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Research Article

Propranolol Induces Apoptosis in Epithelial Ovarian Cancer Cells by Inhibiting the AKT Signaling Pathway

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Abstract:

Objective: This study aimed to investigate whether propranolol affects ovarian cancer (OV) cell viability and apoptosis through inhibition of the AKT signaling pathway.

Methods: SKOV3 human epithelial ovarian cancer cells were treated with 0, 25, 50, 100, 200, and 400 μM propranolol hydrochloride for 0, 24, 48, and 72 hours. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8). After treating SKOV3 cells with 0, 50, 100, and 200 μM propranolol for 24 hours, quantitative real-time PCR (qRT-PCR) was used to measure mRNA levels of BCL-2, BAX, and AKT. Western blotting was performed to detect caspase-3 and AKT protein expression. Additionally, SKOV3 cells were co-treated with propranolol and either the AKT activator N-oleoyl glycine or the AKT inhibitor AZD5363 for 24 hours. AKT protein expression and cell proliferation were evaluated by Western blot and CCK-8 assay, respectively.

Results: Propranolol significantly inhibited SKOV3 cell proliferation in a concentration- and time-dependent manner ($P < 0.001$) and induced apoptosis, as evidenced by upregulation of BAX mRNA and caspase-3 protein, and downregulation of BCL-2 mRNA and AKT mRNA/protein ($P < 0.01$). The AKT inhibitor synergistically enhanced propranolol-induced suppression of cell viability and AKT protein expression ($P < 0.001$), whereas the AKT activator significantly reversed the inhibitory effects of propranolol on AKT and cell viability ($P < 0.001$).

Conclusions: Propranolol-induced apoptosis of OV is mediated by inhibiting the AKT signaling pathway.

Keywords: Apoptosis, AKT signaling pathway, Ovarian cancer, Propranolol, SKOV3

1. Introduction

Ovarian cancer (OV) is one of the most common gynecologic malignancies [1], with 324,398 newly diagnosed ovarian cancer cases and 206,839 deaths due to ovarian cancer worldwide in 2022 [2]. Despite the use of increasingly more chemotherapeutic drugs, the overall 5-year survival rate is still less than 30–40% [3]. The high mortality is largely attributed to late diagnosis, frequent recurrence, and the development of chemoresistance [4]. Therefore, it is urgently needed to develop novel therapeutic strategies to improve efficacy and prolong patient survival.

Propranolol is a non-selective β-blocker widely used in the treatment of cardiovascular diseases such as hypertension, chronic heart failure, and arrhythmia. Interestingly, since Léauté-Labrèze et al. first reported the successful application of propranolol in infantile hemangioma in 2008 [5], accumulating evidence has revealed its anti-tumor potential in various cancers. Studies have indicated that propranolol can suppress the occurrence, progression, and metastasis of breast cancer and melanoma [6,7]. Preclinical research

further suggests that propranolol exerts anti-tumor effects through multiple mechanisms, including inhibition of the MAPK and AKT signaling pathways, suppression of angiogenesis, and modulation of the tumor microenvironment [8].

However, the role of propranolol in ovarian cancer and its underlying mechanisms remain incompletely understood. Given the critical involvement of AKT signaling in ovarian cancer cell proliferation, apoptosis, and chemoresistance, we hypothesized that propranolol may inhibit OV progression by targeting the AKT pathway. Therefore, this study aims to investigate the therapeutic effect of propranolol on ovarian cancer cells and to elucidate whether its action is mediated through regulation of the AKT signaling pathway, thereby providing a potential novel adjuvant strategy for the treatment of ovarian cancer.

Methods and Materials

2.1 Cell Culture and Proliferation

The human OV cell SKVO-3 obtained from Xiangya Medical College of South Chian University was cultured in RPMI 1640 medium (containing 100 U/mL penicillin, 100 μg/mL streptomycin) containing 10% fetal bovine serum in an incubator (37 °C, 5% CO2). Cells (1 x 105 cells/mL) in the logarithmic growth phase were placed in a 96-well plate (200 μL/well) and allowed to adhere. The cells were treated with propranolol (final concentration of 25, 50, 100, 200, and 400 μM) and cultured for 0, 24, 48, and 72 h. Five replicates were set up for each concentration with normal untreated cells used as control and the same volume of RPMI 1640 medium used as the blank. CCK-8 reagent (10 μl/well) was added to the wells 2 h before the end of the experiment. The absorbance (A) was measured at 450 nm on a microplate reader and the cell proliferation was calculated as (A experiment-A blank)/(A control-A blank).

