

Original Article



Anticancer effect of the antipsychotic agent penfluridol on epithelial ovarian cancer

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ABSTRACT

Objective: Chemoresistant-epithelial ovarian cancer (EOC) has a poor prognosis, prompting the search for new therapeutic drugs. The diphenylbutylpiperidine (DPBP) class of antipsychotic drugs used in schizophrenia has shown anticancer effects. This study aimed to investigate the preclinical efficacy of penfluridol, fluspirilene, and pimozide (DPBP) using in vitro and in vivo models of EOC.

Methods: Human EOC cell lines A2780, HeyA8, SKOV3ip1, A2780-CP20, HeyA8-MDR, and SKOV3-TR were treated with penfluridol, fluspirilene, and pimozide, and cell proliferation, apoptosis, and migration were assessed. The preclinical efficacy of DPBP was also investigated using in vivo mouse models, including cell lines and patient-derived xenografts (PDX) of EOC.

Results: DPBP drugs significantly decreased cell proliferation in chemosensitive (A2780, HeyA8, and SKOV3ip1) and chemoresistant (A2780-CP20, HeyA8-MDR, and SKOV3-TR) cell lines. Among these drugs, penfluridol exerted a relatively stronger cytotoxic effect on all cell lines. Penfluridol significantly increased apoptosis and inhibited migration of EOC cells. In the cell line xenograft mouse model with HeyA8, the penfluridol group showed significantly decreased tumor weight compared with the control group. In the paclitaxel-resistant model with HeyA8-MDR, the penfluridol group had significantly decreased tumor weight compared with the paclitaxel or control groups. Penfluridol exerted anticancer effects on the PDX model.

Conclusion: Penfluridol exerted significant anticancer effects on EOC cells and xenograft models, including PDX. Thus, penfluridol therapy, as a drug repurposing strategy, might be a potential therapeutic for EOCs.

Keywords: Penfluridol; Drug Repurposing; Epithelial Ovarian Cancer; Diphenylbutyl; Patient-Derived Xenograft; Cell Line Xenograft

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Data Availability

The data generated in this study are available upon request from the corresponding author.

Author Contributions

Conceptualization: L.J.W.; Data curation: C.Y.J.; Formal analysis: K.W.J., C.C.S.; Investigation: R.J.Y, C.Y.J, H.J.R.; Methodology: H.J.R., C.J.Y.; Resources: C.J.J.; Supervision: L.J.W.; Visualization: R.J.Y.; Writing - original draft: K.W.J., C.C.S.; Writing - review & editing: R.J.Y., L.J.W.

Synopsis

Penfluridol decreased the cell viability of both chemosensitive and chemoresistant epithelial ovarian cancer (EOC) cells. Penfluridol exerted an anti-cancer effect on orthotopic xenograft and high grade serous ovarian patient-derived xenografts mouse models. Antiproliferative effect of penfluridol is related to the modification of the MAPK pathway via the activation of protein phosphatase 2A. Repurposing of penfluridol has the potential as a therapeutic drug for EOC.

INTRODUCTION

Epithelial ovarian cancer (EOC) is the second most prevalent and leading cause of death among gynecologic cancers [1]. Over 80% of EOC cases are diagnosed at an advanced stage, leading to a poor disease prognosis with a 5-year survival rate of <45% [2]. Maximal cytoreductive surgery, followed by adjuvant combination chemotherapy with taxane- and platinum-based agents, is the standard treatment for EOC. With therapeutic advances, bevacizumab and poly-ADP-ribose polymerase inhibitors are widely used in therapeutic and maintenance settings [3]. Despite progress in therapeutic approaches and a good response to primary treatment, patients with advanced EOC frequently experience relapse and ultimately die of it. Drug resistance is a major obstacle in overcoming this poor prognosis [4,5]; thus, new therapeutic drugs for EOC are needed.

The challenges in discovering novel anticancer drugs lead to the concept of drug repurposing, that is, the development of new uses of a drug outside its original use or initial approved indication, which emerged as a promising strategy. Repurposing can help identify new therapies for disease at a lower cost and in a shorter time, particularly when preclinical safety profiles have already been identified [6]. Previous studies have investigated the incidence of cancer in patients with psychiatric disorders, such as schizophrenia, who are undergoing treatment with antipsychotic drugs. There are contradictory reports suggesting that users of psychiatric drugs exhibit a lower cancer risk compared to the general population [6,7]. Conclusive results on this issue are difficult to obtain due to potential confounding factors. Although the relationship between antipsychotic drugs and cancer risk remains unclear, several reports have demonstrated that antipsychotic drugs induce apoptosis and inhibit metastasis in various types of cancer. Additionally, research has been conducted on the functional mechanisms underlying their cytotoxic activities, suggesting the potential repurposing of antipsychotic drugs as cancer therapeutics.

The diphenylbutylpiperidine (DPBP) class of antipsychotics includes penfluridol, fluspirilene, and pimozide. Compared with other neuroleptics, these drugs are used for treating psychosis and have demonstrated superior efficacy in reversing negative schizophrenic symptoms, such as emotional withdrawal and disrupted affect [6]. Preclinical studies have explored the potential anticancer effects of DPBP drugs on various cancers. For example, fluspirilene exhibits antiproliferative effects on hepatic cancer, colon cancer, and glioma [7-9], and pimozide exerts antiproliferative effects on breast, lung, prostate, and pancreatic cancers [10-16]. Studies have investigated the anticancer effects of penfluridol on breast, lung, and colon cancer [17-19]. Moreover, several studies have suggested that penfluridol and existing anticancer drugs may have synergistic effects on pancreatic cancer [20] and glioblastoma [21], and it could overcome paclitaxel resistance in metastatic breast cancer [22].

While the mechanism through which penfluridol exhibits its anticancer effects is not well known, studies have indicated that penfluridol induced apoptosis by activating protein phosphatase 2A (PP2A) to deactivate downstream AKT and MAPKs (ERK and JNK). PP2A-C phosphorylation attenuates PP2A activity [23,24].

However, the anticancer effects of DPBP on ovarian gynecologic cancer have not been examined. Based on previous literature, we hypothesized that DPBP drugs may be effective in patients with EOC. Thus, this study aimed to investigate the anticancer effects of DPBP on EOC cell lines in in vitro and in vivo mouse models.

MATERIALS AND METHODS

1. Cell lines and treatments

Human EOC cell lines, HeyA8, HeyA8-MDR, SKOV3ip, SKOV3-TR, and A2780-CP20 were gifts from Dr. Anil K. Sood, Department of Cancer Biology, University of Texas MD Anderson Cancer Center (Houston, TX, USA). The A2780 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The histological features of A2780 and A2780-CP20 cells were originated from ovarian endometrioid adenocarcinoma. SKOV3ip1 and SKOV3-TR cells were from ovarian serous cystadenocarcinoma and HeyA8 and HeyA8-MDR from high-grade ovarian serous adenocarcinoma. All human EOC cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and 0.1% gentamicin sulfate (Gemini Bioproducts, Calabasas, CA, USA) in 5% CO₂ at 37°C. DPBP drugs, namely, penfluridol, fluspirilene, and pimozone, were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2. Cell proliferation assay

Cells were plated in a 96-well plate at a density of 3×10^3 cells/well in the culture medium. After 24 hours, human EOC cell lines were treated with penfluridol, fluspirilene, or pimozone, and assays were performed after 48 and 72 hours. For proliferation assays, cells were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Amresco, Solon, OH, USA). After 4 hours of incubation, the culture medium was discarded, 100 μ L of acidic isopropanol (0.1 N HCL in absolute isopropanol) was added, and the plate was gently shaken. Absorbance was measured using an enzyme-linked immunosorbent assay (ELISA) reader with a test wavelength of 540 nm.

