



Editor-in-Chief:

Miaoqing Zhao, PhD, MD (Shandong First Medical University, Jinan, China)
He Wang, MD, PhD (Yale University School of Medicine, New Haven, Connecticut, USA)

Founding Editor & Editor-in-chief Emeritus:

Vinod B. Shidham, MD, FIAC, FRCPath (WSU School of Medicine, Detroit, USA)



Research Article

Mechanistic insights into the effect of midazolam on the malignant progression of ovarian cancer

Xiaokun Sun, ^{BD}¹, Haifang Yu, ^{MBBS}¹, Bing Fu, ^{MM}², Yun Zhang, ^{MM}^{1*}

Departments of ¹Anesthesiology Surgery, ²Blood Transfusion, Qingdao Traditional Chinese Medicine Hospital, Qingdao Hiser Hospital Affiliated of Qingdao University, Qingdao, China.

*Corresponding author:



Yun Zhang,
Department of Anesthesiology
Surgery, Qingdao Traditional
Chinese Medicine Hospital,
Qingdao Hiser Hospital
Affiliated of Qingdao
University, Qingdao, China.
asdfg123450126@126.com

Received: 07 February 2025

Accepted: 07 May 2025

Published: 04 November 2025

DOI

10.25259/Cytojournal_25_2025

Quick Response Code:



ABSTRACT

Objective: As the first gynecological tumor, ovarian cancer seriously threatens women's lives and health. Here, the possible mechanism of midazolam against ovarian cancer progression was investigated.

Material and Methods: OVCAR3 and SKOV3 cells were treated with midazolam for 24 h, and cell activity was subsequently detected by cell counting kit-8 assay to screen for the suitable treatment concentrations of midazolam. Cell proliferation was investigated through 5-ethynyl-2'-deoxyuridine staining and cell plate cloning experiments. Apoptosis rate was detected by flow cytometry, and cell invasion and migration ability were examined through transwell test and scratch test. Western blot was adopted to explore the expression changes of apoptotic proteins, cell cycle-related proteins, and extracellular signal-regulated kinase/c-Jun N-terminal kinase (ERK/JNK) pathway-related proteins.

Results: After midazolam intervention, the OVCAR3 and SKOV3 cells showed decreased proliferation, invasion, and migration abilities and accelerated apoptosis rate. Western blot results displayed that midazolam downregulated the phosphorylation levels of ERK, JNK, and P38. Anisomycin reversed the effect of midazolam on the ERK/JNK pathway and cell proliferation, apoptosis, cell cycle, invasion, and migration.

Conclusion: Midazolam can block the development of ovarian cancer, and the relevant mechanism may be realized through the ERK/JNK pathway.

Keywords: Apoptosis, Midazolam, Ovarian cancer, Proliferation

INTRODUCTION

As the “first gynecological cancer,” ovarian cancer seriously threatens women's health. Globally, over 320 thousand new cases of ovarian cancer and over 200 thousand deaths from ovarian cancer have been reported.^[1] Owing to the location of the ovaries deep in the pelvic cavity and the lack of typical recognition features, the early diagnosis of ovarian cancer is difficult. Therefore, the onset of ovarian cancer is relatively secretive, and three-quarters of patients have entered the advanced stage by the time of diagnosis.^[2] Surgical treatment is mainly used in clinical practice; however, surgical stress can cause the spread of residual cancer cells, which is also one of the reasons for tumor recurrence and metastasis. Anesthetics, such as fentanyl,^[3] ropivacaine,^[4] and lidocaine,^[5] can affect the malignant behavior of cancer cells and the prognosis of patients undergoing cancer surgery. Therefore, the selection of appropriate anesthetics is of great significance in the surgical treatment of ovarian cancer.^[6]

Midazolam is a water-soluble benzodiazepine with anti-anxiety, sedative amnesia, anti-muscle relaxation, and other effects and is mostly used to induce sleep before surgery and to treat insomnia.^[7] By inhibiting synaptic timing in the hippocampal CA1 region and regulating central CaMKII protein expression, this drug exerts sedative effects while reducing negative effects on patients' cognitive ability.^[8] Midazolam is a commonly used sedative drug in clinical surgery with a proven excellent sedative effect.^[9] Rania *et al.*^[10] confirmed that midazolam plus epidural morphine could reduce the total post-operative analgesia in patients undergoing colorectal cancer surgery. Novac *et al.*^[11] evaluated the effects of midazolam for total intravenous anesthesia on inflammatory responses after minimally invasive gynecological surgery and found that it has anti-inflammatory and immunomodulatory effects when combined with fentanyl. Midazolam also reduces bone cancer pain by inhibiting "neuron-astrocyte activation" in the spinal cord.^[12] It prohibits the growth of colon cancer and leukemia cells by activating the intrinsic pathway of mitochondrial apoptosis.^[13] It blocks liver cancer cell metastasis and promotes apoptosis, which is related to miR-217 down-regulation.^[14] Despite its potential anti-tumor effects, only a few reports are available on the correlation between midazolam and ovarian cancer.

In this work, the effect of midazolam on the biological behaviors of ovarian cancer was studied through functional experiments to clarify the mechanism of this drug during the progression of ovarian cancer and provide reference and guidance for subsequent studies.

MATERIAL AND METHODS

Cell culture

Ovarian cancer cells OVCAR3 (BH-C238) and SKOV3 (BH-C124) were bought from Bohui Biotechnology (Guangzhou, China) and subjected to short tandem repeat (STR) identification and mycoplasma detection. The cell culture conditions were as follows: Roswell Park Memorial Institute-1640 medium (PM150110B, Pricella, Wuhan, China) with penicillin/streptomycin solution (1%, 60162ES76, Yeasen, Shanghai, China) and fetal bovine serum (FBS, 10%, C2056-1A, EK-Bioscience, Shanghai, China), 37°C, and 5% carbon dioxide. The experiments were conducted when the cells grew to the logarithmic stage. The cells were treated with midazolam at half the maximal inhibitory concentration (IC₅₀) for 24 h. At 2 h before midazolam (H20031037, NHWA, Xuzhou, China) treatment, 10 μM of anisomycin (HBS-M1117, Huibaishiji, Changsha, China) was added to induce JNK activation *in vitro*.

Cell counting kit-8 (CCK-8) assay

Ovarian cancer OVCAR3 and SKOV3 cells (5×10^4 /well) were inoculated in a 96-well plate and treated with midazolam (0, 10, 30, 50, 100, and 200 μmol/L, H20031037, NHWA, Xuzhou, China) for 24 h. The cells were continuously incubated for 2 h with CCK-8 solution (10 μL, CK04, Dojindo, Shanghai, China). At 450 nm, the absorbance values were measured using an enzyme-labeled instrument.

Cell plate cloning experiment

The cells were inoculated into a six-well plate and treated with midazolam for 24 h after adhesion. After 24 h, the cells were fixed (4% paraformaldehyde, YB-169Z, Ybscience, Shanghai, China) and stained by crystal violet (0.1%, YB-M007, Ybscience, Shanghai, China). The number of clones was counted under a microscope (CX41, Olympus, Japan).

5-ethynyl-2'-deoxyuridine (EdU) staining

The cells were inoculated in a 96-well plate (1×10^4 cells/mL). After cell adhesion, 100 μL of EdU solution was added for 2 h. After washing with phosphate buffered saline (PBS), 1× Apollo and 4,6-diamino-2-phenylindole staining solution were added after fixation and permeability. The EdU positive cell rate was calculated under a fluorescence microscope (DM2500, Leica, Germany).