2.2 Reagents and Antibodies

Propranolol was obtained from Zhuzhou Central Hospital (Bei Jing, China). AZD5363 (CAS No: 1143532-39-1) and N-Oleoyl glycine (CAS No: 2601-90-3) were purchased from MedChem Express. CCK-8 was obtained from Beijing Zhijie Fangyuan Technology Co., Ltd., and Trizol reagent was purchased from Beijing Ovia Biological Company. Takara Reverse Transcription Kit (Code No. RR047A) was purchased from Dalian Bao Biotakara-Baori Biotech (Beijing). AKT antibody, caspase-3 antibody, and β-actin antibody were purchased from Cell Signaling Technology, USA. BCA protein concentration determination kit was obtained from Beijing Soleibao Technology Co., Ltd.

2.3 Apoptosis Assay

The SKOV-3 cells were seeded onto sterile cover glasses in a six-well plate and cultured overnight to 50–80% confluence. The cells were treated with 0, 50, and 100μM propranolol for 24 h. The culture medium was aspirated before adding fixative (0.5 mL) for 10 min. The fixative was removed and the cells were washed twice with PBS or 0.9% NaCl, 3 min each time. All the liquid was aspirated before adding 0.5 mL Hoechst 33258 dye for 5 min, then washed twice with PBS, 3 min each time. The slides were mounted and covered with a coverslip for observation under a fluorescence microscope.

2.4 Quantitative Real-time PCR (Q-PCR) to Quantify BCL-2, BAX, and AKT mRNA Expression

Total RNA was extracted by the Trizol method, then reverse transcribed into cDNA using the Takara Reverse Transcription Kit according to the manufacturer's instructions. The DNA was applied according to the Q-PCR reaction system using the primers shown in Table 1. The fold change in the expression of BCL-2, BAX, and AKT in the experimental group compared to the control group was calculated using the 2-△Ct method.

Table 1, Q-PCR primer information

Gene name	Primer sequence (5'- 3')
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
BCL-2-F	GTCTTCGCTGCGGAGATCAT

BCL-2-R	CATTCCGATATACGCTGGGAC
BAX-F	CATATAACCCCGTCAACGCAG
BAX-R	GCAGCCGCCACAAACATAC

2.5 Western Blotting to Detect Caspase-3 and AKT Protein Expression

SKOV-3 cells were treated with 25, 50, 100, and 200 μM propranolol and phosphate buffer saline (PBS) (blank control), as well as the AKT inhibitor AZD5363 and AKT activator N-Oleoyl glycine for 24 h. The cells were collected and lysed with total protein lysis working solution (mmol/L Tris buffer, 150 mmol/L sodium chloride, 5 mmol/L E3edd DTA, 1% by volume Triton X-100, 1% by volume sodium deoxycholate, 30 mmol/L disodium hydrogen phosphate, 50 mmol/L fluorinated Sodium, and 1 mmol/L sodium vanadate, 1×protease inhibitor). The proteins were quantified using the BCA method and separated by 10% SDS-PAGE before transfer to nitrocellulose membranes (Millipore). The membranes were blocked for 1 h at room temperature before the incubation with rabbit anti-human caspase-3 and AKT antibodies at a dilution of 1:1000 at 4℃ overnight. β-actin was used as the internal control. The membranes were washed three times with TBST (Tris-HCl and polysorbate buffer), and the horseradish-labeled rabbit anti-goat IgG diluted 1:1000 was added, incubated at room temperature for 2 h, and the membrane was washed three times. The protein nads were visualized using ECL luminescent liquid.

2.6 Statistical Analysis

SPSS 19.0 statistical software was used for data analysis and data were expressed as mean ± SD. The comparison between two groups was performed by t-test, between multiple groups by one-way analysis of variance, and the LSD t-test performed a pairwise comparison between groups.

