent experiments. **B-F.** Quantification of the expression of the indicated protein. Data were presented as mean ±SD. **P*<0.05, ***P*<0.01.

increasing the expression level of INPP4B may improve the sensitivity of paclitaxel chemotherapy. Results from the current study suggest that INPP4B might be a promising target for endometrial cancer treatment and could serve as a potential predictor of paclitaxel chemosensitivity.

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Ethics approval and consent to participate. The procedures involving animals and their care were conducted in accordance with ARRIVE guidelines and were approved by the Animal Care and Use Committee of Beijing Shijitan Hospital. Our study covers the 3Rs (refinement, replacement, and reduction) and also outlines the procedures dealing with humane endpoints and pain management.

Consent for publication. Not applicable.

Availability of data and material. The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests. The authors declare that they have no competing interests.

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Authors' contributions. JZ and XM D contributed to the conception and design of the study; WS, ZQ Z, and XH Z performed the experiments, and collected and analyzed data; JZ and XM D wrote the manuscript; JZ and XM D revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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