Cell scratch test

The cells were inoculated into a six-well plate (1×10^6 cells/well). A straight line was drawn with the pipette tip perpendicular to the cell surface, and the different parts were marked. Photographs were taken at 0 and 24 h to calculate the healing efficiency.

Transwell assay

The cells (3×10^3 cells/chamber) in each group were inoculated in transwell chamber and incubated in 37°C incubator for 60 min. The upper chamber was precoated with Matrigel (356234, Solarbio, Beijing, China), and a complete medium was added to the lower chamber. After incubation overnight, polyformaldehyde (4%) and crystal violet (0.1%) were added for fixation and staining, respectively. The transmembrane cells were counted under a microscope (Olympus, Japan).

Western blot assay

Proteins were obtained from the cells using cleavage buffers containing protease inhibitors and quantified in accordance with the instructions for the bicinchoninic acid kit (P0012S, Beyotime, Shanghai, China). The proteins

were added to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gel and then electrically transferred to a polyvinylidene fluoride membrane (IPVH00010, Millipore, Boston, Massachusetts, USA). The cells were added with primary antibodies (anti-extracellular signal-regulated kinase [ERK]: 66192-1-Ig, 1:10000, Sanying, Wuhan, China; anti-ERK1/2: 4696S, 1:2000, Cell signaling technology, Boston, USA; anti-P38: 66234-1-Ig, 1:5000, Sanying, Wuhan, China; anti-p-P38: 9216L, 1:2000, Cell signaling technology, Boston, USA; anti-c-Jun N-terminal kinase [JNK]: 66210-1-Ig, 1:5000, Sanying, Wuhan, China; anti-p-JNK: sc-81502, 1:1000, Santa Cruz Biotechnology, Dallas, USA; and anti-GAPDH: 60004-1-Ig, 1:50000, Sanying, Wuhan, China) and incubated at 4°C overnight, followed by incubation with the secondary antibody (AS003, 1:5000, ABclonal, Wuhan, China). After an enhanced chemiluminescent solution (C500044, Sangon Biotech, Shanghai, China) was added, photographs were taken by a gel imaging analysis system and grayscale analysis was performed by ImageJ software (National Institutes of Health, New York, NY, USA).

Flow cytometry (FCM)

The cells of logarithmic growth stage were treated with midazolam (midazolam group) or equal volume PBS (control group). For the cell apoptosis assay, the cell suspension was incubated with Annexin V-fluorescein isothiocyanate/propidium iodide (PI) solution (BL107B, Jinpan Biotech, Shanghai, China) for 30 min. For the cell cycle experiment, the cells were fixed using 75% alcohol and then treated with 400 μ L of PI and 100 μ L of RNase A (R31498-50T, BIOBOMEI, HeFei, China) under the dark at 4 °C for 30 min. Finally, apoptosis and cell cycle were detected by FCM (CytoFLEX, Beckman Coulter, USA).

Statistical analysis

Data were expressed as mean \pm standard deviation and analyzed by SPSS 16.0 statistical software (the Statistical Package for the Social Sciences Inc., IBM Corporation, Chicago, IL, USA). Tukey's test of one-way analysis of variance was used to determine differences among different groups, and *t*-test was used for pairwise comparison between groups. $P < 0.05$ indicated that the difference was statistically significant. All experiments were repeated 3 times.

RESULTS

Toxicity of midazolam to ovarian cancer cells

SKOV3 and OVCAR3 cells were treated with different midazolam concentrations (0, 10, 30, 50, 100, and 200 μ mol/L) for 24 h to investigate its toxicity to ovarian cancer. As shown in Figure 1 a, the OVCAR3 cells showed dose-dependent inhibition of cell viability after midazolam treatment. The IC_{50} of midazolam on OVCAR3 cells was 73.40 μ mol/L. In SKOV3 cells, different midazolam concentrations also caused a decrease in cell viability, and the IC_{50} was 78.94 μ mol/L [Figure 1a and b]. Therefore, we treated the cells with midazolam at half the concentration of IC_{50} , that is, 36 μ mol/L for OVCAR3 cells and 39 μ mol/L for SKOV3 cells, to achieve the appropriate experimental effect.

Midazolam inhibited the proliferative behavior of ovarian cancer cells

The effect of midazolam on ovarian cancer cell proliferation was observed by cell plate cloning experiments and EdU methods. Experimental results showed that midazolam notably reduced the EdU positive cell rate of OVCAR3

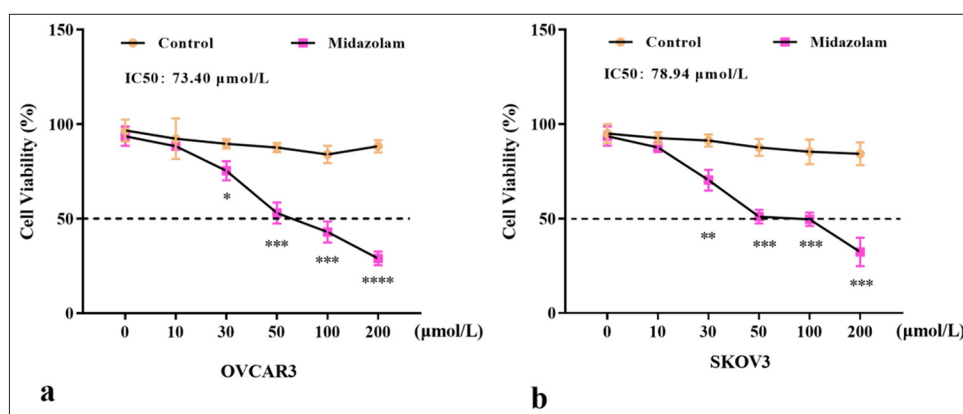


Figure 1: Toxicity of midazolam to ovarian cancer cells. (a) Growth of OVCAR3 cells treated with midazolam at different concentrations as detected by CCK-8 assay. (b) Growth of SKOV3 cells treated with midazolam at different concentrations as detected by CCK-8 assay. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. CCK-8: Cell counting kit-8.

($P < 0.01$) and SKOV3 cells ($P < 0.001$) [Figure 2a and b] and inhibited their clone formation capacity ($P < 0.001$), [Figure 2c and d]. Western blot verified the influence

of midazolam on the expression of cell cycle-related proteins. Midazolam decreased the expression of CDK4 ($P < 0.001$) and cyclin D1 ($P < 0.001$) [Figure 2e-g].