3. Apoptosis assay

The relative percentage of apoptotic cells was assessed after 48 hours using the Annexin V-FITC Apoptosis Detection Kit-1 (BD Pharmingen, San Diego, CA, USA) following the manufacturer's protocol. Briefly, cells were washed twice with phosphate-buffered saline, and the pellet was suspended in annexin V binding buffer at 10^6 cells/mL. Annexin V-FITC and propidium iodide were added (5 μ L to each per 10^5 cells). The mixture was gently mixed, and using a fluorescence-activated cell sorter (FACS), the samples were incubated for 15 min at room temperature in the dark before analysis.

4. Wound-healing and migration assays

Wound-healing and transwell assays were performed to assess the effect of penfluridol on EOC cell migration. In the wound-healing assay, HeyA8 and HeyA8-MDR cell lines were seeded into dishes and cultured overnight. Subsequently, to create a wound incubated with or without

penicillin, the cell monolayer was scratched with a sterile pipette tip. After washing with a culture medium, the scratched areas were observed using a microscope after 5 hours. After penfluridol treatment, cell migration was also evaluated using the CytoSelect 24-Well Cell Migration Assay Kit, which contains polycarbonate membrane inserts (Cell Biolabs, San Diego, CA, USA). The number of cells that passed through the polycarbonate membrane was measured by colorimetry (560-nm absorbance) using a plate reader. To visualize the migrated cells, representative photomicrographs were taken, which were stained with cell stain solution from the kit.

5. ELISA

To analyze the secretion of matrix metalloproteinase 9 (MMP-9), the culture media were harvested and centrifuged to remove cellular debris. The media were concentrated by centrifugal filtration at 4,000 rpm for 20 minutes using an Amicon Ultra 10K centrifugal filter tube (Millipore, Billerica, MA, USA). To measure the concentrations of human MMP-9 (R & D Systems, Minneapolis, MN, USA), ELISA kits were used as described by the manufacturer. Samples were measured in triplicate.

6. Western blot analysis

Cells were lysed in PRO-PRE Protein Extraction Solution (Intron Biotechnology, Seongnam, Korea). Protein concentrations were analyzed using a Bradford assay kit (Bio-Rad, Hercules, CA, USA). By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, cells (40 µg of total protein) were separated on 7.5% or 10% acrylamide gels and transferred to Immobilon-P transfer membranes (Merck Millipore, Burlington, MA, USA). Membranes were blocked for 1 hour with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 at room temperature. The protein bands were probed with total-PP2A and phospho-PP2A (p-PP2A), total-AKT and phospho-AKT, total-JNK and phospho-JNK, total-ERK, and phospho-ERK (Cell Signaling Technology, Danvers, MA, USA) antibodies at 1:1,000 dilutions and β-actin (Santa Cruz Biotechnology, Dallas, TX, USA) antibodies at a 1:2,000 dilution and then tagged with horseradish peroxidase-conjugated antirabbit antibody (GE Healthcare, Piscataway, NJ, USA). Bands were visualized by enhanced chemiluminescence using an ECL kit according to the manufacturer's protocol (Amersham Biosciences, Buckinghamshire, UK).

7. Animal care and development of in vivo models, including established orthotopic cell lines and patient-derived xenografts (PDX)

Female BALB/c nude mice were purchased from Orient Bio (Sunngam, Korea). This study was reviewed and approved by the Institutional Animal Care and Use Committee of the Samsung Biomedical Research Institute (protocol No. H-A9-003), which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International and abides by the guidelines of the Institute of Laboratory Animal Resources. In this study, 6–8-week-old mice were used in these experiments. To establish orthotopic models, HeyA8 (2.5×10^5 cells/0.2 mL Hanks' Balanced Salt Solution [HBSS]) or HeyA8-MDR (1×10^6 cells/0.2 mL HBSS) cells were implanted into the peritoneal cavity of female nude mice.

To generate PDX models of EOC, tumor specimen was cut into small pieces (<2–3 mm) and transplanted into the subrenal capsule of the left mouse kidney [25,26]. A PDX of the chemosensitive high-grade serous carcinoma sample (OV-179) was selected from a 53-year-old woman with Federation of Gynecology and Obstetrics stage IIIC [27]. The patient underwent primary cytoreductive surgery and received 6 cycles of paclitaxel–carboplatin combination chemotherapy. No residual tumor was found after primary surgery, and the progression-free survival was 45 months. Penfluridol was orally administered (10 mg/kg,

daily). Paclitaxel was intraperitoneally injected (6 mg/kg once weekly). The mice (n=10 per group) were monitored daily for tumor development and were sacrificed either on days 30–35 after cancer cell injection or on days 60–90 (PDX model) or when any of the mice appeared moribund. The total body weight and tumor weight at the time of sacrifice were recorded. Tumors were fixed in formalin and embedded in paraffin or snap-frozen in OCT compound (Sakura Finetek Japan, Tokyo, Japan) in liquid nitrogen.

8. Immunohistochemistry

Formalin-fixed, paraffin-embedded, 4 µm-thick tissue sections were subjected to immunohistochemical studies. Immunostaining for Ki-67 (NB600-1252; NOVUS Biologicals, Centennial, CO, USA) was performed using a BOND-MAX™ automated immunostaining device (Leica Biosystems, Melbourne, Australia) and the Bond™ Polymer purification detection kit (Vision Biosystems, Melbourne, Australia). Briefly, antigen retrieval was performed in an ER1 buffer at 97°C for 20 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase for 10 minutes, and the antibody was diluted at room temperature at 1:200 for 15 minutes. Apoptotic-positive cells were analyzed by the TUNEL assay using the ApopTag Peroxidase In Situ Apoptosis Detection Kit (Merck Millipore) as described above. p-PP2A protein expression was detected using rabbit polyclonal anti-p-PP2A (Tyr307) antibody (Invitrogen, PA5-36874). The primary antibody was diluted 1:100 and incubated for 45 minutes. Antigen–antibody complexes were detected with Dako EnVision+ System HRP-labeled polymer following the manufacturer's instructions (K4003, Dako). Two pathologists blindly reviewed slides and evaluated immunohistochemical staining.

9. Statistical analysis

Significance and differences among the groups for in vitro and in vivo assays were evaluated using the Mann–Whitney U test. All statistical tests were 2-sided, and p-values <0.05 were considered statistically significant. GraphPad Prism (San Diego, CA, USA) was used for all statistical analyses.

