Submitted: 29 January, 2024 Accepted: 29 February, 2024 Published: 15 June, 2024 DOI:10.22514/ejgo.2024.049

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

Taraxasterol inhibits cell proliferation and angiogenesis in ovarian cancer by targeting EphA2

Chunhui Wang[1,*], Suwei Lan[1], Bing Han[1], Xingcha Wang[1]

[1]Department of Gynecology, Affiliated Hospital of Chengde medical university, 067000 Chengde, Hebei, China

***Correspondence**
chunhui1233@163.com
(Chunhui Wang)

Abstract

Ovarian Cancer (OC) is a prevalent gynecological malignancy, underscoring the urgency to discover novel therapeutic approaches that enhance both clinical efficacy and safety. Taraxasterol (TA), an important component of dandelion, has been reported to exhibit inhibitory effects on the progression of various tumors. However, its specific role in OC is yet to be reported. In this present study, we observed that TA significantly inhibits the proliferation of OC cells, promotes apoptosis and reduces OC cell migration and angiogenesis. Mechanistically, TA targets the EPH receptor A2 (EphA2) receptor, thereby inhibiting OC progression. In conclusion, the ability of TA to suppress both cell proliferation and angiogenesis in OC through EphA2 targeting suggests its potential utility as a therapeutic agent for OC.

Keywords

Taraxasterol (TA); Ovarian cancer (OC); Apoptosis; Angiogenesis; EPH receptor A2 (EphA2)

1. Introduction

Ovarian cancer (OC) stands as a leading cause of morbidity and mortality among gynecological malignancies [1], with its mortality rate ranking highest among gynecological tumors, thereby representing a significant health risk for women worldwide [2]. The onset of ovarian cancer is often insidious, and most patients have no obvious symptoms in the early stages [3]. The insidious onset of OC often results in a lack of early-stage symptoms, leading to many patients being diagnosed at an advanced stage of the disease [3], which significantly impacts their prognosis, resulting in an overall five-year survival rate of approximately 40%, which further declines to 20–30% for those diagnosed with advanced-stage cancer [4]. Evidence suggests that OC patients adhering to treatment guidelines can benefit from improved five-year survival rates [5]. However, most patients with epithelial OC eventually experience relapse post-initial treatment, and currently, no curative treatments are available [5–7]. Furthermore, conventional treatment modalities, such as surgery and chemotherapy, have not significantly improved the survival rates of patients with this disease [8]. Therefore, the identification of novel early detection methods and the development of individualized treatment strategies are crucial for improving the clinical efficacy and safety outcomes for OC patients.

Taraxasterol (TA) is considered to be an important component of dandelion [9, 10]. It is a pentacyclic triterpenoid compound isolated from Taraxacum officinale and exhibits significant physiological and pharmacological activities, including anti-inflammatory, antioxidant and anticancer effects [11, 12]. For example, in rheumatoid arthritis, it has been demonstrated that TA can reduce inflammation by inhibiting cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8, thereby attenuating IL-1β-induced inflammatory responses [11, 12]. Additionally, TA has been shown to alleviate liver damage through modulation of the Toll-like receptors (TLR)/nuclear factor kappa-B (NF-κB) and Bax/Bcl-2 pathways [13]. In the context of oncology, TA has played a pivotal role in inhibiting tumor progression [14, 15]. It has been found to suppress the RNF31/p53 axis in colorectal cancer through RNF31 targeting, leading to reduced tumor cell proliferation [16]. In liver cancer research, TA inhibited tumor growth both *in vivo* and *in vitro* by enhancing Hint1 expression [17]. Moreover, TA has been effective in countering Transforming growth factor beta 1 (TGF-β1)-induced epithelial-mesenchymal transition (EMT) in thyroid papillary cancer cells through the regulation of the Wnt/β-catenin pathway, thus impeding tumor migration [15]. An important aspect of TA's mechanism involves targeting the EphA2 protein, a receptor tyrosine kinase within the Eph family, known for its role in tumor progression through ligand binding and activation of downstream signaling pathways [15]. Despite these findings, the specific impact and underlying mechanisms of TA on OC remain unexplored, presenting a gap in current scientific knowledge.

This study aims to elucidate the effects of TA in OC. Our research demonstrates that TA can inhibit OC cell proliferation, migration and angiogenesis and could induce apoptosis by specifically targeting the EphA2 receptor. Based on these findings, TA could be considered a promising candidate for

improving OC treatment.