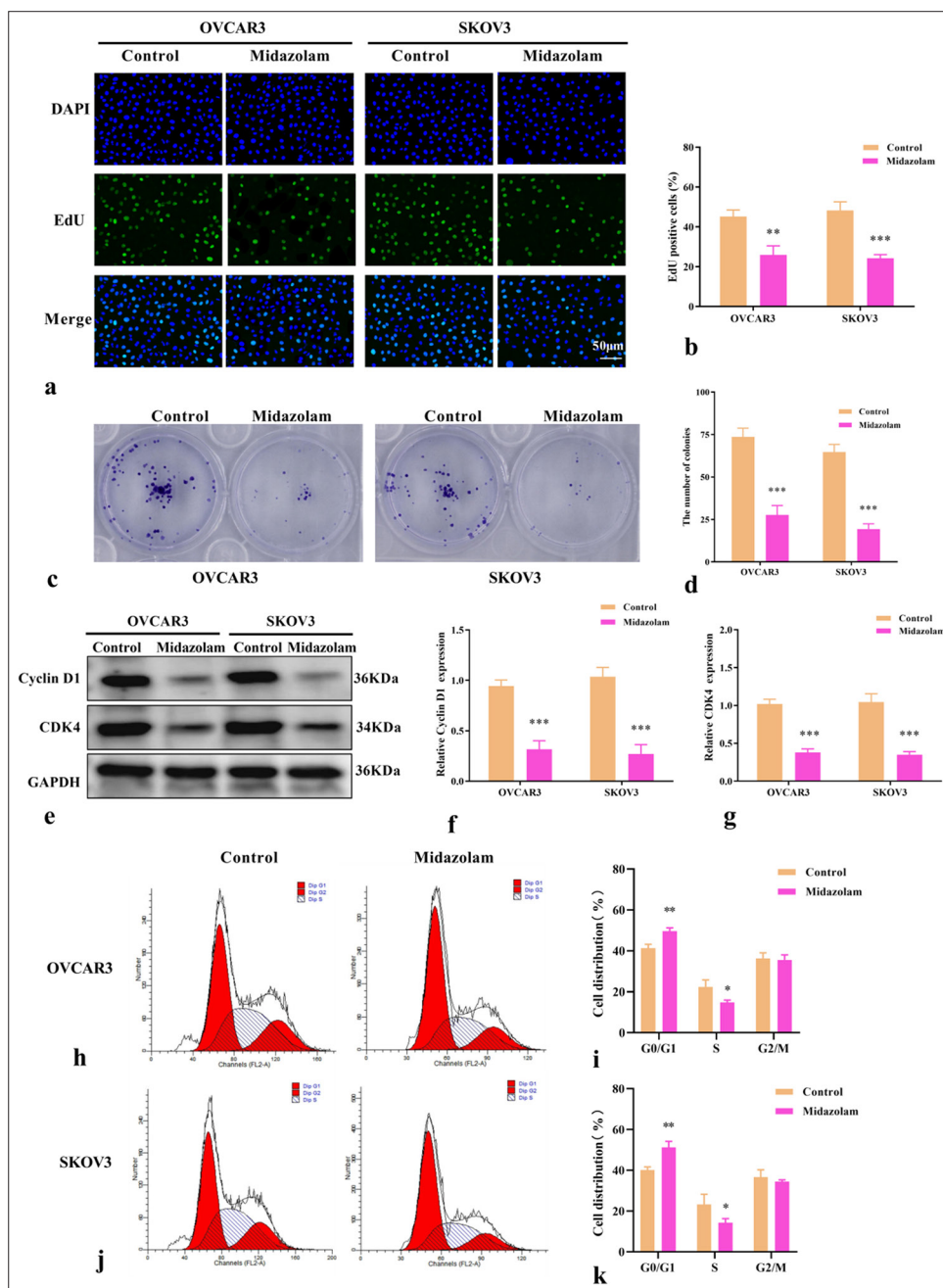


Figure 2: Midazolam inhibited the proliferative behavior of ovarian cancer cells. (a and b) Effect of midazolam on ovarian cell proliferation as confirmed by EdU experiment (scale bar: 50 μ m, magnification: $\times 200$). (c and d) Effect of midazolam on the clone formation capacity was confirmed by cell plate cloning experiment. (e-g) Effect of midazolam on the level of cell cycle-related proteins as confirmed by Western blot assay. (h-k) Effect of midazolam on cell cycle as confirmed by FCM. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. DAPI: 4,6-diamino-2-phenylindole, EdU: 5-ethynyl-2'-deoxyuridine, CDK4: Cyclin-dependent kinase 4, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, FCM: Flow cytometry.

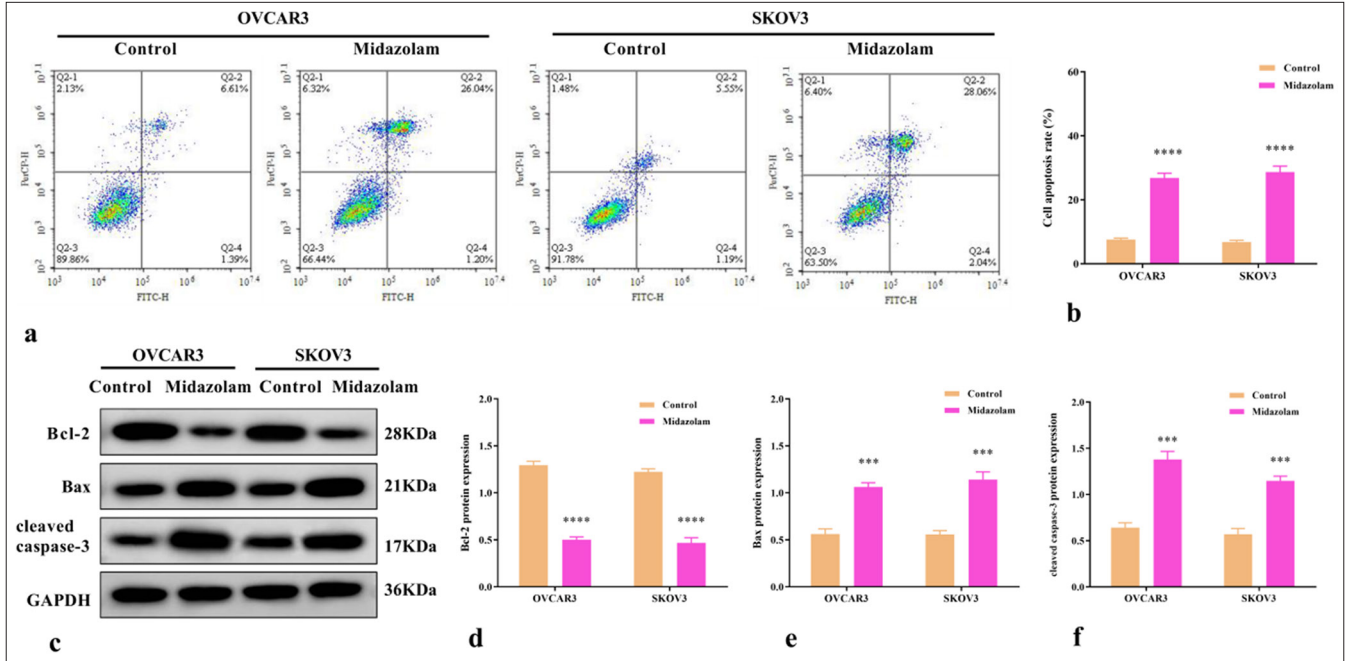


Figure 3: Midazolam induced the apoptosis of ovarian cancer cells. (a and b) FCM confirmed the effect of midazolam on the apoptosis of OVCAR3 and SKOV3 cells. (c-f) Western blot experiment confirmed the effects of midazolam on apoptosis-promoting and apoptosis-inhibiting proteins. $n = 3$, $***P < 0.001$, $****P < 0.0001$. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, FCM: Flow cytometry.