3. Results

3.1 Propranolol inhibits SKOV3 cell proliferation in a concentration- and time-dependent manner To investigate the effect of propranolol on cell viability, SKOV3 cells were treated with various concentrations (0–400μM) of propranolol for 24, 48, and 72 hours. CCK-8 assays revealed that propranolol significantly reduced the viability of SKOV3 cells in a concentration- and time-dependent manner (Figure 1A, $P < 0.001$). Specifically, the cell viability in the 50μM group was significantly higher than that in the 100μM group at all time points. No significant differences were observed among the 0, 25, and 50μM groups, or among the 100, 200, and 400μM groups. In addition, Hoechst 33258 staining confirmed a significant increase in apoptotic cells following treatment with 100μM and 200μM propranolol for 24 hours compared to the control and lower concentration groups (0, 25, and 50μM) (Figure 1B, $P < 0.001$).

Apoptotic cells are indicated by red arrows in Figure 1B.

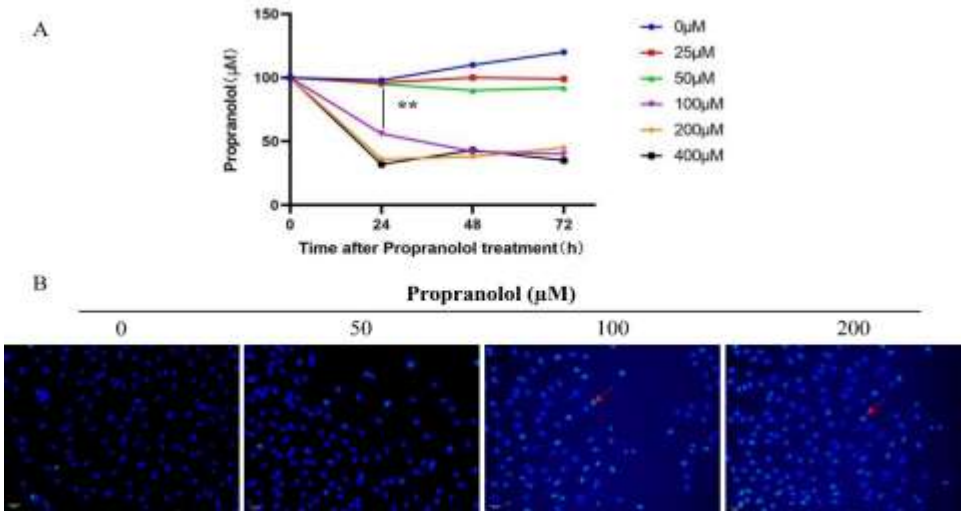

Figure 1 Propranolol inhibits viability and induces apoptosis in SKOV-3 cells

(A) Cell viability was assessed by CCK-8 assay after treatment with the indicated concentrations of propranolol for 24, 48, and 72 h.
(B) Apoptotic cells were detected by Hoechst 33258 staining following 24 h propranolol treatment. Arrows indicate representative apoptotic cells.

3.2 Propranolol Modulates the Expression of Apoptosis-Related Genes and Proteins

To explore the pro-apoptotic effect of propranolol, SKOV-3 cells were treated with increasing concentrations (0–200μM) of propranolol for 24 hours. qRT-PCR analysis showed that propranolol significantly inhibited the mRNA expression of the anti-apoptotic gene Bcl-2 (0μM vs 100μM, 50μM vs 100μM, and 50μM vs 200μM, $P < 0.01$; Figure 2A) and promoted the mRNA expression of the pro-apoptotic gene Bax (0μM vs 100μM, 50μM vs 100μM, and 50μM vs 200μM, $P < 0.01$; Figure 2B). Consistent with these findings, Western blot analysis demonstrated that propranolol treatment significantly increased the protein expression of cleaved caspase-3 (50μM vs 100μM, $P < 0.05$; 50μM vs 200μM, $P < 0.01$; Figure 2C).