2. Materials and methods

2.1 Cell culture

Human SK-OC-3 and SW626 cells were purchased from the Chinese Academy of Sciences. SK-OC-3 cells were cultured in McCoy's 5A (Gbico, USA) complete medium, while SW626 cells were cultured in DMEM (Gbico, USA) complete medium. Both cell lines were treated with TA (Sigma) for 24 h at the concentration of 0, 5, 10 and 20 μM.

2.2 Western blotting

OC cells were lysed using RIPA lysis buffer (P0013K, Beyotime, Shanghai, China) to extract proteins, which were then quantified using Bicinchoninic Acid (BCA) assay (Beyotime, China). The proteins were separated using 8% Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and transferred to Polyvinylidene fluoride (PVDF) membranes for 2 h. The membranes were blocked with tris buffered saline tween (TBST) containing 5% milk for 1 h before incubation with primary antibodies: EphA2 (ab273118; 1:500, Abcam), Bax (ab32503; 1:1000), Bcl-2 (ab32124; 1:500), Cleaved caspase-3 (ab32042; 1:1000), and β-actin (ab8226; 1:3000), followed by secondary antibodies for 1 h. Protein band detection was performed using chemiluminescence. All reagents were purchased from Wuhan Google Co., LTD. The blots were quantified using the ImageJ software.

2.3 Cell viability assays

For the cell counting kit-8 (CCK-8) assays, cells were seeded into 96-well plates and incubated at 37 °C. The CCK-8 reagent was added, and the cells were incubated at 37 °C for 4 hours. Relative cell viability was measured using a spectrophotometer at a wavelength of 450 nm (imark, Bio-Rad, CA, USA) for 3 d.

In the colony formation assay, the cells were plated in 6-well plates and cultured in media for 10 days at 37 °C. Then, the cells were fixed with paraformaldehyde (PFA) for 20 minutes and stained with 0.2% crystal violet for 10 minutes before being photographed.

2.4 Transwell-migration or invasion assays

In the migration and invasion assays, BD Falcon cell culture inserts were used as the upper chambers and 24-well plates were used as the lower chambers. For invasion assays, the inserts were pre-coated with 100 μL of Matrigel, diluted 1:3 with serum-free medium, while Matrigel coating was not used for the migration assays. Approximately 1×10^5 OC cells were seeded into each upper chamber. After the migration or invasion experiment period, the cells on the underside of the inserts were fixed with 4% paraformaldehyde, stained with 0.2% crystal violet, and photographed for analysis.

2.5 Cell apoptosis assay

A cell apoptosis kit (C1088, Beyotime, Beijing, China) was used for this experiment. Briefly, the OC cells were initially washed with PBS and then fixed with 70% ethanol at −20 °C for 2 h. Following fixation, the cells were stained with propidium iodide (PI) and Fluorescein Isothiocyanate (FITC)-conjugated Annexin V at 4 °C, and the apoptosis levels were quantified on a FACSCalibur flow cytometer and analyzed with CellQuest Pro software version 5.1 (BD Biosciences, Inc., Franklin Lake, NJ, USA).

2.6 Tube formation assay

OC cells were plated onto 24-well plates pre-coated with 50% matrigel. To examine tube formation, photographs were taken 4 h later using a fluorescence microscope (LSM710, Carl Zeiss Inc, Oberkochen, Germany).

2.7 Statistical analysis

Statistical assessments were performed using the GraphPad v5.0 software (Graphpad, La Jolla, CA, USA). One-way Analysis of Variance (ANOVA) was conducted for between-group comparisons using the Tukey *post hoc* test. Each experiment was repeated thrice, and the data are represented as mean $\pm$ SD. $p < 0.05$ was used to indicate statistical significance.

3. Results

3.1 TA inhibited the proliferation of OC cells

To confirm the effects of TA on the growth of OC, two types of OC cell lines, SK-OC-3 and SW626 cells, were treated with TA at the concentrations of 0, 5, 10 and 20 μM for 24 h. CCK-8 assay results revealed that TA treatment suppressed the viability of SK-OC-3 and SW626 cells at both low and high TA concentrations, as evidenced by decreased optical density at 450 nm (OD450) values (Fig. 1A). Furthermore, colony formation assays demonstrated a reduction in the number of colonies for both cell types following TA treatment, indicating a suppressive effect on OC cell growth (Fig. 1B). Collectively, these results indicate that TA effectively inhibits the proliferation of OC cells.

