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Bicalutamide-Mediated Inhibition of Androgen Receptor Signaling and Its Effect on the Invasion and Migration of Ovarian Cancer Cells

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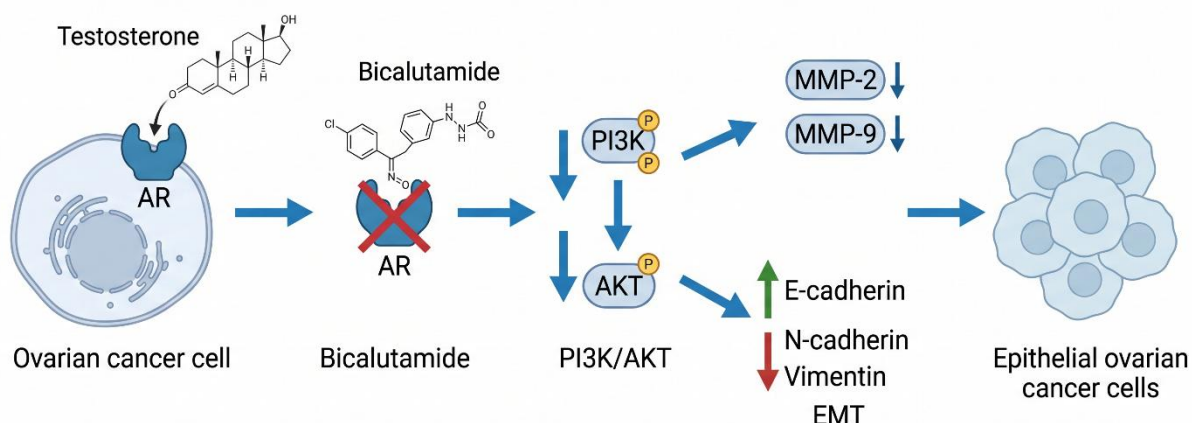
ABSTRACT

Ovarian cancer remains one of the deadliest gynecological malignancies, largely because it is often detected at advanced stages when metastatic spread has already occurred. The androgen receptor (AR), which is expressed in a substantial proportion of epithelial ovarian carcinomas, has been implicated in promoting tumor cell proliferation, migration, and invasion. Bicalutamide, a well-established nonsteroidal antiandrogen primarily used in the management of prostate cancer, competitively blocks AR-mediated transcriptional activity. However, its potential role in suppressing ovarian cancer cell metastatic behavior has not been thoroughly explored. In this study, we investigated the effects of bicalutamide on the invasion and migration of SKOV-3 and OVCAR-3 ovarian cancer cell lines. Cells were treated with varying concentrations of bicalutamide (10, 25, and 50 μ M) for 24 and 48 hours. Cell viability was assessed using the MTT assay, while migration and invasion were evaluated by wound healing and Matrigel-coated Transwell assays, respectively. Western blot analysis was carried out to determine the expression levels of AR, MMP-2, MMP-9, E-cadherin, N-cadherin, and vimentin. Our findings indicate that bicalutamide significantly reduced cell migration and invasion in a dose- and time-dependent manner. These effects were accompanied by downregulation of MMP-2, MMP-9, N-cadherin, and vimentin, alongside upregulation of E-cadherin, suggesting suppression of epithelial-mesenchymal transition (EMT). Furthermore, bicalutamide attenuated PI3K/AKT phosphorylation downstream of AR signaling. These data collectively suggest that bicalutamide may represent a promising adjunctive therapeutic approach against AR-positive ovarian cancers by limiting their metastatic potential.

Highlights

- Bicalutamide dose-dependently inhibits the migratory and invasive capabilities of SKOV-3 and OVCAR-3 ovarian cancer cells.
- The antiandrogen suppresses EMT markers including N-cadherin and vimentin while restoring E-cadherin expression.
- MMP-2 and MMP-9 protein levels are significantly reduced following bicalutamide treatment.
- Bicalutamide attenuates PI3K/AKT pathway activation downstream of the androgen receptor.
- These results suggest a potential therapeutic relevance for AR-targeting strategies in epithelial ovarian cancer.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Ovarian cancer stands as one of the most lethal malignancies affecting women globally, with approximately 313,959 new cases and 207,252 deaths reported in 2020 according to GLOBOCAN estimates [1]. High mortality rates for this disease are mostly due to the asymptomatic early stages of the disease, with about 70% of patients diagnosed at an advanced stage of disease when it has spread beyond the ovary [2]. Despite the fact that cytoreductive surgery and platinum- and taxane-based chemotherapy are still the standard of care, most patients relapse and acquire chemoresistance, thereby generating an urgent and pressing need for innovative therapeutic methods [3].

Recently, increasing data has focused on the role of steroid hormone receptors, especially the androgen receptor (AR) in ovarian cancer aetiology. AR is a member of the nuclear receptor super family and acts as a ligand-dependent transcription factor by binding androgens like testosterone and dihydrotestosterone (DHT) [4]. Immunohistochemistry studies have shown that AR is expressed in roughly 43.5–86% of epithelial ovarian carcinomas, and some biochemical experiments have indicated this may be as high as approximately 90% [5,6]. It has been demonstrated that the AR signalling promotes ovarian cancer cell proliferation through crosstalk with many pathways including TGF- β , IL-6/IL-8, EGFR and PI3K/AKT cascade [7,8]. Furthermore, androgen-induced AR activation has been demonstrated to promote cell motility and invasion in vitro, indicating a direct contribution to the metastatic phenotype [9,10].