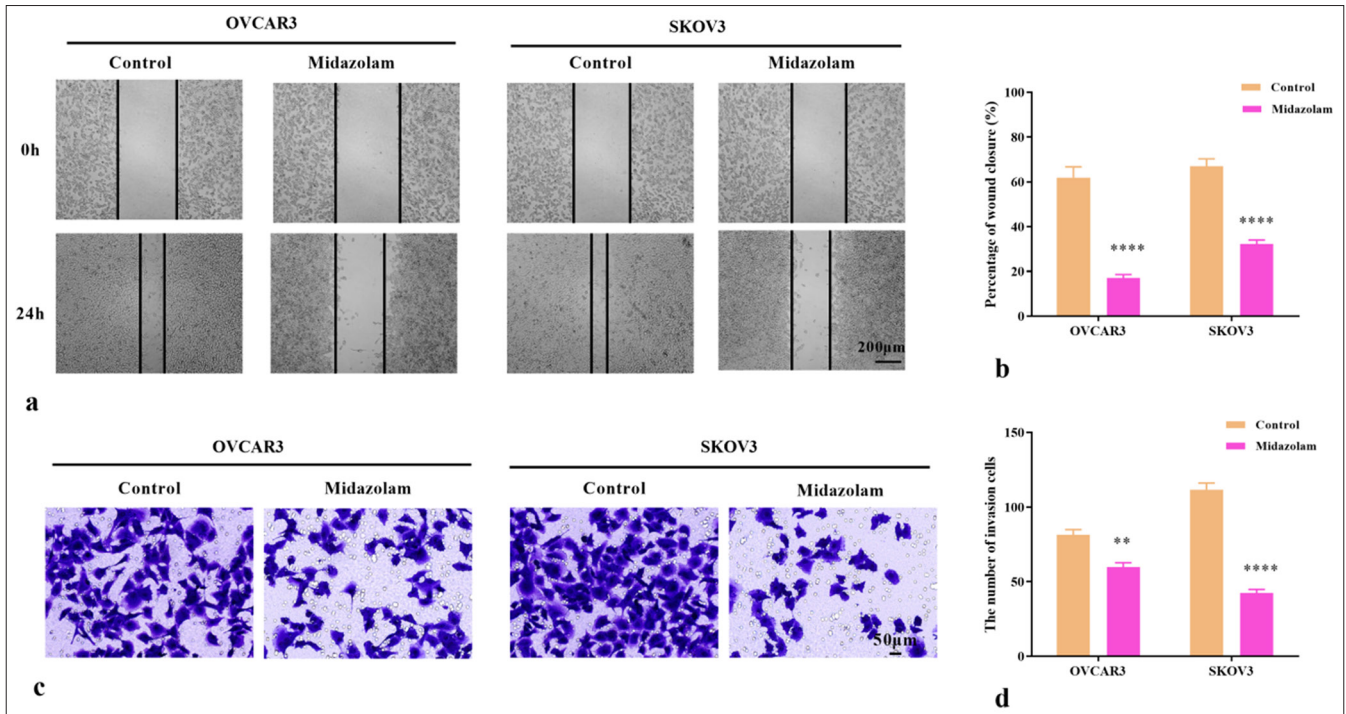


Figure 4: Midazolam hindered the metastatic behavior of ovarian cancer cells. (a and b) Effect of midazolam on the migration ability of OVCAR3 and SKOV3 cells as demonstrated by scratch experiments (scale bar: 200 μm , magnification: $\times 40$). (c and d) Transwell assay showed the effect of midazolam on the invasion ability of OVCAR3 and SKOV3 cells (scale bar: 50 μm , magnification: $\times 200$). $n = 3$, $**P < 0.01$, $****P < 0.0001$.

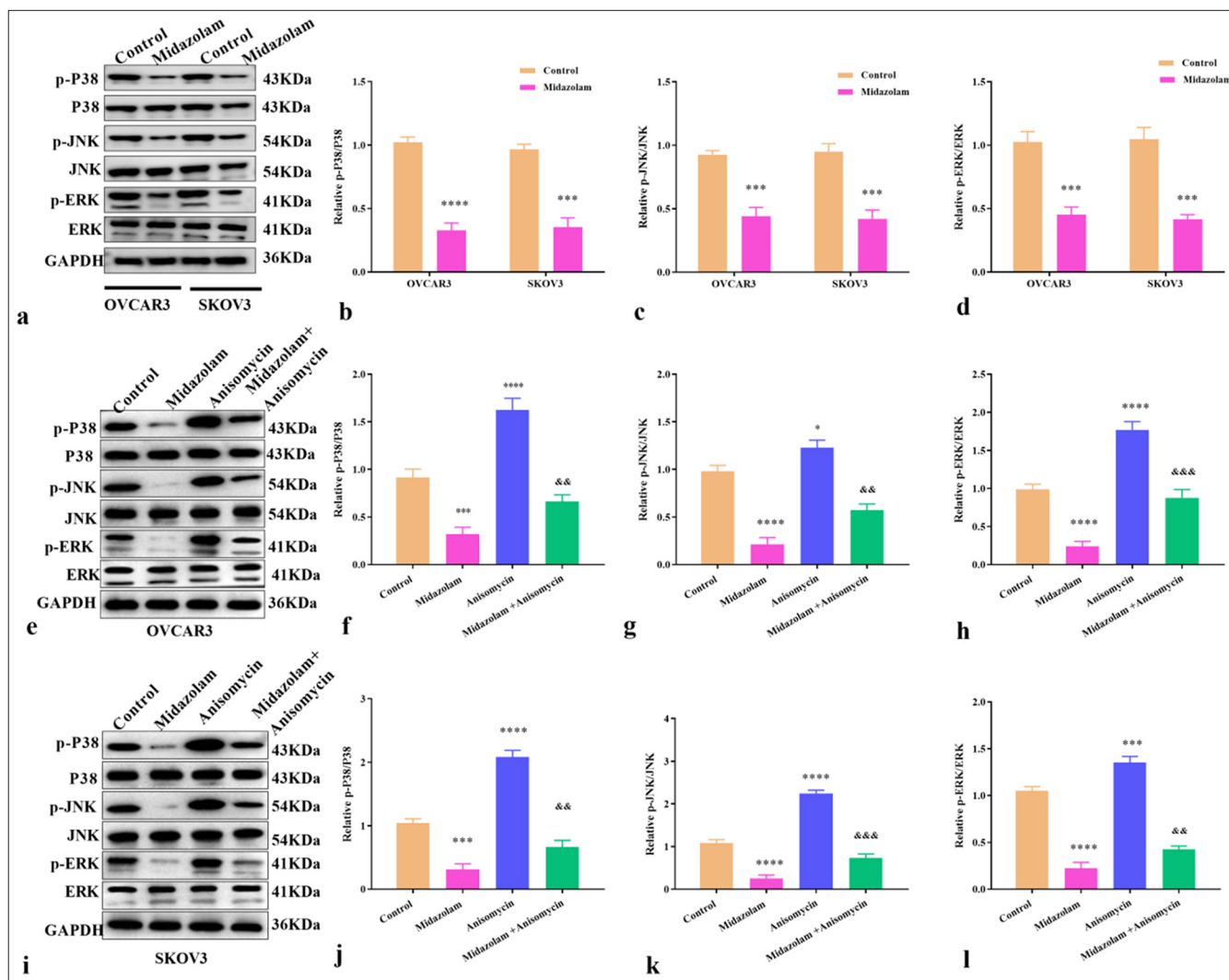


Figure 5: Midazolam blocked the activation of the ERK/JNK signaling pathway in ovarian cancer cells. (a-j) Western blot results showed the effects of midazolam on the expression of total P38, JNK, and ERK and the phosphorylation levels of P38, JNK, and ERK in OVCAR3 and SKOV3 cells. (e-h) Western blot results showed that anisomycin neutralized the effect of midazolam on the ERK/JNK pathway in OVCAR3 cells. (i-l) Western blot results showed that anisomycin neutralized the effect of midazolam on the ERK/JNK pathway in SKOV3 cells. $n = 3$, $*P < 0.05$, $***P < 0.001$, $****P < 0.0001$: Compared with control group; $\&\&P < 0.01$, $\&\&\&P < 0.001$: Compared with midazolam group. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, ERK: Extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinase.