3.2 TA promoted OC cell apoptosis

Next, the influence of TA on the apoptosis of OC cells was investigated. Flow cytometry assays revealed that TA treatment at concentrations of 5, 10 and 20 μM significantly induced apoptosis in both SK-OC-3 and SW626 cells, as indicated by an increased percentage of apoptotic cells (Fig. 2A). Immunoblotting assays were conducted to assess the impact of TA on the expression levels of key apoptosis-related proteins. The results showed that TA treatment upregulated the expression of Bax and cleaved caspase-3 while downregulating Bcl-2 expression in both SK-OC-3 and SW626 cells (Fig. 2B). These findings suggest that TA effectively promotes apoptosis in OC cells.

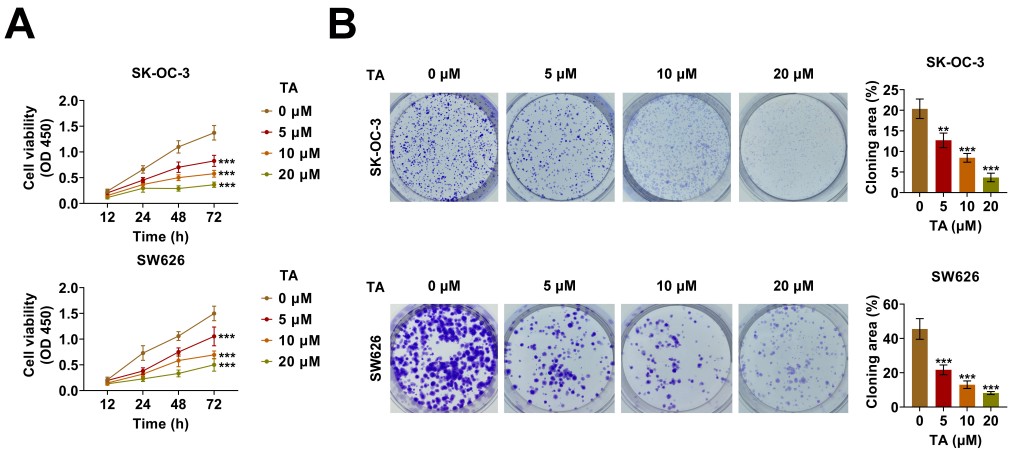

FIGURE 1. TA inhibited the proliferation of OC cells. (A) CCK-8 assays demonstrate TA's impact on SK-OC-3 and SW626 cell viability at concentrations of 0, 5, 10 and 20 μM over 24 hours, as indicated by OD450 values. (B) Colony formation assays reveal TA's influence on the proliferative capacity of SK-OC-3 and SW626 cells at the same concentrations. Colonies were counted per field. Experiments were performed in triplicate. Data are presented as mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$. TA: Taraxasterol; OD: optical density.