Bicalutamide is a second-generation nonsteroidal antiandrogen that competitively inhibits the binding of androgens to the ligand-binding domain of the AR. It was designed for treatment of prostate cancer and was approved by FDA in 1995 [11,12]. The medication inhibits nuclear translocation and subsequent transcriptional activity of AR, therefore inhibiting androgen-driven gene expression programs [13]. Anti-androgen approaches have been investigated in a few clinical trials in

ovarian cancer, including a phase II trial of goserelin and bicalutamide [14] and a promising case report of AR-targeted therapy in recurrent high-grade serous ovarian cancer [15]. Yet, the specific molecular mechanisms through which bicalutamide can affect the invasiveness and migratory properties of ovarian cancer cells are not yet fully understood. Therefore, the present investigation was aiming to analyse the effect of bicalutamide on the invasion and migration of ovarian cancer cells and to elucidate the underlying signalling pathways, including EMT markers and the PI3K/AKT pathway.

2. MATERIALS AND METHODS

2.1. Cell Lines and Culture Conditions

Human ovarian cancer cell lines SKOV-3 and OVCAR-3 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). SKOV-3 cells were cultured in McCoy's 5A modified medium, and OVCAR-3 cells were cultured in RPMI-1640 media. Both media were supplemented with 10% foetal bovine serum (FBS; Gibco, Grand Island, NY, USA), 100 U/mL penicillin and 100 μ g/mL streptomycin. The cells were cultivated under conventional conditions, 37 °C in a humidified environment of 5% CO₂. Cells were subcultured at roughly 80–85% confluency and used for assays within passages 5–20. The two cell lines were selected due to their well established variations in levels of AR expression and invasive potential with SKOV-3 widely considered to be a more aggressive line than OVCAR-3 [9,16].

2.2. Drug Preparation and Treatment

Bicalutamide (purity $\geq$ 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO) to prepare a 100 mM stock solution, which was stored at –20 °C. Working concentrations of 10, 25, and 50 μ M were freshly prepared by dilution in the appropriate culture medium immediately before use. The final DMSO concentration in all treatment groups did not exceed 0.1% (v/v), and an equivalent volume of DMSO was added to the control wells as vehicle control. Cells were treated with bicalutamide or vehicle for 24 or 48 hours depending on the specific assay being conducted.

Dihydrotestosterone (DHT; 10 nM) was co-administered with bicalutamide in selected experiments to assess the ability of bicalutamide to counteract androgen-stimulated effects [12,17].

2.3. Cell Viability Assay (MTT)

Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded at a density of 5×10^3 cells per well in 96-well plates and allowed to adhere overnight. Following incubation with bicalutamide at the stated doses for 24 or 48 h, 20 μ L of MTT solution (5 mg/mL in PBS) was added to each well and the plates incubated further at 37 °C for 4 h. One hundred and fifty microlitres of DMSO was added to dissolve the formazan crystals and absorbance was measured at 570 nm on a microplate reader (Bio-Tek Instruments, Winooski, VT, USA). Cell viability was indicated as % compared to vehicle-treated control group. All experiments were done in duplicate and repeated independently at least 3 times.

2.4. Wound Healing (Scratch) Assay

Migration potential of ovarian cancer cells was tested by wound healing assay. Cells were plated in 6-well plates and cultivated to near confluency (~90%). A sterile 200 μ L pipette tip was used to create a uniform scratch across the cell monolayer. The detached cells were gently washed away with phosphate-buffered saline (PBS), and the medium was replaced with serum-reduced medium (2% FBS) containing either bicalutamide (10, 25, or 50 μ M) or vehicle control. Images of the wound area were captured at 0, 24, and 48 h using an inverted phase-contrast microscope (Olympus, Tokyo, Japan) at $\times 40$ magnification. The wound closure rate was quantified using ImageJ software (NIH, Bethesda, MD, USA) and calculated as follows: wound closure (%) = [(wound area at 0 h – wound area at time t) / wound area at 0 h] $\times 100$ [18].

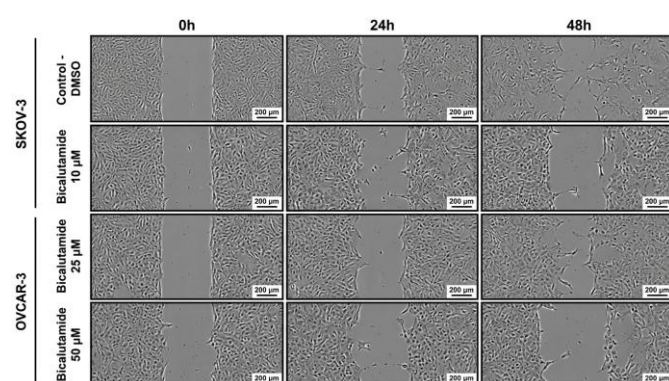


Figure 1. Representative images of wound healing assay showing migration of SKOV-3 and OVCAR-3 cells treated with bicalutamide (10, 25, 50 μ M) or vehicle control at 0, 24, and 48 h. Scale bar = 200 μ m.