RESULTS

1. DPBP drugs significantly inhibit EOC cell proliferation

The in vitro anticancer effects of DPBP drugs, including penfluridol, fluspirilene, and pimozone, on EOC cell lines were examined. The MTT assay was performed to investigate whether the DPBP drugs inhibited EOC cell proliferation. Penfluridol time- and dose-dependently decreased the cell viability of chemosensitive (A2780, HeyA8, and SKOV3ip-1) and chemoresistant (A2780-CP20, HeyA8-MDR, and SKOV3-TR) EOC cells (**Fig. 1A**). Fluspirilene and pimozone also showed similar antiproliferative effects on A2780, HeyA8, A2780-CP20, and HeyA8-MDR but were not effective in SKOV3ip1 and SKOV3-TR (**Fig. 1B and C**). The IC₅₀ of each drug on EOC cells is described in **Table S1**.

2. Penfluridol significantly increased EOC cell apoptosis

Among the 3 drugs, penfluridol was more potent and showed consistent effects on all EOC cells, including drug-resistant lines, in the MTT assay. Therefore, further tests with penfluridol were performed. To assess apoptosis, FACS analysis of EOC cells was performed using penfluridol. Apoptotic cells were counted by annexin V incorporation after 48 hours of treatment with penfluridol (5 µM). The proportion of apoptotic cells significantly increased in penfluridol-treated A2780, A2780-CP20, HeyA8, HeyA8-MDR, SKOV3ip1, and SKOV3-TR cells (**Fig. 2**).

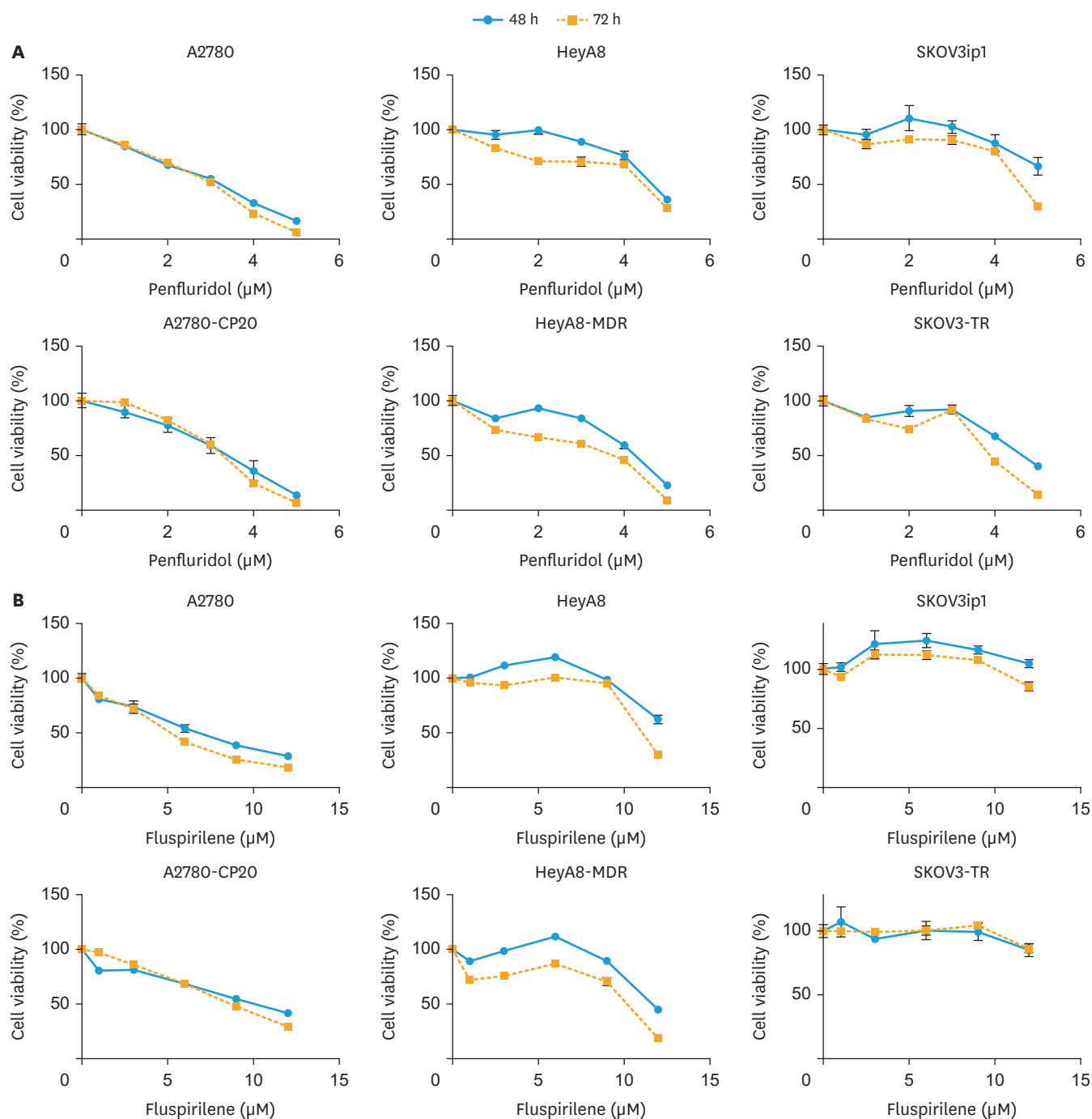


Fig. 1. MTT assay of cell proliferation after treatment of EOC cells with DPBP drugs. Cell proliferation was reduced in EOC cell lines treated with (A) penfluridol, (B) fluspirilene, and (C) pimoide.

DPBP, diphenylbutylpiperidine; EOC, epithelial ovarian cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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3. Penfluridol inhibits EOC cell migration via MMP-9 downregulation

The effect of penfluridol on HeyA8 and HeyA8-MDR cell migration was examined using wound-healing and Transwell chamber cell migration assays. In the wound-healing assay, the cell migration rate decreased in HeyA8 and HeyA8-MDR cells compared with those in the control group, and the migration capability also decreased significantly after treatment