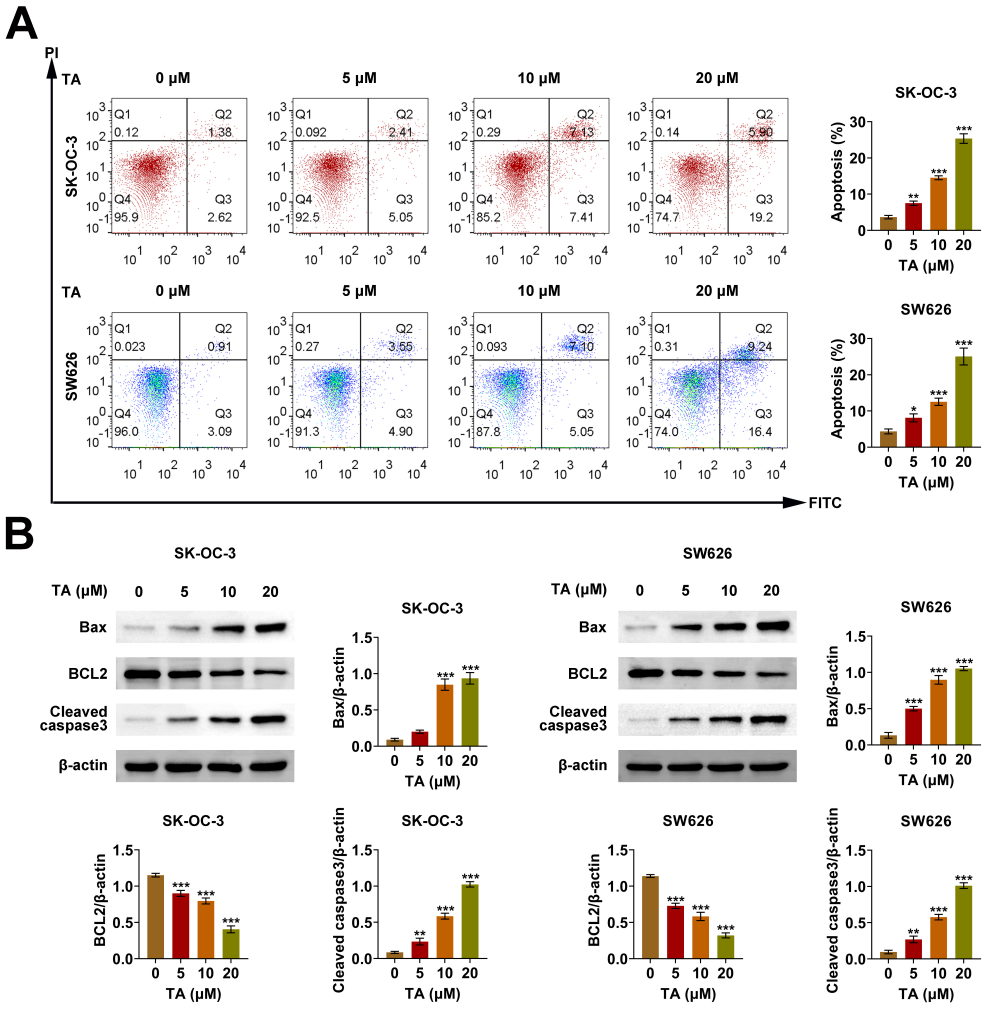

FIGURE 2. TA promoted OC cell apoptosis. (A) FCM assays showing the effects of TA on cell apoptosis at 0, 5, 10 and 20 μM concentrations for 24 h in SK-OC-3 and SW626 cells. The percentage of apoptosis cells was calculated. (B) Immunoblotting reveals changes in the expression levels of apoptosis-related proteins Bax, Bcl-2 and cleaved caspase-3 in SK-OC-3 and SW626 cells treated with varying concentrations of TA. Experiments were replicated three times. Data were represented as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. TA: Taraxasterol; FITC: Fluorescein Isothiocyanate.

3.3 TA inhibited OC cell migration and angiogenesis

Here, we assessed the impact of TA on the motility of OC cells. Transwell assays demonstrated that TA treatment significantly inhibited the migration and invasion of both SK-OC-3 and SW626 cells, evidenced by a reduced number of cells migrating (Fig. 3A, upper panel) or invading (Fig. 3A, lower panel). Additionally, tube formation assays were conducted after treating SK-OC-3 and SW626 cells with TA for 24 hours at concentrations of 0, 5, 10 and 20 μM, and the results indicated that TA treatment effectively reduced angiogenesis in both cell lines, as shown by a decrease in the number of branch points (Fig. 3B). Thus, TA was found to impede OC cell migration and angiogenesis.

3.4 TA could target EphA2 in OC cells

Subsequent analyses assessed the impact of Taraxasterol (TA) on EphA2 expression in ovarian cancer (OC) cells, given EphA2's role in OC progression. Immunoblot assays revealed that TA treatment led to a reduction in EphA2 expression levels in both SK-OC-3 and SW626 cells (Fig. 4). Thus, TA's ability to target EphA2 in OC cells contributed to the suppression of OC progression.

4. Discussion

OC ranks as the third most common malignancy of the female reproductive system, following cervical and endometrial cancers, comprising approximately 4% of all female malignancies [8]. Despite its comparatively lower incidence, OC is the leading cause of death among patients with gynecological cancers, significantly impacting women's health [18]. Current treatments, including surgery and chemoradiotherapy, show limited effectiveness against advanced ovarian cancer stages, underscoring the urgent need for more effective therapeutic options [18]. Herein, our findings suggest that TA inhibits OC through various pathways, highlighting its potential as a novel therapeutic agent.