2.5. Transwell Invasion Assay

Cell invasion was assessed using 24-well Transwell chambers (8 μ m pore size; Corning Costar, Cambridge, MA, USA) with inserts pre-coated with 50 μ L of Matrigel (1:3 dilution in serum-free medium; BD Biosciences, Franklin Lakes, NJ, USA). A total of 1×10^5 cells were resuspended in 200 μ L of serum-free medium containing the specified concentration of bicalutamide and seeded into the upper chamber. The lower chamber was filled with 600 μ L of complete medium containing 20% FBS as a chemoattractant. Following incubation for 24 h at 37 °C, non-invading cells on the upper surface of the filter were carefully removed with cotton swabs. The cells that had invaded through the Matrigel-coated membrane were fixed with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet for 20 min. Invaded cells were counted in five randomly selected fields under a light microscope at $\times 200$ magnification [16,19].

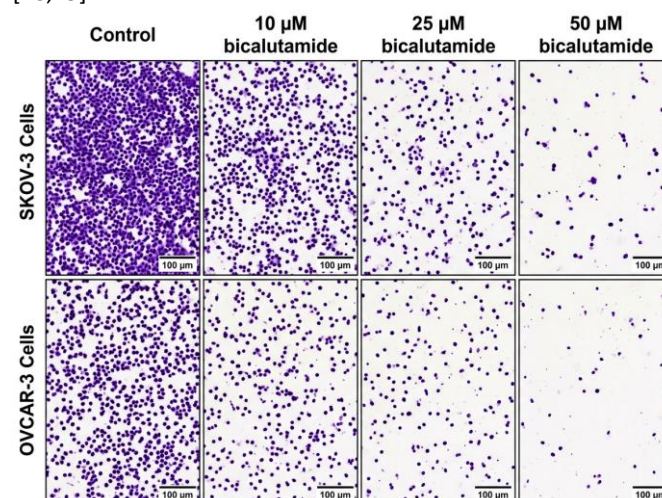


Figure 2A. Representative photomicrographs of crystal violet-stained invaded cells in Transwell assay for SKOV-3 and OVCAR-3 cells treated with bicalutamide (10, 25, 50 μ M) or vehicle control.

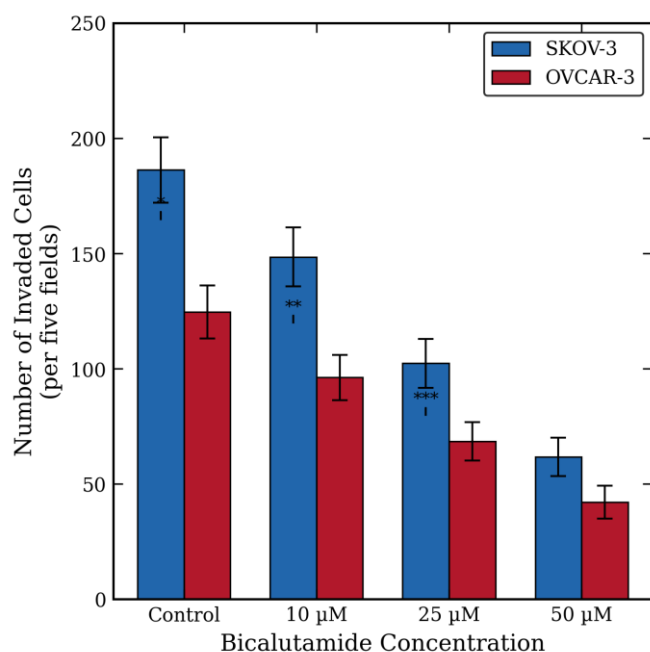


Figure 2B. Quantitative analysis of invaded cell numbers. Data are presented as mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ vs. control.

2.6. Western Blot Analysis

Following the designated treatments, cells were harvested and lysed in RIPA buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease and phosphatase inhibitor cocktails (Roche, Basel, Switzerland). Total protein concentrations were quantified using the Bradford assay. Equal amounts of protein (30 μ g per lane) were separated by 10% SDS-PAGE and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Burlington, MA, USA). Membranes were blocked with 5% non-fat milk in TBST for 1 h at room temperature and then incubated overnight at 4 °C with primary antibodies against the following targets: AR (1:1000), MMP-2 (1:1000), MMP-9 (1:1000), E-cadherin (1:1000), N-cadherin (1:1000), vimentin (1:1000), PI3K (1:1000), p-PI3K (1:1000), AKT (1:1000), p-AKT (Ser473) (1:1000), and β -actin (1:5000) as loading control. All primary antibodies were purchased from Cell Signalling Technology (Danvers, MA, USA), unless otherwise indicated. The membranes were washed three times with TBST, then incubated with the appropriate HRP-conjugated secondary antibodies (1:5000) for 1 h at room temperature. Protein bands were visualised with the enhanced chemiluminescence (ECL) detection method and densitometric quantification was performed using ImageJ software. [20].