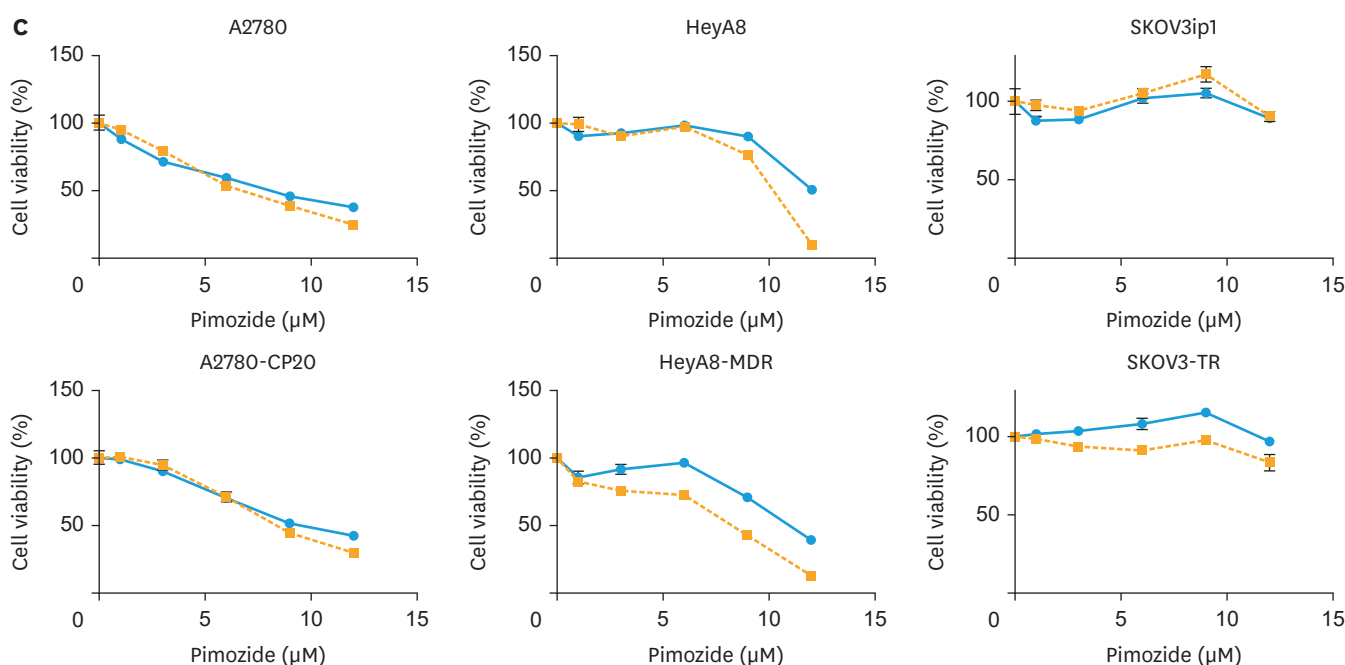


Fig. 1. (Continued) MTT assay of cell proliferation after treatment of EOC cells with DPBP drugs. Cell proliferation was reduced in EOC cell lines treated with (A) penfluridol, (B) fluspirilene, and (C) pimozide.
DPBP, diphenylbutylpiperidine; EOC, epithelial ovarian cancer; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

with 5 μ M penfluridol in both cell lines (**Fig. 3A and B**). The level of MMP-9 protein, which is associated with tumor migration and invasion in several cancers [28], significantly decreased after treatment with penfluridol, indicating its potential role as an underlying mechanism for the anticancer effects (**Fig. 3C**).

4. In vivo efficacy of penfluridol in mouse xenograft models containing PDX

In vivo experiments were performed to confirm the potential clinical relevance of the results from the in vitro experiments and compare the efficacy of penfluridol on tumor growth in orthotopic cell lines or PDX mouse models. In the HeyA8 model, compared with the control treatment, penfluridol (10 mg/kg) significantly decreased the tumor weight (**Fig. 4A**, left). Moreover, in the HeyA8-MDR model, penfluridol led to a significant decrease in tumor weight compared with paclitaxel or control treatment (**Fig. 4A**, right).

These results were quite similar to the in vitro data. Moreover, the body weight of the penfluridol group did not differ from that of the control or paclitaxel group, which suggests the absence of significant side effects on mice. When applied to the chemosensitive PDX model, penfluridol significantly reduced the tumor weight compared with the control treatment (**Fig. 4B**), without a significant effect on body weight. Using the harvested tumor tissue, the inhibitory effect of penfluridol was also confirmed; Ki-67, a cell proliferation marker, was significantly decreased after penfluridol treatment, whereas TUNEL, a marker of apoptosis, was significantly increased. Consistent results were observed in mouse models using HeyA8 and HeyA8-MDR cells and in the PDX model.

5. Potential mechanism for the anticancer effect of penfluridol on EOC cells

To identify the effects of penfluridol on the regulation of cell proliferation or apoptosis, the PP2A-modulated MAPK pathway in EOC cells was evaluated using Western blot analysis.

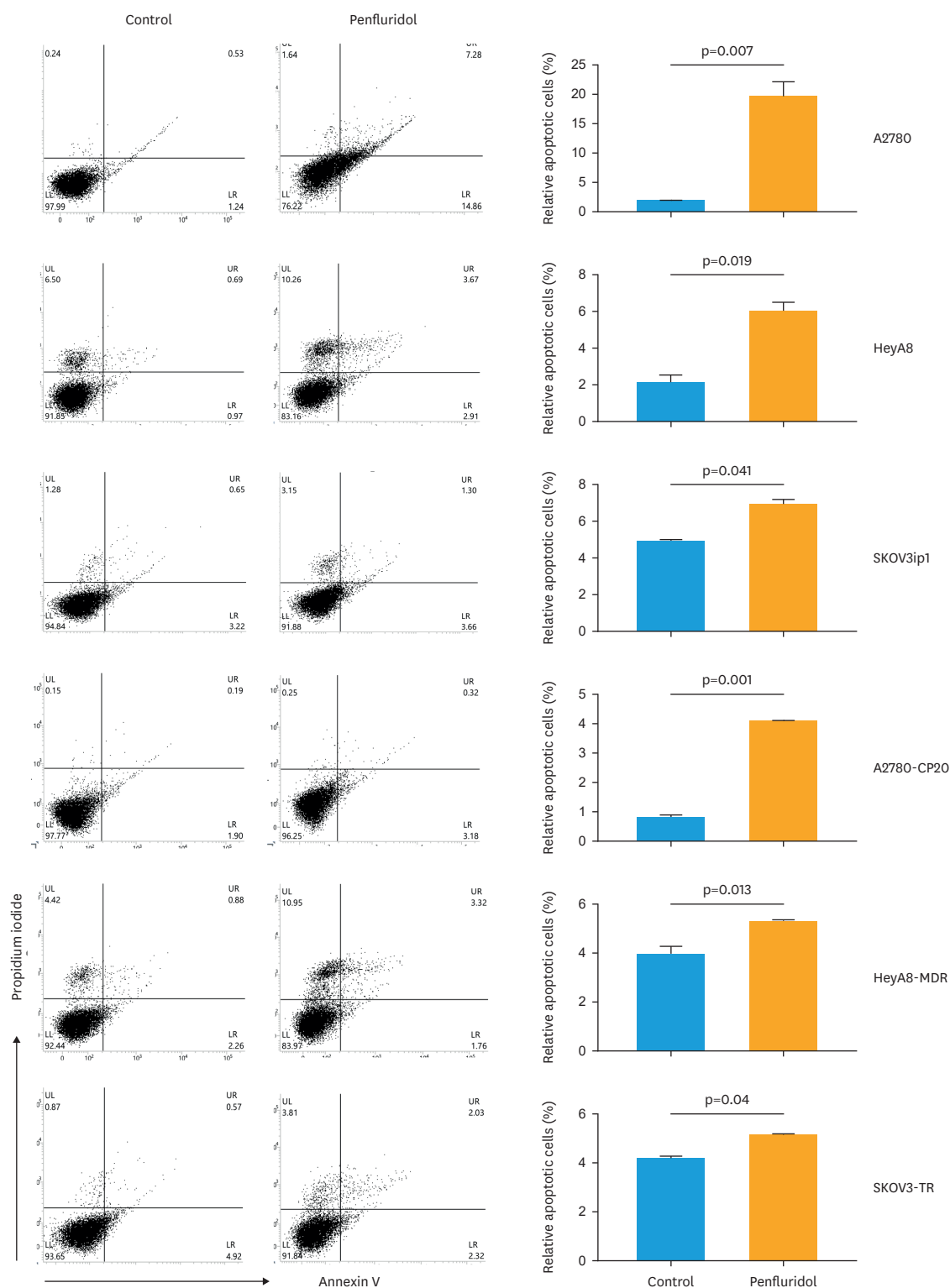


Fig. 2. Apoptosis assay of EOC cells treated with penfluridol. After 48 hours of treatment, apoptotic cell death was measured by fluorescence-activated cell sorter analysis detecting annexin V. The apoptotic EOC cell population significantly increased after penfluridol treatment. EOC, epithelial ovarian cancer.