Moreover, midazolam significantly increased the proportion of G0/G1 phase ($P < 0.01$) and decreased the proportion of the S phase ($P < 0.05$) [Figure 2h-k]. Therefore, midazolam prohibited the proliferation of ovarian cancer cells to some extent.

Midazolam induced the apoptosis of ovarian cancer cells

After treatment with midazolam, the proportion of early and late apoptotic cells increased significantly ($P < 0.0001$), [Figure 3a and b]. Western blot results showed that midazolam notably down-regulated Bcl-2 ($P < 0.0001$)

and up-regulated Bax ($P < 0.001$) and cleaved caspase-3 ($P < 0.001$) [Figure 3c-f]. These results suggested that midazolam induced the apoptosis of ovarian cancer cells.

Midazolam hindered the metastatic behavior of ovarian cancer cells

Scratch healing experiment verified the change in cell migration ability after midazolam treatment. Midazolam showed a significant migration inhibitory effect on OVCAR3 and SKOV3 cells ($P < 0.0001$), [Figure 4a and b]. Transwell assay displayed that the invasion ability of OVCAR3 cells

($P < 0.01$) and SKOV3 cells ($P < 0.0001$) was inhibited after midazolam treatment [Figure 4c and d]. Hence, we concluded that midazolam could hinder the metastatic behavior of ovarian cancer cells to some extent.

Midazolam blocked the activation of ERK/JNK pathway

We examined the expression of ERK/JNK pathway protein markers after treating ovarian cancer cells with midazolam.

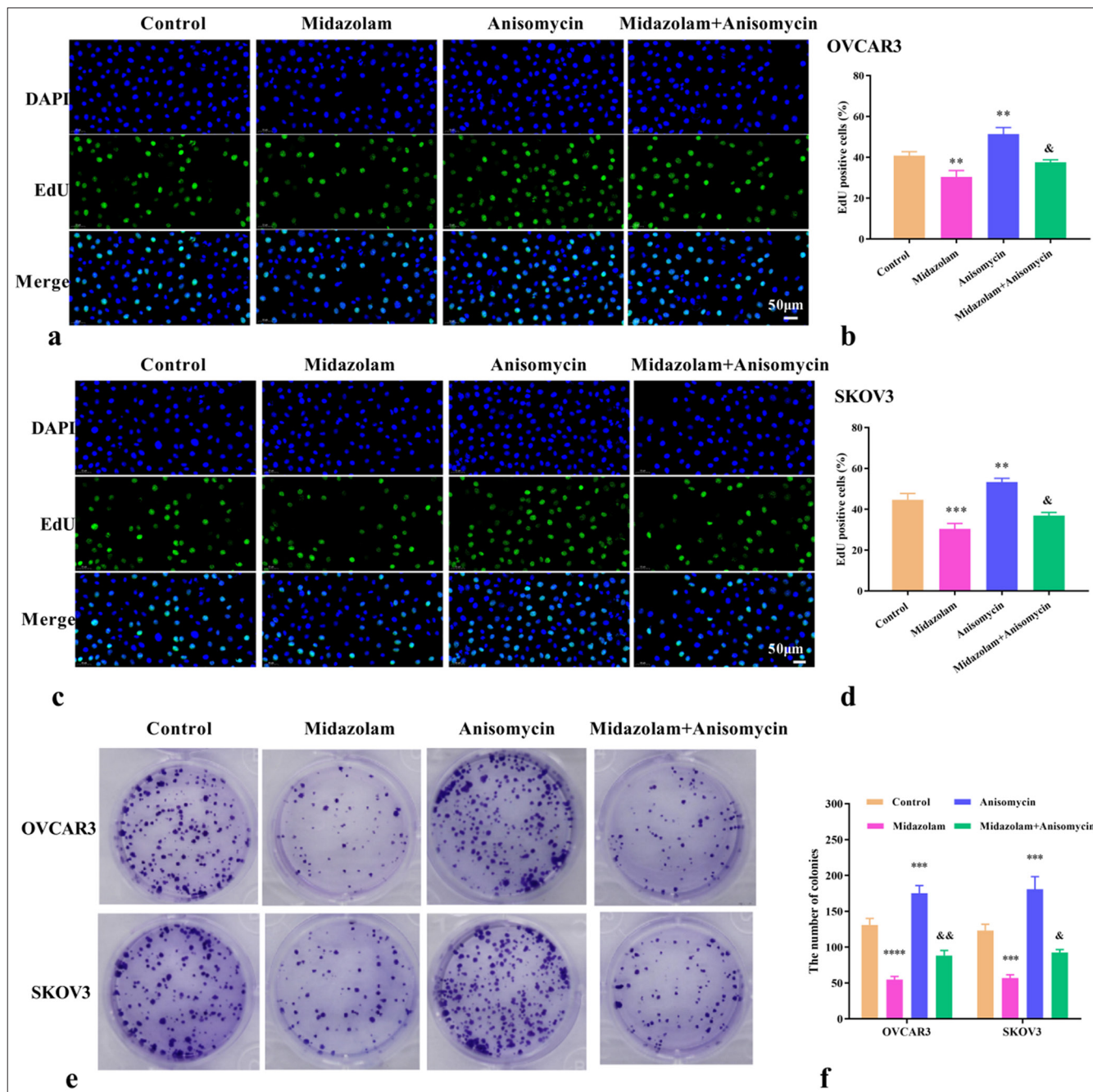


Figure 6: Anisomycin neutralized the effect of midazolam on cell proliferation. (a and b) EdU experiments confirmed that the inhibitory effect of midazolam on the proliferation of OVCAR3 cells was neutralized by anisomycin (scale bar: 50 μm, magnification: ×200). (c and d) EdU experiments confirmed that the inhibitory effect of midazolam on the proliferation of SKOV3 cells was neutralized by anisomycin (scale bar: 50 μm, magnification: ×200). (e and f) Anisomycin neutralized the inhibitory effect of midazolam on the clone formation capacity of OVCAR3 and SKOV3 cells. $n = 3$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$: compared with control group; & $P < 0.05$, && $P < 0.01$: compared with midazolam group. DAPI: 4,6-diamino-2-phenylindole, EdU: 5-ethynyl-2'-deoxyuridine.

Western blot results displayed that the expression levels of total P38, JNK, and ERK were not affected by midazolam. Meanwhile, the phosphorylation levels of P38 (OVCAR: $P < 0.0001$; SKOV3: $P < 0.001$), JNK (OVCAR: $P < 0.001$; SKOV3: $P < 0.001$), and ERK (OVCAR: $P < 0.001$; SKOV3: $P < 0.001$) were significantly reduced [Figure 5a-d]. Anisomycin reversed the effect of midazolam on the ERK/JNK pathway [Figures 5e-l], neutralized its inhibition of the cell proliferation of SKOV3 and OVCAR3 cells (both $P < 0.05$) [Figure 6a-d], and counteracted its inhibitory effect on the clone formation capacity of OVCAR3 ($P < 0.01$) and SKOV3 cells ($P < 0.05$) [Figure 6e and f]. The promoting effect of midazolam on G1 phase was reversed by anisomycin (OVCAR: $P < 0.05$; SKOV3: $P < 0.01$) [Figure 7a-e]. Moreover, the effects of midazolam on cell apoptosis and migration [Figure 8a-d] were also neutralized by anisomycin (all $P < 0.001$). Moreover, the effects of midazolam on the cell invasion of OVCAR3 cells and SKOV3 cells (both $P < 0.01$) were

counteracted by anisomycin [Figure 8e and f]. Therefore, we suggested that midazolam affected ovarian cancer progression by blocking the activation of the ERK/JNK pathway.