2.7. Statistical Analysis

All quantitative results are expressed as mean $\pm$ standard deviation (SD) of three independent experiments ($n = 3$), each performed in triplicate. Prior to parametric testing, the normality of data distribution was verified using the Shapiro–Wilk test, and homogeneity of variances was confirmed by Levene’s test; all datasets met the assumptions for parametric analysis. Student’s t-test was used for statistical comparisons between two groups. One-way analysis of variance (ANOVA)

followed by Tukey’s post hoc test was employed for multiple group comparisons ($n = 3$ per group). A P -value < 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism software (version 9.0; GraphPad Software, San Diego, CA, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of Bicalutamide on Cell Viability

It was important to determine the range of concentrations that did not cause significant cytotoxicity before assessing the anti-migratory and anti-invasive effects of bicalutamide so that any observed effects on migration and invasion would be primarily attributed to the drug’s effects on motility pathways rather than cell death. The MTT experiment showed that bicalutamide at 10 and 25 μ M exerted rather minor effects on the viability of both SKOV-3 and OVCAR-3 cells following a 24 h treatment, with cell viability staying above 85% in both lines. A more substantial drop was found at 50 μ M but cell viability was still over 70% after 24 h. A dose-dependent drop in viability was seen following 48 h of treatment, with the 50 μ M concentration reducing viability to about 62–68% in the two cell lines. These data suggest that bicalutamide does have some growth inhibitory effects particularly at higher concentrations and longer incubation times, but that the concentrations used for the migration and invasion assays (10–50 μ M) fall within a range that allows the majority of cells to remain viable, so that motility-related endpoints can be meaningfully assessed.

Table 1. Cell viability (% of control) of SKOV-3 and OVCAR-3 cells treated with bicalutamide at different concentrations and time points (mean $\pm$ SD, $n = 3$).

Concentration	SKOV-3 24 h	SKOV-3 48 h	OVCAR-3 24 h	OVCAR-3 48 h
Control (0.1% DMSO)	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
10 μ M	92.40 $\pm$ 3.20	84.60 $\pm$ 4.10	94.10 $\pm$ 2.80	87.30 $\pm$ 3.50
25 μ M	86.70 $\pm$ 3.80*	76.20 $\pm$ 3.60**	88.30 $\pm$ 3.40*	78.80 $\pm$ 4.20**
50 μ M	73.50 $\pm$ 4.50**	62.10 $\pm$ 5.30***	76.20 $\pm$ 4.10**	67.80 $\pm$ 4.80***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

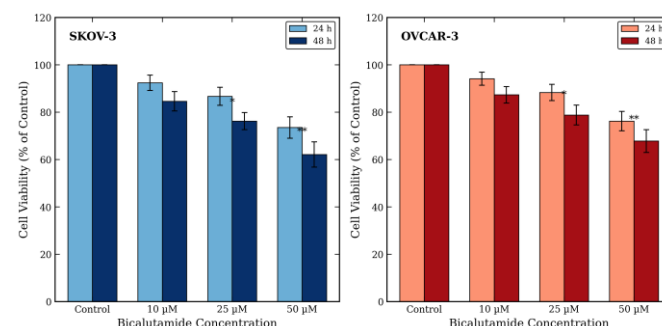


Figure S1. Cell viability (MTT assay) of SKOV-3 and OVCAR-3 cells treated with bicalutamide at different concentrations for 24 and 48 h. Data are mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ vs. control.

3.2. Bicalutamide Suppresses Migration of Ovarian Cancer Cells

The influence of bicalutamide on migratory potential of SKOV-3 and OVCAR-3 cells was measured by the wound healing assay. Both cell lines in the control group displayed significant wound closure by 24 h. The SKOV-3 cells migrated at a significantly faster rate than the OVCAR-3 cells, corresponding with the reported greater migratory phenotype of the SKOV-3 line [16]. Bicalutamide treatment led to a dose-dependent decrease of wound closure in both cell lines. At 10 μ M, the inhibitory impact was low but apparent, with wound closure reduced by about 15–20 % compared to controls at 24 h. The migration was more remarkably inhibited at 25 μ M. The closure of the wound was inhibited by around 45–55% at 50 μ M compared with the vehicle group at the same time point.

The changes were considerably more striking at the 48 h time point. The control cells had nearly or completely closed the wound gap, whereas bicalutamide-treated cells displayed dose-dependent gaps that remained largely unfilled, especially at 25 and 50 μ M. Interestingly, when cells were co-treated with 10 nM DHT and bicalutamide, the antiandrogen substantially abolished the DHT-induced enhancement of migration, providing evidence that the anti-migratory effect of bicalutamide is at least partly mediated through AR antagonism. These observations align with previous reports demonstrating that androgen/AR signaling promotes ovarian cancer cell migration. For instance, Ligr et al. (2011) proved that synthetic androgen could accelerate the invasion of OVCAR-3 and SKOV-3 cells in Matrigel invasion experiments [9], while Zhu et al. (2016) found that AR overexpression could promote the proliferation and migration of OVCAR-3 and SKOV-3 cells [10].