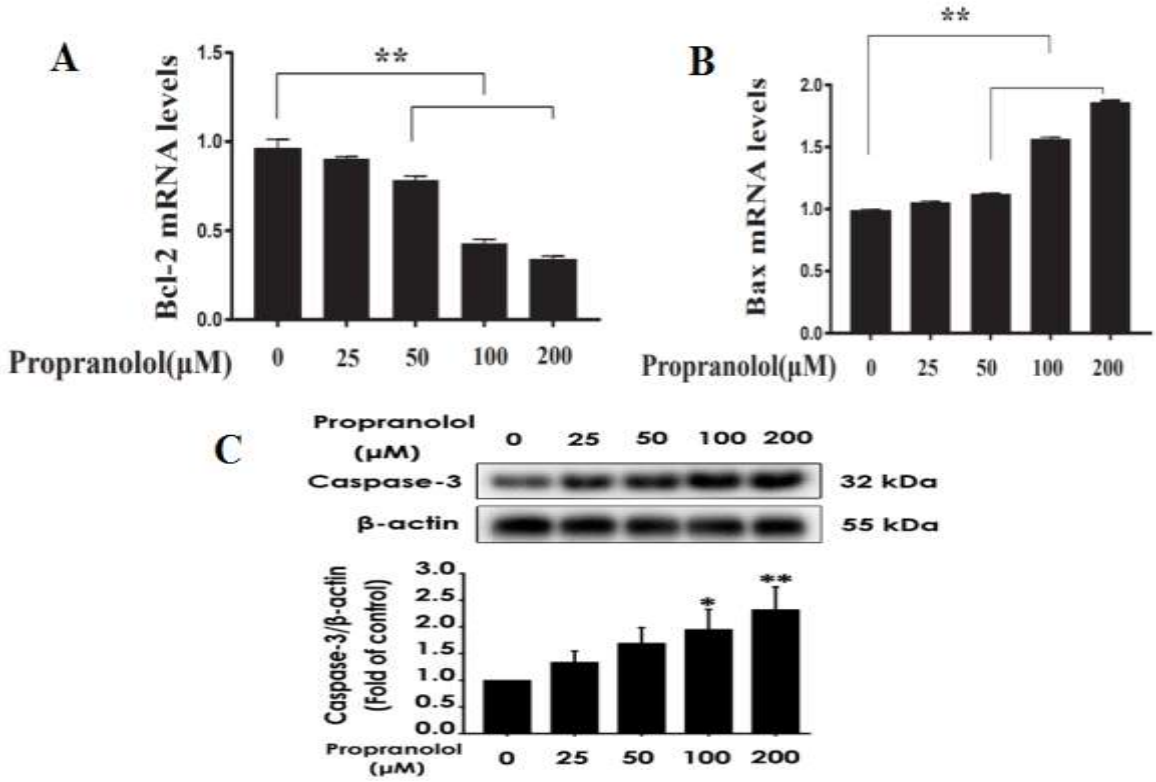

Figure 2. Propranolol modulates the expression of apoptosis-related markers in SKOV-3 cells. Cells were treated with the indicated concentrations of propranolol for 24 h.
(A, B) mRNA expression levels of Bcl-2 and Bax were determined by qRT-PCR.
(C) Protein expression of cleaved caspase-3 was analyzed by Western blot. β-actin served as a loading control.

3.3 Propranolol Suppresses AKT Expression at Both mRNA and Protein Levels

We further examined whether the AKT signaling pathway is involved in propranolol-induced effects. As shown in Figure 3, propranolol treatment for 24 hours resulted in a significant inhibition of AKT protein expression (0μM vs 50μM, $P = 0.071$; 50μM vs 100μM, $P < 0.05$; 50μM vs 200μM, $P < 0.01$; Figure 3A). Similarly, the mRNA expression of AKT was significantly suppressed (50μM vs 100μM and 50μM vs 200μM, $P < 0.001$; Figure 3B).