Our comprehensive *in vitro* analyses revealed that TA, a pentacyclic triterpenoid derived from Taraxacum mongolicum, effectively hampers the progression of OC by inhibiting cell growth, motility, and angiogenesis [11, 12]. Recognized for its metabolite and anti-inflammatory properties, TA has previously demonstrated significant therapeutic potential in modulating inflammatory responses in lipopolysaccharide-stimulated RAW 264.7 macrophages and preventing concanavalin A-induced acute hepatic injury in mice through the regulation of TLRs/NF-κB and Bax/Bcl-2 pathways [19, 20]. Beyond its anti-inflammatory effects, TA's anticancer efficacy across various types, including breast, lung and liver cancers, has been documented [15–17]. It can inhibit the proliferation of liver cancer cells and also enhance the immunomodulatory responses and immune reactions against tumors [15]. TA can promote apoptosis in tumor cells *via* the mitochondrial pathway, triggering biochemical reactions such as mitochondrial membrane potential loss, cytochrome c release, caspase-3 activation, and Bcl-2 downregulation [14, 17]. This study also aims to explore TA's impact on OC cells' mitochondrial function. Moreover, TA's ability to suppress TPC-1 cell migration and invasion by modulating EMT-related genes and reducing Matrix Metallopeptidase-2 (MMP-2) and MMP-9 expression is noteworthy [15]. Furthermore, in human colon cancer

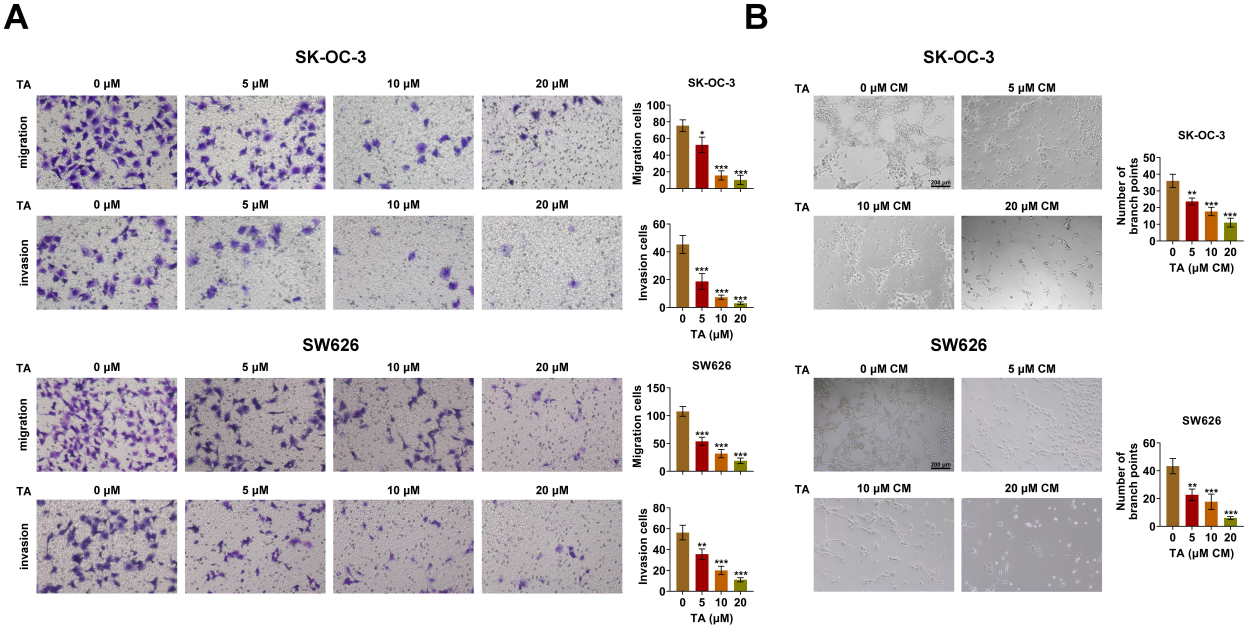

FIGURE 3. TA inhibited OC cell migration as well as angiogenesis. (A) Transwell assays showing the effects of TA on the cell motility at the concentration of 0, 5, 10 and 20 μM for 24 h in SK-OC-3 and SW626 cells. The invasive cell number was counted. (B) Tube formation assays illustrate TA's inhibition of angiogenesis in SK-OC-3 and SW626 cells at similar concentrations, assessed by counting branch points. Experiments are performed in triplicate. Data are presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. TA: Taraxasterol.