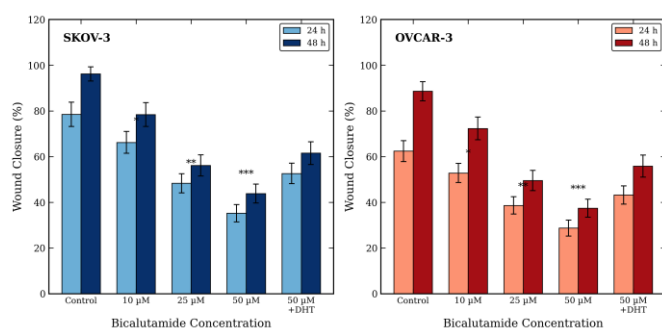


Figure 3. Quantitative analysis of wound closure rates (%) in SKOV-3 and OVCAR-3 cells treated with vehicle control, bicalutamide alone (10, 25, 50 μ M), or bicalutamide (50 μ M) + DHT (10 nM) at 24 and 48 h. Data are mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

3.3. Bicalutamide Inhibits Invasion of Ovarian Cancer Cells

Having established that bicalutamide might impair cell migration, we next examined whether the drug also affected the invasive capacity of ovarian cancer cells using the Matrigel-coated Transwell assay, which more closely resembles the process of tumour cell penetration through extracellular matrix barriers. In the absence of therapy, the number of invaded cells

was much larger in SKOV-3 cells than in OVCAR-3 cells, which is consistent with the reported higher invasive potential of SKOV-3 [16,21]. Treatment with bicalutamide led to significant and dose-dependent reduction in the number of invading cells in both cell lines. At 10 μ M the reduction was about 20–25 % in both lines relative to the control. The 25 μ M dose was around 40–50% inhibition while the 50 μ M dose was the most suppressive, reducing invading cell counts to approximately 60–70% of the untreated control.

These findings are particularly important since the metastatic spread of ovarian cancer, which occurs mainly by peritoneal dissemination rather than through the blood, depends on the ability of cancer cells to breakdown and traverse the extracellular matrix and mesothelial layer of the peritoneal lining. The ability of bicalutamide to inhibit this Matrigel-invasive behaviour implies that antagonism of AR could be a potential method to interfere with this important step of ovarian cancer progression. These results are consistent with the general evidence reviewed by Kossai et al. (2017) and Chung et al. (2021) implicating AR signalling in ovarian cancer invasion, and expand on the earlier observations by Du et al. (2016) that moderately aggressive mouse ovarian cancer cells overexpressing AR demonstrated enhanced invasive phenotypes [22]. [7,8].

Table 2. Number of invaded cells (per five fields) in the Transwell assay for SKOV-3 and OVCAR-3 cells following bicalutamide treatment (mean $\pm$ SD, n = 3).

Concentration	SKOV-3	OVCAR-3
Control (0.1% DMSO)	186.3 $\pm$ 14.2	124.7 $\pm$ 11.5
10 μ M	148.5 $\pm$ 12.8*	96.2 $\pm$ 9.8*
25 μ M	102.4 $\pm$ 10.6**	68.5 $\pm$ 8.4**
50 μ M	61.7 $\pm$ 8.3***	42.1 $\pm$ 7.2***

*P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

3.4. Bicalutamide Downregulates MMP-2 and MMP-9 Expression

Matrix metalloproteinases, particularly MMP-2 (gelatinase A) and MMP-9 (gelatinase B), play central roles in tumor invasion by degrading components of the basement membrane and extracellular matrix [23]. To determine whether the anti-invasive effects of bicalutamide were connected with alterations in MMP expression, we conducted western blotting analysis of cell lysates from cells treated with bicalutamide for 48 h. Bicalutamide therapy lowered the protein levels of MMP-2 and MMP-9 in SKOV-3 and OVCAR-3 cells in a dose-dependent manner. This reduction was more appreciable at 50 μ M than the control group, where the expression of MMP-2 and MMP-9 was decreased by about 55–65% and 50–60%, respectively. These results offer a mechanistic explanation for the decreased Matrigel invasion since the capacity of cells to breakdown reconstituted basement membrane matrix is directly related to the activity of these gelatinases. Previously, androgens have been associated with modulation of MMP expression in several cancer types. The PI3K/AKT and NF- κ B pathways are recognised

as crucial upstream regulators of MMP-2 and MMP-9 transcription in ovarian cancer [24,25]. The ability of bicalutamide to lower the levels of these proteases via its inhibition of AR signalling reveals an indirect but mechanistically plausible relationship between AR antagonism and impairment of extracellular matrix breakdown.