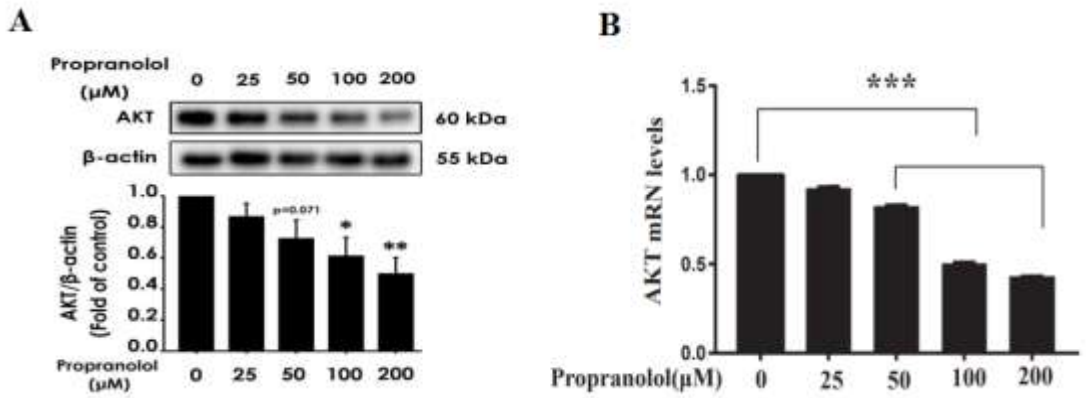

Figure 3. Propranolol suppresses AKT expression in SKOV-3 cells.
Cells were treated with the indicated concentrations of propranolol for 24 h.
(A) AKT protein expression was analyzed by Western blot.
(B) AKT mRNA levels were determined by qRT-PCR.
β-actin and GAPDH served as loading controls for Western blot and qRT-PCR, respectively.

3.4 Pharmacological Modulation of AKT Signaling Alters Propranolol's Anti-Tumor Effects

To determine whether propranolol exerts its anti-tumor effects through the AKT pathway, SKOV-3 cells were co-treated with propranolol and either the AKT inhibitor AZD5363 (0.5μM) or the AKT activator N-Oleoyl glycine (15μM) for 24 hours. As demonstrated in Figure 4, the combination of propranolol (50μM or 100μM) with AZD5363 synergistically enhanced the inhibition of AKT protein expression and further reduced cell viability ($P < 0.001$; Figures 4A and 4B). In contrast, the addition of N-Oleoyl glycine significantly attenuated the propranolol-induced suppression of AKT expression and cell viability at the 100μM propranolol concentration ($P < 0.001$; Figures 4A and 4C). However, no significant difference in cell viability was observed when 50μM propranolol was combined with N-Oleoyl glycine compared to 50μM propranolol alone.

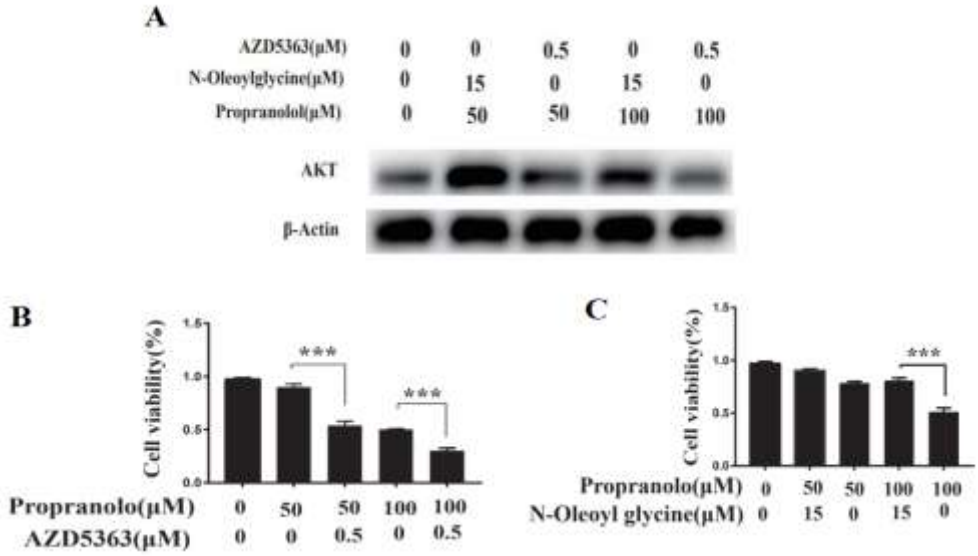

Figure 4. Pharmacological modulation of AKT signaling alters propranolol's effects on SKOV-3 cells.
Cells were treated with propranolol alone or in combination with the AKT inhibitor AZD5363 (0.5μM) or AKT activator N-Oleoyl glycine (15μM) for 24 h.
(A) AKT protein expression was analyzed by Western blot.
(B, C) Cell viability was assessed by CCK-8 assay.
β-actin served as a loading control.