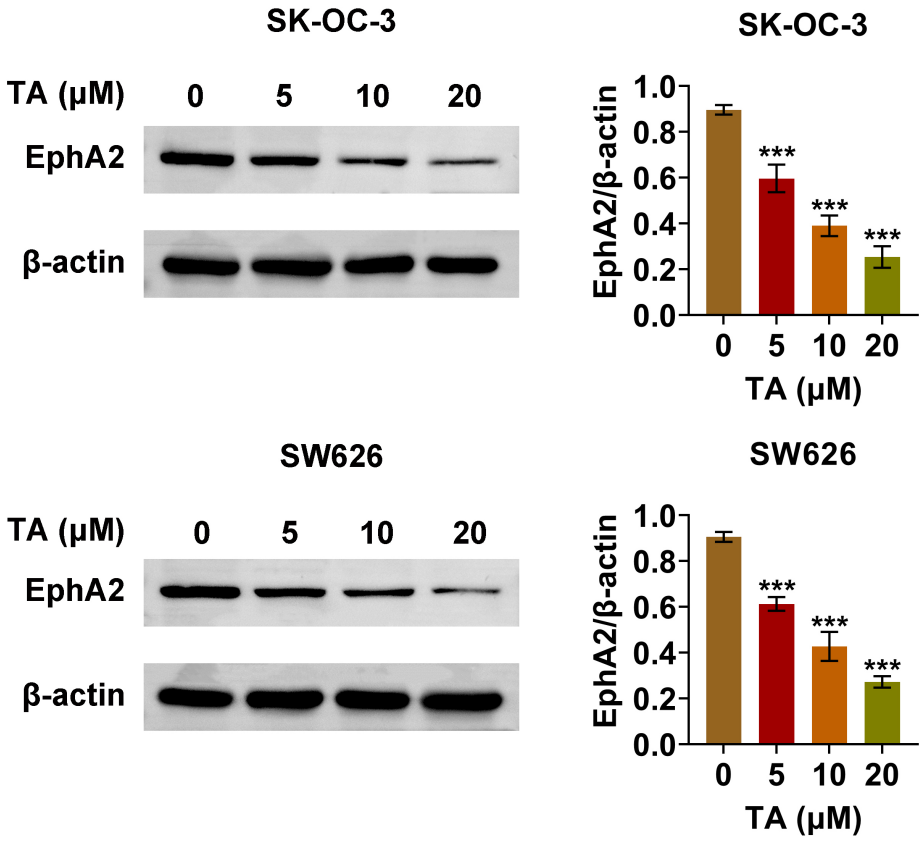

FIGURE 4. TA targets EphA2 in OC cells. Immunoblot analysis demonstrates the downregulation of EphA2 expression in SK-OC-3 and SW626 cells after 24 h of TA treatment at concentrations of 0, 5, 10 and 20 μM. Experiments are replicated three times. Data are shown as mean $\pm$ SD. ***$p < 0.001$. TA: Taraxasterol.

HCT116 and SW480 cells, TA inhibits RNF31 and induces autophagy by facilitating the ubiquitination and degradation of the p53 protein [16], highlighting its multifaceted therapeutic potential against OC.

Angiogenesis is an important process in tumor growth and metastasis, as it provides nutrients for tumor cells to accelerate their growth [21]. This complex process encompasses the proliferation, mobility and differentiation of endothelial cells into capillary structures [21, 22]. Consequently, targeting angiogenesis presents a viable strategy for curtailing tumor expansion and dissemination. Correspondingly, our findings showed that TA can impede OC progression by inhibiting angiogenesis, substantiating its potential as a therapeutic agent in obstructing the angiogenic pathways critical for OC advancement.