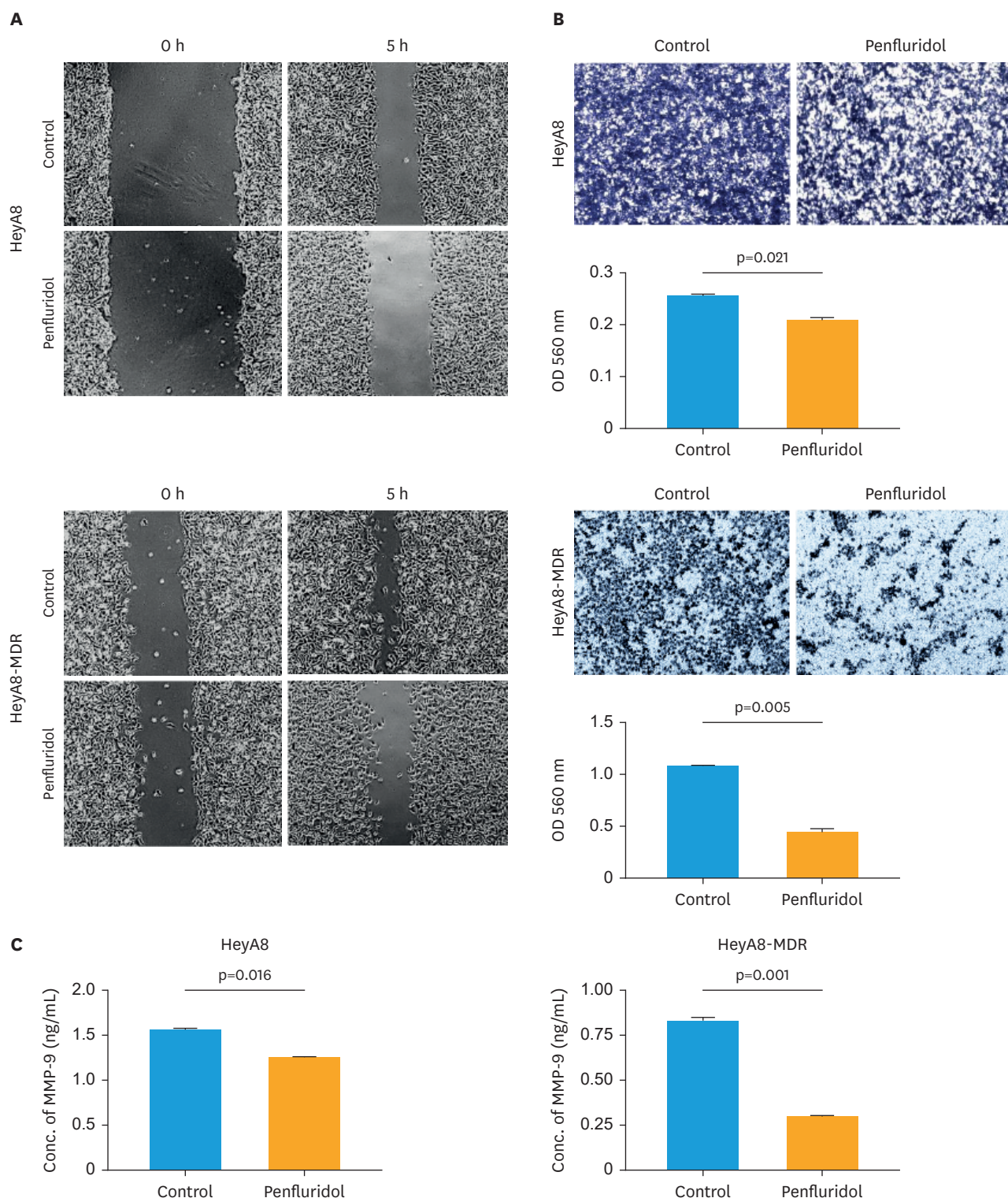


Fig. 3. Effect of penfluridol on HeyA8 and HeyA8-MDR cell migration. Penfluridol significantly inhibited cell migration and MMP-9 expression. (A) Wound-healing assay after treating HeyA8 and HeyA8-MDR cells with penfluridol (5 μ M). Penfluridol interfered with the migratory ability of EOC cells. All photographs were taken at 100 $\times$ magnification. (B) HeyA8 and HeyA8-MDR cells were placed into a Transwell chamber containing 5 μ M penfluridol and incubated for 24 hours. Migrated cells were stained and measured by optical density at 560 nm. (C) The expression of MMP-9 was measured by ELISA. A conditioned medium (24 hours) was used for the MMP-9 ELISA in penfluridol-treated HeyA8 and HeyA8-MDR cell lines. ELISA, enzyme-linked immunosorbent assay; EOC, epithelial ovarian cancer; MMP-9, matrix metalloproteinase 9.