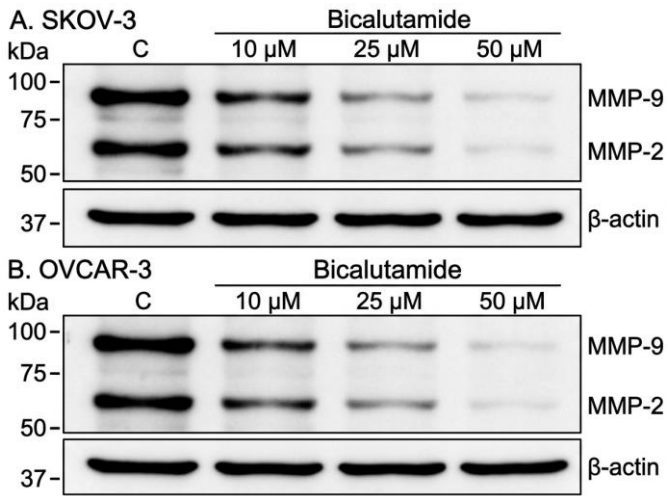


Figure 4A. Representative western blot images of MMP-2 and MMP-9 protein expression in SKOV-3 and OVCAR-3 cells following 48 h treatment with bicalutamide (10, 25, 50 μ M). C = Control.

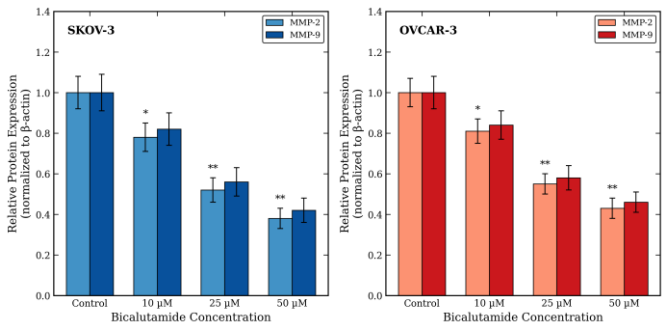


Figure 4B. Densitometric quantification of MMP-2 and MMP-9 expression normalized to β -actin. Data are mean $\pm$ SD (n = 3). *P < 0.05, **P < 0.01 vs. control.

3.5. Bicalutamide Reverses Epithelial-Mesenchymal Transition (EMT) Markers

Epithelial-mesenchymal transition (EMT) is a well-known biological process in which epithelial cells acquire mesenchymal features such as improved motility, invasiveness and apoptosis resistance. EMT is reported to be crucial in the development and spread of ovarian cancer [26,27]. Since androgen/AR signalling has been demonstrated to trigger EMT in prostate cancer cells [28] and the influence of bicalutamide on migration and invasion of ovarian cancer cells, we investigated if bicalutamide could modulate the expression of key EMT markers.

Western blot analysis showed dose-dependent effects of bicalutamide therapy on EMT marker expression. In particular, we found a considerable upregulation of the epithelial marker

E-cadherin, whose downregulation is a hallmark of EMT, after bicalutamide therapy, with the highest rise at 50 μ M. On the other hand, mesenchymal markers N-cadherin and vimentin were significantly downregulated in a dose-dependent manner. These shifts in the E-cadherin/N-cadherin balance, together with the decrease in vimentin, strongly suggest that bicalutamide promotes a reversal of the mesenchymal phenotype toward a more epithelial state, thereby limiting the capacity of ovarian cancer cells for migration and invasion. To our knowledge, this is among the earliest demonstrations that AR blockade by bicalutamide can reverse EMT-associated protein expression changes in ovarian cancer cells. In prostate cancer, Zhu and Kyprianou (2010) showed that androgens could induce EMT via Snail activation, and that low AR content was associated with the EMT phenotype [28]. In the ovarian cancer setting, the relationship between AR and EMT has been less explored, but the present findings suggest that a similar cross-talk may exist and that pharmacological AR inhibition can interfere with it.

Table 3. Relative protein expression levels of EMT markers (E-cadherin, N-cadherin, vimentin) in SKOV-3 and OVCAR-3 cells after 48 h bicalutamide treatment, normalized to β -actin (mean $\pm$ SD, n = 3).

Marker	Cell Line	Control	10 μ M	25 μ M	50 μ M
E-cadherin	SKOV-3	1.00 $\pm$ 0.08	1.28 $\pm$ 0.11*	1.64 $\pm$ 0.14**	2.18 $\pm$ 0.17***
E-cadherin	OVCAR-3	1.00 $\pm$ 0.07	1.22 $\pm$ 0.10*	1.53 $\pm$ 0.12**	1.96 $\pm$ 0.15***
N-cadherin	SKOV-3	1.00 $\pm$ 0.09	0.78 $\pm$ 0.08*	0.52 $\pm$ 0.07**	0.31 $\pm$ 0.06***
N-cadherin	OVCAR-3	1.00 $\pm$ 0.08	0.82 $\pm$ 0.09*	0.58 $\pm$ 0.08**	0.36 $\pm$ 0.07***
Vimentin	SKOV-3	1.00 $\pm$ 0.10	0.74 $\pm$ 0.09*	0.48 $\pm$ 0.07**	0.28 $\pm$ 0.05***
Vimentin	OVCAR-3	1.00 $\pm$ 0.09	0.80 $\pm$ 0.08*	0.55 $\pm$ 0.08**	0.34 $\pm$ 0.06***