EphA2, a receptor tyrosine kinase in the ephrin family, also known as epithelial cell kinase (Eck), plays a pivotal role in neuronal migration during development and is generally not expressed in most normal adult tissues [23]. Aberrant expression of EphA2 has been implicated in the progression of OC, with studies indicating that its overexpression contributes to increased microvascular density (MVD) and, consequently, tumor metastasis [24, 25]. Based on these findings, we suggest that TA can exert anticancer effects by targeting EphA2. Our results validate this hypothesis, demonstrating that TA modulates EphA2 expression, which in turn influences OC progression, identifying EphA2 as a potential therapeutic target for TA. Although the precise underlying mechanisms remain to be elucidated, future research could focus on assessing TA's impact on OC tumor growth in mouse models to elucidate the underlying mechanism through comprehensive multi-omics analyses and additional experimental approaches.

5. Conclusions

In summary, our findings reveal that TA could significantly inhibit OC cell proliferation, migration and angiogenesis and enhance apoptosis at 5, 10 and 20 μM concentrations by targeting EphA2. These results suggest TA's therapeutic potential for OC patients. Future studies should explore TA's mechanisms and its clinical applicability for OC treatment.

AVAILABILITY OF DATA AND MATERIALS

The authors declare that all data supporting the findings of this study are available within the paper and any raw data can be obtained from the corresponding author upon request.

AUTHOR CONTRIBUTIONS

CHW—designed the study and carried them out; prepared the manuscript for publication and reviewed the draft of the manuscript; CHW, SWL, BH and XCW—supervised the data collection, CHW, SWL—analyzed the data; BH and XCW—

interpreted the data. All authors have read and approved the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

ACKNOWLEDGMENT

Not applicable.

FUNDING

This work was supported by Chengde City science and technology plan project (Grant No. 202109A073).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1] Dong L, Wang H, Gao Y, Wang S, Wang W. Long non-coding RNA PVT1 promotes the proliferation, migration and EMT process of ovarian cancer cells by regulating CTGF. Oncology Letters. 2023; 25: 71.

[2] Pecorino B, Lagana AS, Chiantera V, Ferrara M, Di Stefano AB, Di Donna MC, *et al.* Progression free survival, overall survival, and relapse rate in endometrioid ovarian cancer and synchronous endometrial-ovarian endometrioid cancer (SEO-EC): results from a large retrospective analysis. Medicina. 2022; 58: 1706.

[3] Wempe SL, Gamarra-Luques CD, Telleria CM. Synergistic lethality of mifepristone and LY294002 in ovarian cancer cells. Cancer Growth and Metastasis. 2013; 6: 1–13.

[4] Li C, Liu VWS, Chan DW, Yao KM, Ngan HYS. LY294002 and metformin cooperatively enhance the inhibition of growth and the induction of apoptosis of ovarian cancer cells. International Journal of Gynecologic Cancer. 2012; 22: 15–22.

[5] Burnell M, Gentry-Maharaj A, Glazer C, Karpinskyj C, Ryan A, Apostolidou S, *et al.* Serial endometrial thickness and risk of non-endometrial hormone-dependent cancers in postmenopausal women in UK collaborative trial of ovarian cancer screening. Ultrasound in Obstetrics & Gynecology. 2020; 56: 267–275.

[6] Li J, Yan W, Ren F, Sang H. Tectorigenin inhibits inflammation in keratinocytes by inhibition of NLRP3 inflammasome regulated by the TLR4/NF-κB pathway. Allergologia et Immunopathologia. 2023; 51: 82–89.

[7] Liu L, Huan L, Zhang Y, Wei W, Chen Z, Xu D, *et al.* Ubiquitin-specific protease 8 inhibits lipopolysaccharide-triggered pyroptosis of human bronchial epithelial cells by regulating PI3K/AKT and NF-κB pathways. Allergologia et Immunopathologia. 2022; 50: 96–103.

[8] Ghoneum A, Gonzalez D, Afify H, Shu J, Hegarty A, Adisa J, *et al.* Compound C inhibits ovarian cancer progression *via* PI3K-AKT-mTOR-NFκB pathway. Cancers. 2022; 14: 5099.

[9] Li M, He Y, Zhang W, Yin Y, Jiang Q, Loor JJ, *et al*. Taraxasterol alleviates fatty acid-induced lipid deposition in calf hepatocytes by decreasing ROS production and endoplasmic reticulum stress. Journal of Animal Science. 2023; 101: skad015.

[10] Sang R, Ge B, Li H, Zhou H, Yan K, Wang W, *et al*. Taraxasterol alleviates aflatoxin B_1-induced liver damage in broiler chickens *via* regulation of oxidative stress, apoptosis and autophagy. Ecotoxicology and Environmental Safety. 2023; 251: 114546.