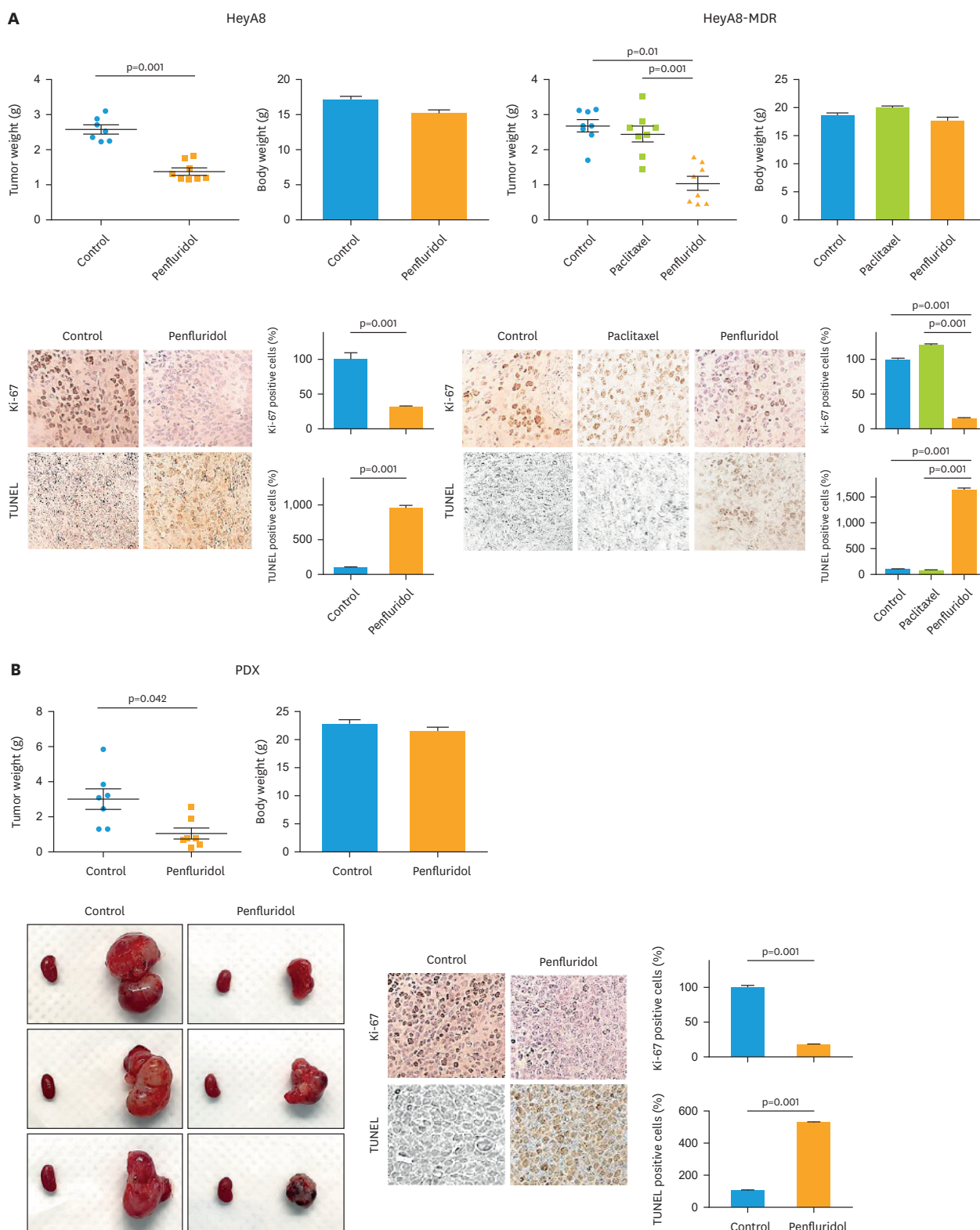


Fig. 4. In vivo efficacy of penfluridol in cell line xenograft and PDX models of EOC. (A) In the cell line xenograft models using HeyA8 and HeyA8-MDR, the penfluridol group had significantly inhibited tumor growth compared with the paclitaxel or control group. The body weight of the penfluridol group was not different from that of the control group. Tumor cell proliferation based on Ki-67 immunohistochemical analysis of harvested tumor tissues was significantly decreased in the penfluridol group. Conversely, the TUNEL assay showed higher apoptosis rates in the penfluridol group. (B) Results were consistent with those in the PDX mouse model. Compared with the control group, the penfluridol group had significantly reduced tumor weight and size. EOC, epithelial ovarian cancer; PDX, patient-derived xenografts.

After treatment of HeyA8 and HeyA8-MDR cells with penfluridol, the expression levels of p-PP2A (at Tyr 307), AKT and ERK significantly decreased compared with the control treatment (**Fig. 5A**). These results suggest that penfluridol regulates cell proliferation by activating PP2A and modulating the MAPK pathway.

DISCUSSION

In this study, in vitro and in vivo analyses were performed to evaluate the anticancer effect of penfluridol on EOC cells. In the in vitro study, DPBP drugs, particularly penfluridol, significantly decreased cell proliferation and increased apoptosis in EOC cells. The anticancer effect of penfluridol was confirmed in vivo using orthotopic xenografts prepared with HeyA8 and HeyA8-MDR cells, as well as a PDX mouse model of EOC. These findings suggest the potential of penfluridol as a novel therapeutic agent, particularly effective in paclitaxel-resistant and chemosensitive EOC. In our study, we also conducted in vivo experiments using A2780 and A2780-CP20 orthotopic xenografts. However, penfluridol did not significantly inhibit tumor growth in these models (data not shown). Taken together, penfluridol may exert a cytotoxic effect against high-grade ovarian serous adenocarcinoma.

Studies have proposed that the mechanism of action of penfluridol as an antipsychotic agent is the blockade of D₂ dopamine receptor, particularly the postsynaptic D₂ receptor and inhibits the release of hypothalamic and hypophyseal hormones [29-32]. Many studies have reported on the anticancer effects of antipsychotic drugs such as penfluridol, pimozide, and fluspirilene [33]. In this study, we investigated the cytotoxic effects of these drugs on EOC cells. Fluspirilene inhibited the proliferation of hepatocellular carcinoma, colon cancer, and glioma cells by targeting CDK2-, p53-MDM2-, and STAT3-related signaling pathways, respectively. The anticancer effects of pimozide have been reported in various cancers, including gliomas, breast, pancreatic, and prostate cancers. The suggested molecular targets of pimozide were STAT5, STAT3, Ca²⁺ T-channels, PI3K/AKT, and DNA damage/repair-related proteins. Cytotoxic effects of penfluridol have demonstrated in various cancers by targeting diverse signaling pathways such as apoptosis, migration/invasion, cholesterol homeostasis, and DNA damage. In this study, results of MTT assays demonstrated that penfluridol exhibited more effective cytotoxic activity than fluspirilene and pimozide in EOC cells. Taken together, each drug may utilize a distinct pathway for inhibiting cell growth, depending on the cancer type and cellular context. Studies have reported that penfluridol induces the tumor suppressor PP2A in pancreatic cancer and leukemia cells, leading to AKT deactivation and resulting in apoptosis [20,34]. In addition, either reactive oxygen species mediated penfluridol-induced autophagy [17,34]. The inhibition of the integrin signaling pathway by penfluridol was also reported in triple-negative breast cancer [18]. Furthermore, penfluridol blocks the human epidermal growth receptor-2/ β -catenin signaling pathway, which is increased in paclitaxel-resistant breast cancer cells [22]. These mechanisms appear to interact intricately with no direct target for the drug.