DISCUSSION

Surgery is the preferred treatment for most primary tumors, and more than 80% of patients need to be diagnosed and treated through anesthesia. Ovarian cancer is mainly treated through surgery, which can remove the tumor as much as possible and create conditions for post-operative chemotherapy or radiotherapy to improve the curative effect.^[15] However, surgery may also activate the sympathetic nervous system and certain tumor-derived factors, resulting in tumor micrometastasis and clinical metastasis.^[16] Anesthetics can affect the prognosis of patients undergoing surgery, especially those undergoing tumor surgery.

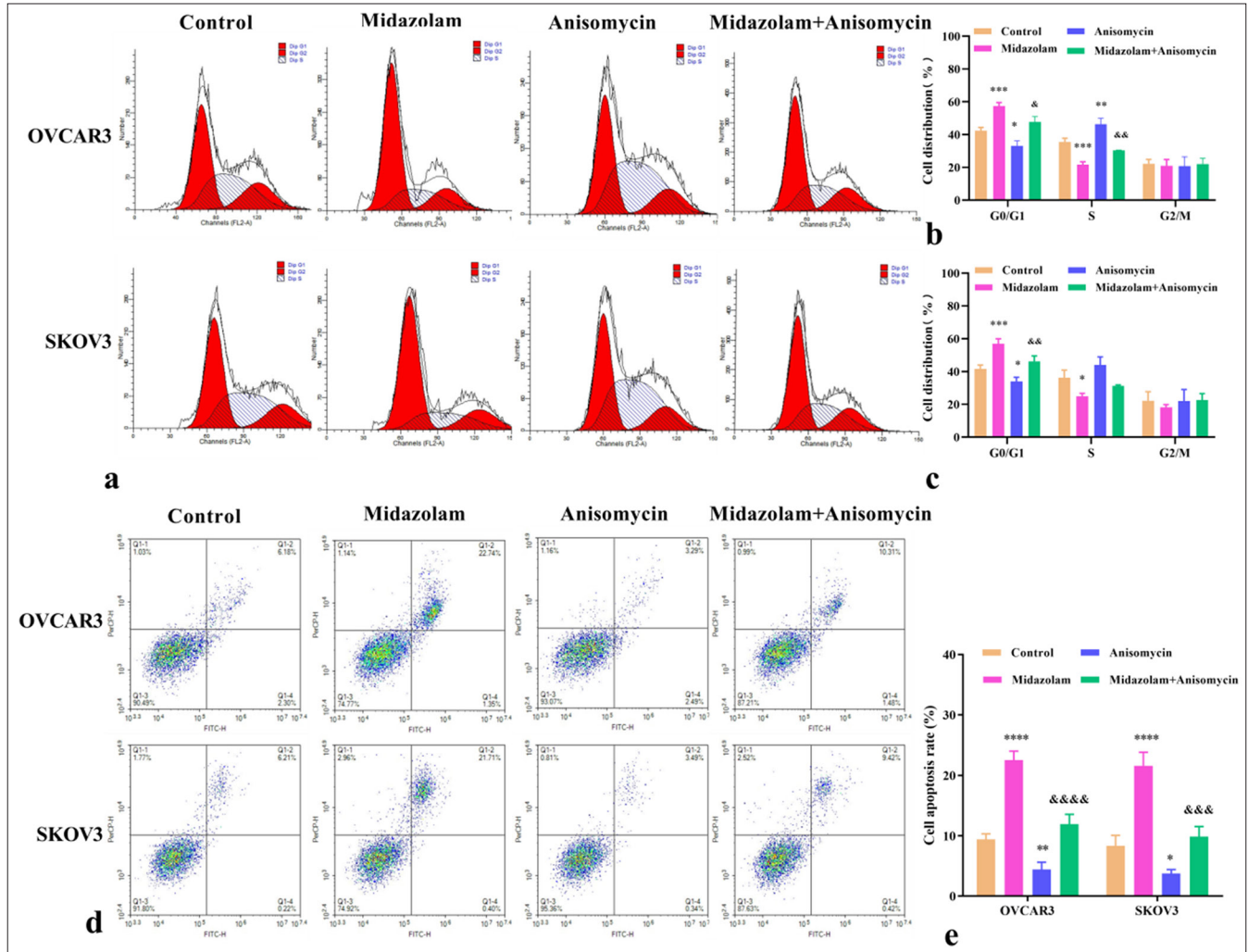


Figure 7: Anisomycin neutralized the effects of midazolam on cell cycle and apoptosis. (a-c) Anisomycin neutralized the effects of midazolam on cell cycle. (d and e) Anisomycin neutralized the effects of midazolam on cell apoptosis. $n = 3$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$: compared with control group; $&P < 0.05$, $&&P < 0.01$, $&&&P < 0.001$, $&&&&P < 0.0001$: compared with midazolam group.