*P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

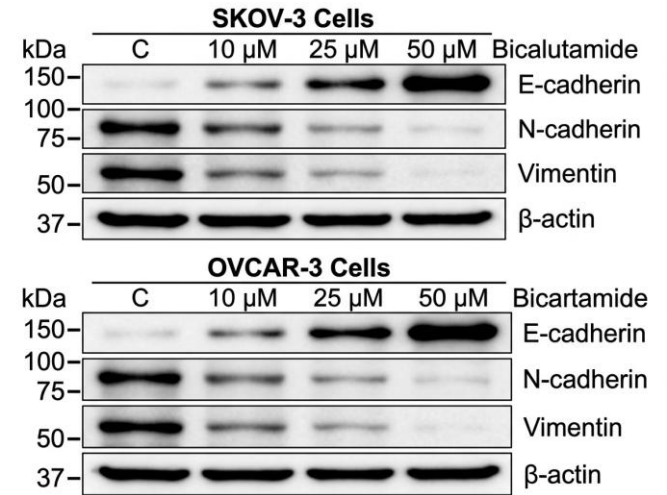


Figure 5A. Representative western blots for E-cadherin, N-cadherin, vimentin, and β -actin in SKOV-3 and OVCAR-3 cells treated with bicalutamide (10, 25, 50 μ M) for 48 h. C = Control.

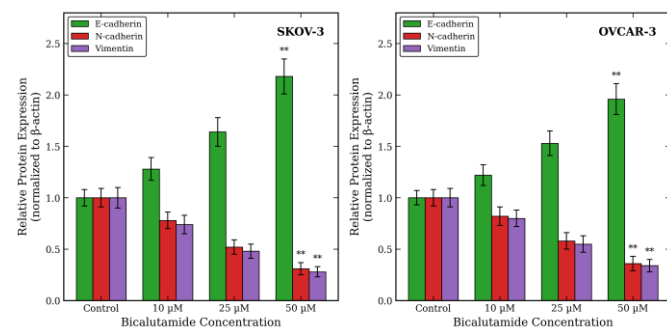


Figure 5B. Densitometric analysis of EMT marker expression normalized to β -actin. Data are mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ vs. control.

3.6. Bicalutamide Attenuates PI3K/AKT Signaling Pathway

The PI3K/AKT signaling pathway is one of the most frequently dysregulated cascades in epithelial ovarian cancer, with PTEN loss and PI3K amplification being commonly observed genetic alterations in this malignancy [29,30]. Moreover, the PI3K/AKT axis has been directly linked to the regulation of cell migration, invasion, EMT, and MMP expression in ovarian cancer [24,31]. Given the well-established cross-talk between AR signaling and the PI3K/AKT pathway in other cancer types—particularly prostate cancer—we next examined whether bicalutamide affected the activation status of key components in this cascade.

Western blotting revealed that treatment with bicalutamide for 48 h resulted in a dose-dependent reduction in the phosphorylation levels of both PI3K (p-PI3K) and AKT (p-AKT at Ser473) in both SKOV-3 and OVCAR-3 cells, while total PI3K and AKT protein levels remained largely unchanged. This observation implies that bicalutamide reduces the activation but not the overall amount of these signalling molecules. The reduction in PI3K/AKT phosphorylation is mechanistically consistent with the observed reduction in MMP-2/MMP-9 production and EMT marker alterations, since PI3K/AKT signalling is a well-established upstream regulator for both processes.

According to the study by Sun et al. (2021), androgens had carcinogenic effects in an AR-dependent manner via the PI3K/AKT signalling pathway in ovarian cancer [32]. Similarly, Vasiliou et al. (2019) reported that VEPH1 suppressed the activation of AR by reducing the activation of SMAD3 and AKT in ovarian cancer cells [33]. Our observations contribute to this body of literature by demonstrating that pharmacological AR inhibition with bicalutamide can suppress PI3K/AKT signalling output, presumably explaining the reduced invasive and migratory phenotype we observed.

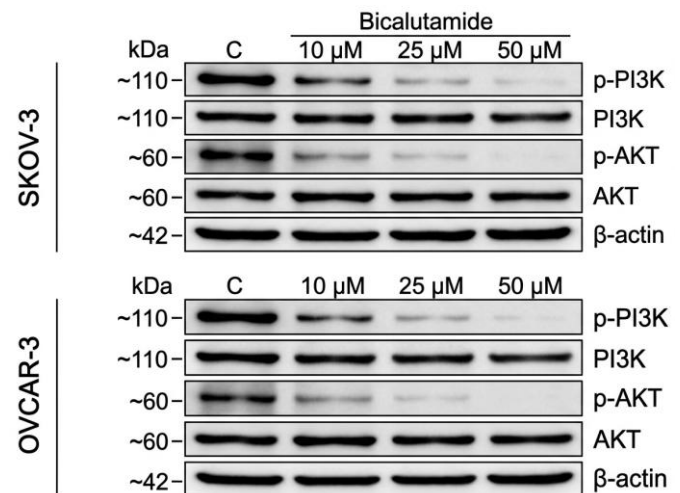


Figure 6A. Representative western blots for p-PI3K, PI3K, p-AKT (Ser473), AKT, and β -actin in SKOV-3 and OVCAR-3 cells treated with bicalutamide (10, 25, 50 μ M) for 48 h. C = Control.