In this study, the cytotoxic effect of penfluridol may mediated by PP2A activation and thereby inhibits AKT and ERK activity. This suggests that PP2A activation and modulated MAPK pathway could be potential mechanisms of action for penfluridol. PP2A helps regulate cell growth and has been associated with autophagy activation [35]. It was suggested as an antitumor target [36]; however, a clear target for the action of penfluridol is still unknown, indicating the need for more investigations. When compared with paclitaxel, which is a

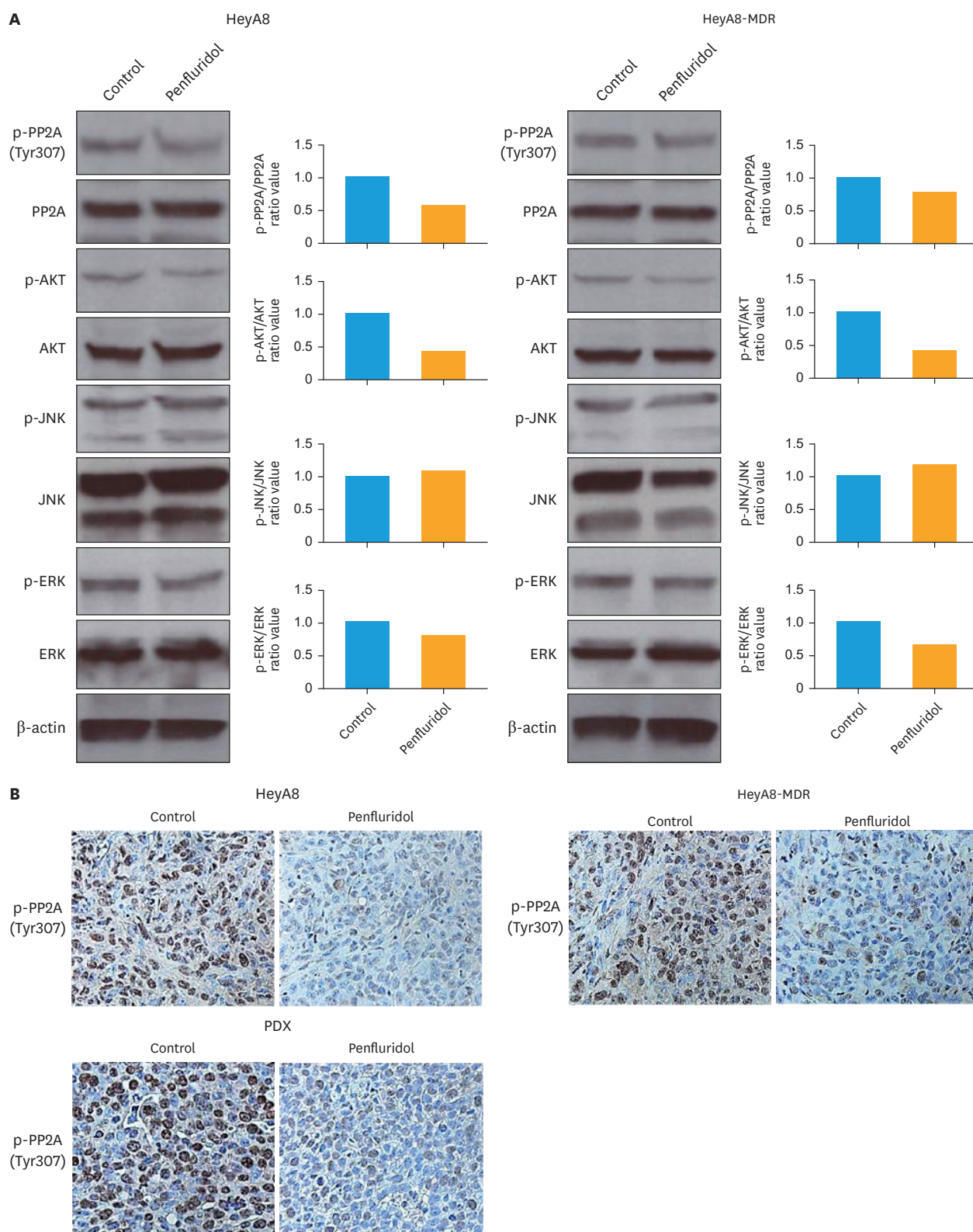


Fig. 5. Effect of penfluridol on the PP2A-MAPK pathway in EOC cells. (A) Cells were incubated with penfluridol (5 μ M) for 1 hour, harvested, and subjected to Western blot analysis of p-PP2A, p-AKT, p-JNK, and p-ERK levels. (B) Consistent results were observed in cell line xenograft and PDX models of EOC. The expression of p-PP2A, measured by IHC, significantly decreased compared to the control.

EOC, epithelial ovarian cancer; IHC, immunohistochemistry; p-AKT, phospho-AKT; PDX, patient-derived xenografts; p-ERK, phospho-ERK; p-JNK, phospho-JNK; PP2A, protein phosphatase 2A; p-PP2A, phospho-PP2A.

microtubule-targeting drug, or other targeted cancer drugs, penfluridol lacks a clearly defined target, and the absence of a specific target may limit drug development. If a direct target can be identified, studies of penfluridol derivatives may gain further momentum.

In summary, this study demonstrated the anticancer effect of penfluridol on EOC in vitro and in vivo. In the quest for potential agents to manage patients with “untreatable cancer,” penfluridol has emerged as a potent anticancer agent in in vitro and in vivo models with strong suppressive effects on the characteristic features of cancer. To the best of our knowledge, this study is the first to report the anticancer effect of penfluridol on EOC. As a drug repurposing strategy, penfluridol therapy may be a potential therapeutic drug for EOC, particularly in paclitaxel-resistant EOC.

SUPPLEMENTARY MATERIAL

Table S1

IC₅₀ values of each drug for cell proliferation in epithelial ovarian cancer cell lines

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;70:7-30. [PUBMED](#) | [CROSSREF](#)
2. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-86. [PUBMED](#) | [CROSSREF](#)
3. Kuroki L, Guntupalli SR. Treatment of epithelial ovarian cancer. *BMJ* 2020;371:m3773. [PUBMED](#) | [CROSSREF](#)
4. Ozols RF, Bookman MA, Connolly DC, Daly MB, Godwin AK, Schilder RJ, et al. Focus on epithelial ovarian cancer. *Cancer Cell* 2004;5:19-24. [PUBMED](#) | [CROSSREF](#)
5. Landen CN Jr, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. *J Clin Oncol* 2008;26:995-1005. [PUBMED](#) | [CROSSREF](#)
6. Baker NC, Ekins S, Williams AJ, Tropsha A. A bibliometric review of drug repurposing. *Drug Discov Today* 2018;23:661-72. [PUBMED](#) | [CROSSREF](#)
7. Shi XN, Li H, Yao H, Liu X, Li L, Leung KS, et al. In silico identification and in vitro and in vivo validation of anti-psychotic drug fluspirilene as a potential CDK2 inhibitor and a candidate anti-cancer drug. *PLoS One* 2015;10:e0132072. [PUBMED](#) | [CROSSREF](#)
8. Patil SP, Pacitti MF, Gilroy KS, Ruggiero JC, Griffin JD, Butera JJ, et al. Identification of antipsychotic drug fluspirilene as a potential p53-MDM2 inhibitor: a combined computational and experimental study. *J Comput Aided Mol Des* 2015;29:155-63. [PUBMED](#) | [CROSSREF](#)
9. Dong Y, Furuta T, Sabit H, Kitabayashi T, Jiapaer S, Kobayashi M, et al. Identification of antipsychotic drug fluspirilene as a potential anti-glioma stem cell drug. *Oncotarget* 2017;8:111728-41. [PUBMED](#) | [CROSSREF](#)
10. García-Santisteban I, Peters GJ, Giovannetti E, Rodríguez JA. USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. *Mol Cancer* 2013;12:91. [PUBMED](#) | [CROSSREF](#)
11. Strobl JS, Kirkwood KL, Lantz TK, Lewine MA, Peterson VA, Worley JF 3rd. Inhibition of human breast cancer cell proliferation in tissue culture by the neuroleptic agents pimozide and thioridazine. *Cancer Res* 1990;50:5399-405. [PUBMED](#)
12. Jandaghi P, Najafabadi HS, Bauer AS, Papadakis AI, Fassin M, Hall A, et al. Expression of DRD2 is increased in human pancreatic ductal adenocarcinoma and inhibitors slow tumor growth in mice. *Gastroenterology* 2016;151:1218-31. [PUBMED](#) | [CROSSREF](#)
13. Fortney K, Griesman J, Kotlyar M, Pastrello C, Angeli M, Sound-Tsao M, et al. Prioritizing therapeutics for lung cancer: an integrative meta-analysis of cancer gene signatures and chemogenomic data. *PLoS Comput Biol* 2015;11:e1004068. [PUBMED](#) | [CROSSREF](#)