4. Discussion

Propranolol has been depicted to have a wide range of anti-tumor effects, such as inhibiting the proliferation and promoting the apoptosis of hemangioma, melanoma, breast cancer, and liver cancer cells [7-9]. In our study, propranolol inhibited the proliferation and promoted apoptosis of ovarian cancer cells through the AKT signaling pathway. Bcl-2 and Bax genes are the regulatory factors associated with the inhibition and promotion of apoptosis in the Bcl-2 protein family, respectively [10-11]. The death receptor often activates the caspase-3 mediated apoptotic pathway [12]. In the present study, propranolol significantly decreased Bcl-2 mRNA and significantly increased Bax, inducing caspase-3 expression thereby inhibiting the proliferation and promoting the apoptosis of SKVO-3 cells. Zhou et al. [10] displayed that melanoma cells treated with 100 μM propranolol for 24 h had significantly reduced viability, while Liao et al. [11] showed that propranolol could significantly inhibit the proliferation of gastric cancer cells. Zhao S et al. [12] also reported that propranolol significantly reduced the viability of human ovarian cancer cell lines SKOV-3 and A2780 in a dose and time-dependent manner. These reports indicate that propranolol can inhibit tumor cell proliferation and promote apoptosis. Our results are consistent with these reports, confirming that propranolol can inhibit the proliferation and promote apoptosis of human epithelial ovarian cancer cells.

AKT can mediate various biological functions such as cell proliferation, survival, glucose mutability, and inhibition of apoptosis in response to different growth factors and extracellular stimuli [13–15]. Propranolol reduced the expression of AKT mRNA and protein expression in SKVO-3 cells and the addition of the AKT inhibitor A2D5363 significantly reduced SKOV-3 viability. Treatment with the AKT activator N-Oleoyl glycine increased AKT expression, decreasing the inhibition of SKOV-3 cell viability. These results confirmed that the propranolol-induced apoptosis of ovarian cancer is mediated by inhibiting the AKT signaling pathway. Targeting AKT1 with siRNA displayed a substantial decrease in cell proliferation, migration, and invasion in OVCAR-3 OC cells [16]. Sun B et al. [17] also showed that propranolol inhibited cell proliferation and induced cell apoptosis via the AKT pathway in HemECs. However, Zhou et al. [10] reported that propranolol inhibited melanoma in vitro and in vivo by suppressing the AKT and MAPK pathways. Zhao S et al. [12] discovered that propranolol regulated apoptosis and autophagy through the ROS/JNK signal pathway. Ramondetta et al. [18] used propranolol during the primary treatment of ovarian cancer, showing that serum IL-6, IL-8, and IL-10 were decreased, and combined with chemotherapy, propranolol improved the QOL over baseline in OC. Several preclinical models have been developed to understand the potential role of the PI3K/AKT/mTOR signaling pathway in OC. Di Fonte R, et al. [19] found propranolol enhanced trabectedin efficacy, further inducing apoptosis through the involvement of mitochondria, Erk1/2 activation, and the increase of inducible COX-2. Liang et al. [20] showed that propranolol inhibited cell proliferation and promoted apoptosis via the AKT signaling pathway. According to the above literature reports, we know that the AKT signaling pathway affects the proliferation and apoptosis of various tumor cells. In our results, it was shown that propranolol inhibits the proliferation of SKOV3 cells and promotes apoptosis through the AKT signaling pathway.

In this study, our data revealed that propranolol inhibited proliferation and promoted apoptosis of SKOV-3 via the AKT signaling pathway. However, this has only been confirmed in SKOV3 cells, and we should validate it in animal models. Importantly, in clinical trials are needed to confirm that the treatment effect of propranolol in ovarian cancer patients as adjuvant therapy.

Authors' Contribution

Xian Zhu and Mei Qin participated in the design, performed statistical analyses, and drafted the manuscript. Xi He and Yin Tao complete cells experiment and helped to draft the manuscript and performed statistical analyses. Xian Zhu and Mei Qin conceived and design the study, revise the manuscript. All authors read and approved the final manuscript.

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