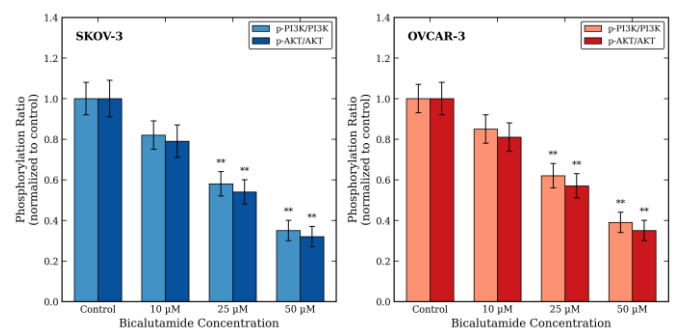


Figure 6B. Quantification of p-PI3K/PI3K and p-AKT/AKT ratios. Data are mean $\pm$ SD ($n = 3$). * $P < 0.05$, ** $P < 0.01$ vs. control.

3.7. Proposed Mechanism and Clinical Implications

The data provided here collectively support a scenario whereby bicalutamide competes with AR-ligand binding and this hinders the nuclear translocation of the receptor and activation of downstream transcriptional processes. Among others, the PI3K/AKT pathway represents a hub connecting AR signalling to the modulation of MMP expression and EMT. Bicalutamide indirectly lowers MMP-2 and MMP-9 levels by inhibiting PI3K/AKT activation, resulting in decreased capacity of ovarian cancer cells to breakdown extracellular matrix barriers. The inhibition of EMT as indicated by the overexpression of E-cadherin and downregulation of N-cadherin and vimentin, also inhibits the inherent motility of the cancer cells, which further explains the observed reductions in migration and invasion. These results are of interest from a clinical point of view for various reasons. First, bicalutamide is a licensed and well-tolerated drug with a long-standing safe profile in prostate cancer patients [11]. Repurposing it for AR-positive ovarian tumours could potentially skip the lengthy and costly process of designing wholly new targeted medicines. Second, Vasylyev et al. (2020) showed that AR-directed therapy with bicalutamide achieved prolonged disease control in a case of recurrent high-grade serous ovarian cancer over 30 months [15], raising the possibility that the preclinical findings reported here may have

real clinical correlates. However, it must be remembered that past clinical trials have had inconsistent results. In a phase II investigation of patients with epithelial ovarian cancer in remission, the combination of goserelin and bicalutamide did not significantly improve progression-free life (Levine et al. 2007) [14]. The lack of patient classification based on AR expression status, underlined by the authors of the case report of Vasyliov et al. as possibly crucial for therapeutic success [15], could at least partially explain this.

There are some limitations of the present study worth mentioning. Experiments were performed only in vitro. While cell culture models are very useful for mechanistic interrogation, they do not fully recapitulate the complexity of the tumour microenvironment, including the influence of stromal cells, immune cells, and the three-dimensional architecture of peritoneal metastatic deposits. Additionally, SKOV-3 is now considered to be derived from a non-serous carcinoma, which raises questions about its representativeness for the most common subtype of ovarian cancer, high-grade serous carcinoma [34]. Future studies should expand these investigations to additional cell lines representing different ovarian cancer subtypes, employ three-dimensional culture systems and animal models, and ultimately design clinical trials with robust AR-based patient selection criteria.

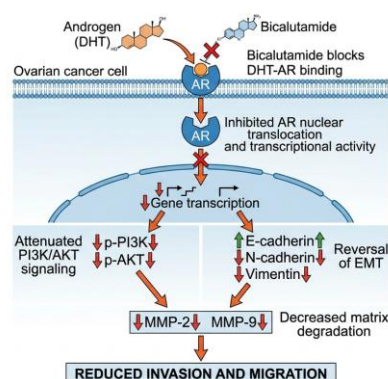


Figure 7. Schematic diagram summarizing the proposed mechanism by which bicalutamide inhibits ovarian cancer cell invasion and migration. AR = androgen receptor; DHT = dihydrotestosterone; EMT = epithelial-mesenchymal transition; MMP = matrix metalloproteinase.

4. CONCLUSION

This study demonstrates that bicalutamide, a nonsteroidal AR antagonist, effectively inhibits the invasion and migration of SKOV-3 and OVCAR-3 ovarian cancer cells in a dose-dependent manner. The underlying mechanisms appear to involve suppression of EMT, as evidenced by increased E-cadherin and reduced N-cadherin and vimentin expression, along with downregulation of the matrix-degrading enzymes MMP-2 and MMP-9. These effects are linked to inhibition of PI3K/AKT pathway activation downstream of AR signalling. This study provides a preclinical basis for the evaluation of bicalutamide as a potential supplementary treatment drug in AR-positive

epithelial ovarian malignancies, especially as a strategy to prevent metastatic progression. Given the favourable safety profile and clinical use of bicalutamide, further validation in vivo models and well-designed clinical studies with AR-stratified patient selection is recommended. If proven, the AR-targeted therapy using bicalutamide may provide an innovative and readily available way to improve outcomes in a condition that nonetheless carries a bleak prognosis for far too many women.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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