14. Zhou W, Chen MK, Yu HT, Zhong ZH, Cai N, Chen GZ, et al. The antipsychotic drug pimozide inhibits cell growth in prostate cancer through suppression of STAT3 activation. *Int J Oncol* 2016;48:322-8. [PUBMED](#) | [CROSSREF](#)
15. Mohanty SK, Yagiz K, Pradhan D, Luthringer DJ, Amin MB, Alkan S, et al. STAT3 and STAT5A are potential therapeutic targets in castration-resistant prostate cancer. *Oncotarget* 2017;8:85997-6010. [PUBMED](#) | [CROSSREF](#)
16. Rondanin R, Simoni D, Maccesi M, Romagnoli R, Grimaudo S, Pipitone RM, et al. Effects of pimozide derivatives on pSTAT5 in K562 cells. *ChemMedChem* 2017;12:1183-90. [PUBMED](#) | [CROSSREF](#)
17. Hedrick E, Li X, Safe S. Penfluridol represses integrin expression in breast cancer through induction of reactive oxygen species and downregulation of Sp transcription factors. *Mol Cancer Ther* 2017;16:205-16. [PUBMED](#) | [CROSSREF](#)
18. Ranjan A, Gupta P, Srivastava SK. Penfluridol: an antipsychotic agent suppresses metastatic tumor growth in triple-negative breast cancer by inhibiting integrin signaling axis. *Cancer Res* 2016;76:877-90. [PUBMED](#) | [CROSSREF](#)
19. Wu L, Liu YY, Li ZX, Zhao Q, Wang X, Yu Y, et al. Anti-tumor effects of penfluridol through dysregulation of cholesterol homeostasis. *Asian Pac J Cancer Prev* 2014;15:489-94. [PUBMED](#) | [CROSSREF](#)
20. Chien W, Sun QY, Lee KL, Ding LW, Wuensche P, Torres-Fernandez LA, et al. Activation of protein phosphatase 2A tumor suppressor as potential treatment of pancreatic cancer. *Mol Oncol* 2015;9:889-905. [PUBMED](#) | [CROSSREF](#)
21. Kim H, Chong K, Ryu BK, Park KJ, Yu MO, Lee J, et al. Repurposing penfluridol in combination with temozolomide for the treatment of glioblastoma. *Cancers (Basel)* 2019;11:1310. [PUBMED](#) | [CROSSREF](#)
22. Gupta N, Gupta P, Srivastava SK. Penfluridol overcomes paclitaxel resistance in metastatic breast cancer. *Sci Rep* 2019;9:5066. [PUBMED](#) | [CROSSREF](#)
23. Chen J, Martin BL, Brautigan DL. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science* 1992;257:1261-4. [PUBMED](#) | [CROSSREF](#)
24. Guo H, Damuni Z. Autophosphorylation-activated protein kinase phosphorylates and inactivates protein phosphatase 2A. *Proc Natl Acad Sci U S A* 1993;90:2500-4. [PUBMED](#) | [CROSSREF](#)
25. Jeong SY, Cho YJ, Ryu JY, Choi JJ, Hwang JR, Kim B, et al. Establishment and preclinical application of a patient-derived xenograft model for uterine cancer. *Gynecol Oncol* 2021;162:173-81. [PUBMED](#) | [CROSSREF](#)
26. Lee CH, Xue H, Sutcliffe M, Gout PW, Huntsman DG, Miller DM, et al. Establishment of subrenal capsule xenografts of primary human ovarian tumors in SCID mice: potential models. *Gynecol Oncol* 2005;96:48-55. [PUBMED](#) | [CROSSREF](#)
27. Chang CS, Ryu JY, Choi JK, Cho YJ, Choi JJ, Hwang JR, et al. Anti-cancer effect of fenbendazole-incorporated PLGA nanoparticles in ovarian cancer. *J Gynecol Oncol* 2023;34:e58. [PUBMED](#) | [CROSSREF](#)
28. Bauvois B. New facets of matrix metalloproteinases MMP-2 and MMP-9 as cell surface transducers: outside-in signaling and relationship to tumor progression. *Biochim Biophys Acta* 2012;1825:29-36. [PUBMED](#) | [CROSSREF](#)
29. Santi CM, Cayabyab FS, Sutton KG, McRory JE, Mezeyova J, Hamming KS, et al. Differential inhibition of T-type calcium channels by neuroleptics. *J Neurosci* 2002;22:396-403. [PUBMED](#) | [CROSSREF](#)
30. Shintomi K, Yamamura M. Effects of penfluridol and other drugs on apomorphine-induced stereotyped behavior in monkeys. *Eur J Pharmacol* 1975;31:273-80. [PUBMED](#) | [CROSSREF](#)
31. Kline CLB, Ralff MD, Lulla AR, Wagner JM, Abbosh PH, Dicker DT, et al. Role of dopamine receptors in the anticancer activity of ONC201. *Neoplasia* 2018;20:80-91. [PUBMED](#) | [CROSSREF](#)
32. Tuan NM, Lee CH. Penfluridol as a candidate of drug repurposing for anticancer agent. *Molecules* 2019;24:3659. [PUBMED](#) | [CROSSREF](#)
33. Shaw V, Srivastava S, Srivastava SK. Repurposing antipsychotics of the diphenylbutylpiperidine class for cancer therapy. *Semin Cancer Biol* 2021;68:75-83. [PUBMED](#) | [CROSSREF](#)
34. Wu SY, Wen YC, Ku CC, Yang YC, Chow JM, Yang SF, et al. Penfluridol triggers cytoprotective autophagy and cellular apoptosis through ROS induction and activation of the PP2A-modulated MAPK pathway in acute myeloid leukemia with different FLT3 statuses. *J Biomed Sci* 2019;26:63. [PUBMED](#) | [CROSSREF](#)
35. Bánréti Á, Lukácsovich T, Csikós G, Erdélyi M, Sass M. PP2A regulates autophagy in two alternative ways in *Drosophila*. *Autophagy* 2012;8:623-36. [PUBMED](#) | [CROSSREF](#)
36. Schönthal AH. Role of serine/threonine protein phosphatase 2A in cancer. *Cancer Lett* 2001;170:1-13. [PUBMED](#) | [CROSSREF](#)