[11] Jiao F, Tan Z, Yu Z, Zhou B, Meng L, Shi X. The phytochemical and pharmacological profile of taraxasterol. Frontiers in Pharmacology. 2022; 13: 927365.

[12] Ge B, Sang R, Wang W, Yan K, Yu Y, Kong L, *et al*. Protection of taraxasterol against acetaminophen-induced liver injury elucidated through network pharmacology and *in vitro* and *in vivo* experiments. Phytomedicine. 2023; 116: 154872.

[13] Li Z, Lian Y, Wei R, Jin L, Cao H, Zhao T, *et al.* Effects of taraxasterol against ethanol and high-fat diet-induced liver injury by regulating TLR4/MyD88/NF-κB and Nrf2/HO-1 signaling pathways. Life Sciences. 2020; 262: 118546.

[14] Zhao Y, Zhang L, Guo M, Yang H. Taraxasterol suppresses cell proliferation and boosts cell apoptosis *via* inhibiting GPD2-mediated glycolysis in gastric cancer. Cytotechnology. 2021; 73: 815–825.

[15] Zhu J, Li X, Zhang S, Liu J, Yao X, Zhao Q, *et al.* Taraxasterol inhibits TGF-β1-induced epithelial-to-mesenchymal transition in papillary thyroid cancer cells through regulating the Wnt/β-catenin signaling. Human & Experimental Toxicology. 2021; 40: S87–S95.

[16] Tang CT, Yang J, Liu ZD, Chen Y, Zeng C. Taraxasterol acetate targets RNF31 to inhibit RNF31/p53 axis-driven cell proliferation in colorectal cancer. Cell Death Discovery. 2021; 7: 66.

[17] Bao T, Ke Y, Wang Y, Wang W, Li Y, Wang Y, *et al*. Taraxasterol suppresses the growth of human liver cancer by upregulating Hint1 expression. Journal of Molecular Medicine. 2018; 96: 661–672.

[18] Sun Q, Suo Y, Lv H, Wang Q, Yin H. Porphin e6 complex loaded with gold nanorod mesoporous silica enhances photodynamic therapy in ovarian cancer cells *in vitro*. Lasers in Medical Science. 2023; 38: 115.

[19] Zhang X, Xiong H, Liu L. Effects of taraxasterol on inflammatory responses in lipopolysaccharide-induced RAW 264.7 macrophages. Journal of Ethnopharmacology. 2012; 141: 206–211.

[20] Sang R, Yu Y, Ge B, Xu L, Wang Z, Zhang X. Taraxasterol from *Taraxacum* prevents concanavalin A-induced acute hepatic injury in mice *via* modulating TLRs/NF-κB and Bax/Bc1-2 signalling pathways. Artificial Cells, Nanomedicine, and Biotechnology. 2019; 47: 3929–3937.

[21] Alhasan L, Addai Z. Allicin-induced modulation of angiogenesis in lung cancer cells (A549). Tropical Journal of Pharmaceutical Research. 2018; 17: 2129–2134.

[22] Luan Y, Luan Y, Feng Q, Chen X, Ren K, Yang Y. Emerging role of mitophagy in the heart: therapeutic potentials to modulate mitophagy in cardiac diseases. Oxidative Medicine and Cellular Longevity. 2021; 2021: 3259963.

[23] Wang Y, Gong J, Ding X, Luo S. CircRTTN upregulates EPHA2 to aggravate the malignant process of melanoma *via* sponging miR-890. Histology & Histopathology. 2024; 39: 211–224.

[24] Lin YG, Han LY, Kamat AA, Merritt WM, Landen CN, Deavers MT, *et al*. EphA2 overexpression is associated with angiogenesis in ovarian cancer. Cancer. 2007; 109: 332–340.

[25] Lu C, Shahzad MMK, Wang H, Landen CN, Kim SW, Allen J, *et al*. EphA2 overexpression promotes ovarian cancer growth. Cancer Biology & Therapy. 2008; 7: 1098–1103.

How to cite this article: Chunhui Wang, Suwei Lan, Bing Han, Xingcha Wang. Taraxasterol inhibits cell proliferation and angiogenesis in ovarian cancer by targeting EphA2. European Journal of Gynaecological Oncology. 2024; 45(3): 62-67. doi: 10.22514/ejgo.2024.049.