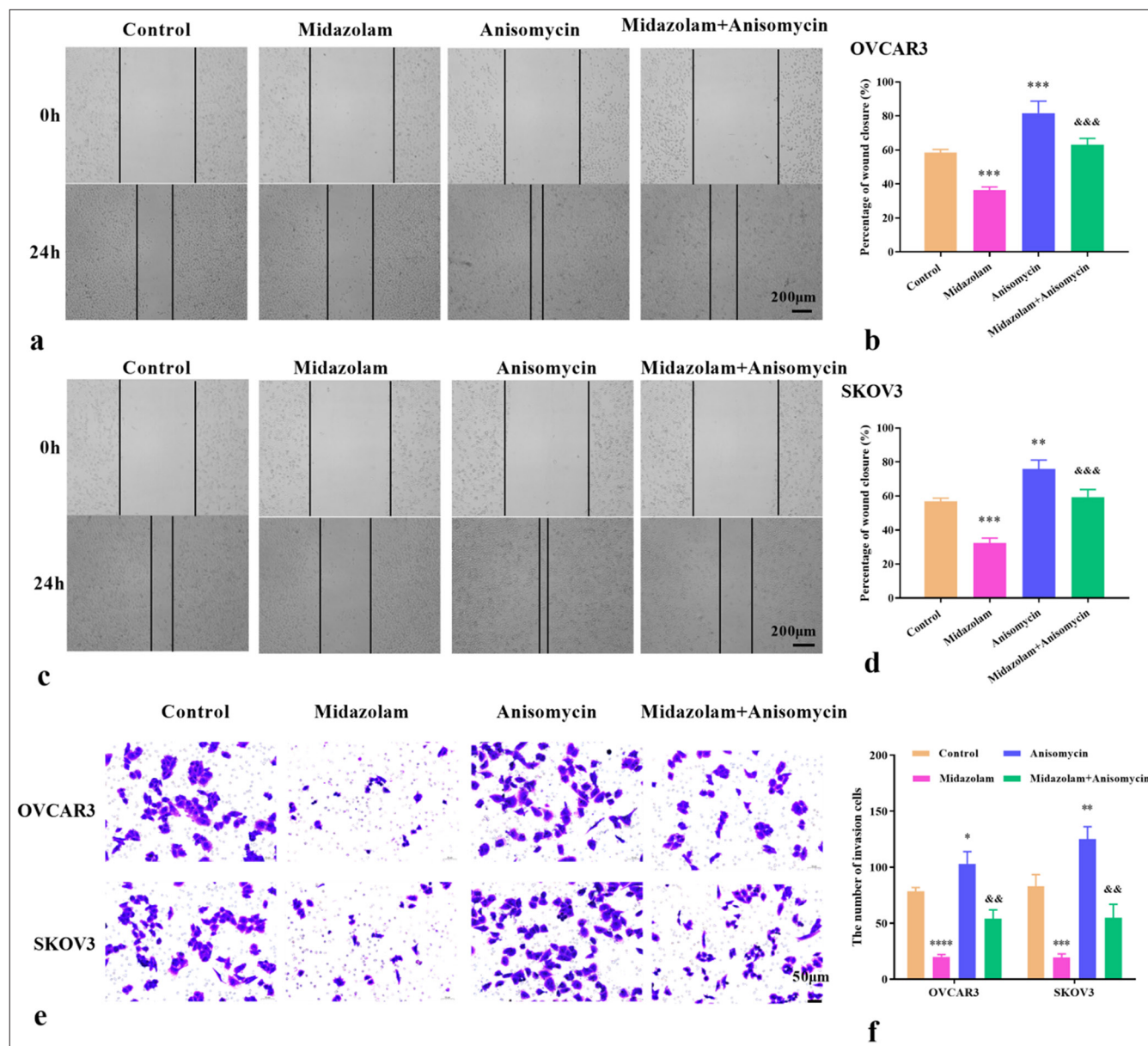


Figure 8: Anisomycin neutralized the effects of midazolam on cell invasion and migration. (a and b) Anisomycin neutralized the effects of midazolam on the migration of OVCAR3 cells (scale bar: 200 μm , magnification: $\times 40$). (c and d) Anisomycin neutralized the effects of midazolam on the migration of SKOV3 cells (scale bar: 200 μm , magnification: $\times 40$). (e and f) Anisomycin neutralized the effects of midazolam on cell invasion (scale bar: 50 μm , magnification: $\times 200$). $n = 3$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$: compared with control group; $&&P < 0.01$, $&&&P < 0.001$: Compared with midazolam group.

As a commonly used intravenous anesthetic, midazolam is frequently applied for the pre-operative, anesthesia induction, and perioperative sedation of patients with tumors due to its rapid effect and significant sedation effect.^[17] This drug is a potent benzodiazepine, which can relieve anxiety in patients with cancer. In clinical settings, midazolam is the choice combination medication for most children because it can significantly reduce the anxiety symptoms and adverse behaviors of children before surgery.^[18] When combined

with ketamine, it can effectively achieve analgesia in children with hematologic tumors undergoing invasive surgery.^[19] Furthermore, patients with cancer experiencing chronic pain are often treated with opioids combined with midazolam and ketamine to reduce their pain.^[20] A recent discovery is that midazolam can hinder the growth of tumor cells and induce apoptosis.^[21,22] Here, we investigated its role in ovarian cancer cell progression. Midazolam reduced the proliferation capacity of ovarian cancer cells, prohibited cell invasion and

migration, and accelerated cell apoptosis rate, indicating its certain effect on the malignant behavior of ovarian cancer cells. Our findings were similar to those reported by other scholars. For instance, midazolam was found to induce apoptosis in A549 cells and hinder cell migration.^[23] In breast cancer, midazolam blocks the levels of mesenchymal proteins, cell migration, invasion, and proliferation.^[24] Furthermore, midazolam induces cell apoptosis and thus impedes cell proliferation, migration, and invasion.^[25] Hence, we concluded that midazolam accelerates apoptosis and blocks the proliferation of ovarian cancer cells.

MAPK is the main carrier of information from the cell surface to the nucleus. Mammals have many MAPK genes, such as ERK, JNK/SAPK, P38MAPK pathway, big MAPkinase-1 (BMK1/ERK5), and ERK3, 4, 6, 7, and 8.^[26] ERK is an important molecule of MAPK, which regulates cell circulation and promotes cell growth and division. JNK and p38 mainly regulate cell stress response and apoptosis.^[27] Anesthetics affect the progression of ovarian cancer cells through the MAPK pathway. For example, propofol exerts anti-cancer effects through the MEK/ERK pathway in ovarian cancer.^[28] Here, we examined the role of midazolam on the ERK/JNK pathway by Western blot assay. After OVCAR3 and SKOV3 cells were treated with midazolam for 24 h, the phosphorylated forms of p-ERK and p-JNK decreased. These inhibitory effects were neutralized by anisomycin. Similarly, midazolam inhibits the development of lung cancer by inactivating the EGFR/MEK/ERK pathway.^[29] and delays the I/R-induced apoptosis of rat cardiomyocytes by reducing the phosphorylation levels of JNK, P38, and ERK.^[30] Anisomycin reversed the effects of midazolam on cell proliferation, apoptosis, cell cycle, invasion, and migration. Thus, we hypothesized that midazolam affects ovarian cancer progression by blocking the activation of the ERK/JNK pathway.

This study still has shortcomings. *In vivo* and immunohistochemical experiments to confirm the function of midazolam in the progression of ovarian cancer are currently lacking. Moreover, the specific mechanism of midazolam for the ERK/JNK pathway in ovarian cancer needs further experimental verification.

SUMMARY

Midazolam plays an anticancer role in ovarian cancer by inhibiting cell proliferation, invasion, and migration, and promoting cell apoptosis. Its mechanism may be related to blocking the ERK/JNK pathway.

AVAILABILITY OF DATA AND MATERIALS

No datasets were generated or analyzed during the current study.

ABBREVIATIONS

CCK-8: Cell counting kit-8
 DAPI: 4,6-diamino-2-phenyl indole
 EdU: 5-ethynyl-2'-deoxyuridine
 ERK/JNK: Extracellular signal-regulated kinase/c-Jun N-terminal kinase
 FBS: Fetal bovine serum
 FCM: Flow cytometry
 GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
 IC₅₀: Half maximal inhibitory concentration

AUTHOR CONTRIBUTIONS

XKS, HFY, and YZ: Designed the study; BF: Collected and analyzed the data; XKS and HFY: Participated in drafting the manuscript. All authors conducted the study and contributed to critical revision of the manuscript for important intellectual content. All authors gave final approval of the version to be published. All authors participated fully in the work, took public responsibility for appropriate portions of the content, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or completeness of any part of the work were appropriately investigated and resolved. All authors meet ICMJE authorship requirements.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval and consent to participate is not required as this study did not involve human participants, animal subjects, or identifiable personal data. All experiments were conducted using commercially available cell lines, which do not require ethical approval or informed consent.

ACKNOWLEDGMENT

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

EDITORIAL/PEER REVIEW

To ensure the integrity and highest quality of CytoJournal publications, the review process of this manuscript was conducted under a **double-blind model** (authors are blinded for reviewers and vice versa) through an automatic online system.

REFERENCES

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, *et al.* Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2024;74:229-63.
- O'Shea AS. Clinical staging of ovarian cancer. *Methods Mol Biol* 2022;2424:3-10.
- Kai X, Zhang Q, Bao L. Fentanyl activates ovarian cancer and alleviates chemotherapy-induced toxicity via opioid receptor-dependent activation of EGFR. *BMC Anesthesiol* 2022;22:268.
- Yi L, Mao J, Xu Y, Pan H, Wang Y, Wei L. Ropivacaine represses the ovarian cancer cell stemness and facilitates cell ferroptosis through inactivating the PI3K/AKT signaling pathway. *Hum Exp Toxicol* 2022;41:9603271221120652.
- Sun M, Huang S, Gao Y. Lidocaine inhibits the proliferation and metastasis of epithelial ovarian cancer through the Wnt/ β -catenin pathway. *Transl Cancer Res* 2022;10:3479-90.
- Kim R. Effects of surgery and anesthetic choice on immunosuppression and cancer recurrence. *J Transl Med* 2018;16:8.
- Gamblin V, Berry V, Tresch-Bruneel E, Reich M, Da Silva A, Villet S, *et al.* Midazolam sedation in palliative medicine: Retrospective study in a French center for cancer control. *BMC Palliat Care* 2020;19:85.
- Balakrishnan S, Pearce RA. Midazolam and atropine alter theta oscillations in the hippocampal CA1 region by modulating both the somatic and distal dendritic dipoles. *Hippocampus* 2014;24:1212-31.
- Xiong H, Liu J, Liu G, Zhang Y, Wei Z, Fan L, *et al.* Effective doses of midazolam oral solution for the prevention of preoperative anxiety in paediatric patients. *Int J Paediatr Dent* 2024;34:621-9.
- Rania MA, Khaled MF, Shereen MK. Effect of combined epidural morphine and midazolam on postoperative pain in patients undergoing major abdominal cancer surgery. *Clin J Pain* 2022;38:693-9.
- Novac MB, Boldeanu L, Rotaru LT, Dijmărescu AL, Șerbănescu MS, Radu L, *et al.* The perioperative effect of anesthetic drugs on the immune response in total intravenous anesthesia in patients undergoing minimally invasive gynecological surgery. *Rom J Morphol Embryol* 2021;62:961-9.
- Guo CH, Bai L, Wu HH, Yang J, Cai GH, Zeng SX, *et al.* Midazolam and ropivacaine act synergistically to inhibit bone cancer pain with different mechanisms in rats. *Oncol Rep* 2016;37:249-58.
- Mishra SK, Kang JH, Lee CW, Oh SH, Ryu JS, Bae YS, *et al.* Midazolam induces cellular apoptosis in human cancer cells and inhibits tumor growth in xenograft mice. *Mol Cells* 2013;36:219-26.
- Shen Q, Xia Y, Yang L, Wang B, Peng J. Midazolam suppresses hepatocellular carcinoma cell metastasis and enhances apoptosis by elevating miR-217. *Comput Math Methods Med* 2022;2022:2813521.
- Ferrari J, De Tommasi O, Noventa M, Spagnol G, Facchetti E, Saccardi C, *et al.* Laparoscopic en-bloc pelvic resection for advanced ovarian cancer. *Gynecol Oncol Rep* 2024;53:101393.
- Zhang X, Moriwaki T, Kawabata T, Goto S, Liu KX, Guo CY, *et al.* Nicaraven attenuates postoperative systemic inflammatory responses-induced tumor metastasis. *Ann Surg Oncol* 2019;27:1068-74.
- Sethi A, Rezk A, Couban R, Chowdhury T. Role of midazolam on cancer progression/survival - an updated systematic review. *Indian J Anaesth* 2023;67:951-61.
- Vaishnavi BD, Goyal S, Sharma A, Kothari N, Kaloria N, Sethi P, *et al.* Comparison of intranasal dexmedetomidine-midazolam, dexmedetomidine-ketamine, and midazolam-ketamine for premedication in paediatric patients: A double-blinded randomized trial. *Anaesthesiol Intensive Ther* 2023;55:103-8.
- Sethupathy A, Gunasekaran V, Chelliah S, Pachamuthu M, Duraisamy S. Efficacy and safety of low dose midazolam and ketamine for sedation during invasive procedures in pediatric hemato-oncology. *Indian J Pediatr* 2024;91:639.
- Mercadante S, Grassi Y, Gebbia V. Cancer survivors: Long-term opioid therapy - the challenge. *BMJ Support Palliat Care* 2024;14:65-7.
- Oshima Y, Sano M, Kajiwara I, Ichimaru Y, Itaya T, Kuramochi T, *et al.* Midazolam exhibits antitumour and anti-inflammatory effects in a mouse model of pancreatic ductal adenocarcinoma. *Br J Anaesth* 2022;128:679-90.
- Kang J, Zheng Z, Li X, Huang T, Rong D, Liu X, *et al.* Midazolam exhibits antitumour and enhances the efficiency of anti-PD-1 immunotherapy in hepatocellular carcinoma. *Cancer Cell Int* 2022;22:312.
- Wang C, Dato T, Zhao H, Wu L, Date A, Jiang C, *et al.* Midazolam and dexmedetomidine affect neuroglioma and lung carcinoma cell biology *in vitro* and *in vivo*. *Anesthesiology* 2018;129:1000-14.
- Lu HL, Wu KC, Chen CW, Weng HK, Huang BM, Lin TY, *et al.* Anticancer effects of midazolam on lung and breast cancers by inhibiting cell proliferation and epithelial-mesenchymal transition. *Life (Basel)* 2021;11:1396.
- Li Y, Tan AP, Zhong YS. Anti-cancer effect of midazolam via downregulating YWHAH in papillary thyroid cancer cells. *Discov Oncol* 2025;16:72.
- Park HB, Baek KH. E3 ligases and deubiquitinating enzymes regulating the MAPK signaling pathway in cancers. *Biochim Biophys Acta Rev Cancer* 2022;1877:188736.
- Ullah R, Yin Q, Snell AH, Wan L. RAF-MEK-ERK pathway in cancer evolution and treatment. *Semin Cancer Biol* 2021;85:123-54.
- Lu H, Zheng G, Gao X, Chen C, Zhou M, Zhang L. Propofol suppresses cell viability, cell cycle progression and motility and induces cell apoptosis of ovarian cancer cells through suppressing MEK/ERK signaling via targeting circVPS13C/miR-145 axis. *J Ovarian Res* 2021;14:30.
- Zhang X, Han Z, Li Z, Wang T. Midazolam impedes lung

- carcinoma cell proliferation and migration via EGFR/MEK/ERK signaling pathway. *Open Med (Wars)* 2023;18:20230730.
30. Zhou W, Cai D. Midazolam suppresses ischemia/reperfusion-induced cardiomyocyte apoptosis by inhibiting the JNK/p38 MAPK signaling pathway. *Can J Physiol Pharmacol* 2022;100:117-24.

How to cite this article: Sun X, Yu H, Fu B, Zhang Y. Mechanistic insights into the effect of midazolam on the malignant progression of ovarian cancer. *CytoJournal*. 2025;22:91. doi: 10.25259/Cytojournal_25_2025

HTML of this article is available FREE at:
https://dx.doi.org/10.25259/Cytojournal_25_2025

The FIRST **Open Access** cytopathology journal
Publish in *CytoJournal* and **RETAIN** your *copyright* for your intellectual property
Become Cytopathology Foundation (CF) Member at nominal annual membership cost
For details visit <https://cytojournal.com/cf-member>

PubMed indexed
FREE world wide **open access**
Online processing with rapid turnaround time.
Real time dissemination of time-sensitive technology.
Publishes as many **colored high-resolution images**
Read it, cite it, bookmark it, use RSS feed, & many----

 **CYTOJOURNAL**
www.cytojournal.com
Peer-reviewed academic cytopathology journal

 **OPEN ACCESS**