Article

Discovery Medicine 2026; 38(212): 2894–2905
https://doi.org/10.24976/Discov.Med.202638212.223

Psoralen Suppresses Bladder Cancer Progression by Targeting AURKA: Inhibition of Proliferation and Promotion of Apoptosis

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Submitted: 17 June 2026 Revised: 20 July 2026 Accepted: 30 July 2026 Published: 23 September 2026

Background: Although psoralen is known for its antitumor and chemosensitizing effects across multiple malignancies, its function and molecular basis in bladder cancer (BC) remain insufficiently understood. Thus, this study examined its anti-cancer actions in BC and identified its potential target.

Methods: Human BC cell lines (5637, T24, UM-UC-3, and J82) and a normal bladder epithelial cell line (SV-HUC-1) were exposed to graded psoralen doses. Cell viability and proliferation were evaluated using Cell Counting Kit-8 (CCK-8) and colony formation assays, while apoptosis was analyzed via flow cytometry. Potential psoralen-related targets and BC-associated genes were identified using public databases and intersected via Venny analysis. The expression of aurora kinase A (AURKA) was analyzed using bioinformatics platforms and validated using quantitative reverse transcription polymerase chain reaction and Western blotting. Functional experiments involving AURKA overexpression were conducted to determine its role in mediating psoralen responses. Expression levels of apoptosis- and proliferation-related proteins, including proliferating cell nuclear antigen (PCNA), B-cell lymphoma-2 (Bcl-2), cleaved caspase-3, and Bcl-2-associated X protein (Bax), were also examined.

Results: Psoralen dramatically decreased BC cell viability and colony-forming ability while markedly promoting apoptosis ($p < 0.05$). Psoralen treatment upregulated cleaved caspase-3 and Bax, and downregulated PCNA and Bcl-2 ($p < 0.01$). Through bioinformatics analysis, AURKA was found to be upregulated in BC tissues and cell lines ($p < 0.05$). Psoralen reduced AURKA expression, and overexpression of AURKA partially attenuated psoralen-induced proliferation inhibition and apoptosis ($p < 0.05$).

Conclusion: *In vitro*, psoralen suppresses BC cell proliferation and promotes apoptosis, and AURKA may be involved in these effects. These results point to psoralen as a promising natural compound for further investigation in BC therapy.

Keywords: psoralen; bladder cancer; proliferation; apoptosis; aurora kinase A; molecular mechanism; target prediction; overexpression

Introduction

Bladder cancer (BC) represents a major global health burden, accounting for nearly 500,000 new diagnoses and 200,000 fatalities annually [1]. BC can cause different disease clinical outcomes, ranging from recurrent tumors that rarely invade deeper and become life-threatening to advanced-stage or metastatic diseases characterized by deep invasion and systemic spread [2]. Therein, non-muscle-invasive bladder cancer (NMIBC) carries a high risk of recurrence, with 20–30% of cases progressing to muscle-invasive bladder cancer (MIBC) over time [3]: MIBC carries a higher risk of metastasis and often incurs therapy resistance; and metastatic bladder cancer is associated with a poor prognosis, with a five-year survival rate of merely 5% [4,5]. Transurethral resection or radical cystectomy, accompanied by chemoradiotherapy or medication, emerges as the leading treatment strategy for early-stage or locally invasive BC [6,7]. Chemotherapy and immunotherapy are commonly used to treat BC with distant metastasis [8]. Therefore, exploring novel therapeutic agents with favorable safety profiles remains an important goal for improving BC patient outcomes.

Suppression of tumor growth through inhibition of proliferation and induction of apoptosis is a major mechanism underlying chemotherapy or immunotherapy [9,10,11]. Proliferation is a fundamental cellular event required for tumorigenesis [12]. Programmed cell death, or apoptosis, is characterized by cell shrinkage, pyknosis, and dense cytoplasm; dysregulated apoptosis results in the development of cancer [13]. Extensive research has demonstrated that traditional Chinese medicines can achieve genuine effects with low toxicity in combating therapy resistance [14,15]. The main bioactive component of *Psoralea corylifolia*, psoralen, has been demonstrated to possess analgesic, anticoagulant, antioxidant, antibacterial, anti-inflammatory, antidiabetic, and anti-neurodegenerative qualities [16]. Beyond these pharmacological functions, psoralen can also exert anticancer effects in a variety of cancer types by inhibiting malignant growth,

triggering cell cycle arrest and death, and regulating cancer cell differentiation [17,18,19,20]. However, the mechanism underlying the antitumor effects of psoralen and its effective and safe dose in BC have not yet been discussed.

The malignant phenotype changes in BC cells are driven by genetic alterations, which harbor predictive, therapeutic and prognostic implications. Recent studies have shown that aurora kinase A (AURKA), a member of the Aurora/Ipl1p kinase family, can promote BC progression and serve as a prognostic biomarker for poor clinical outcomes in BC patients [21]. Targeting AURKA with plant extracts suppresses cancer progression [22], and psoralen has been reported to inhibit RNA expression, thereby reversing docetaxel-induced multidrug resistance and causing cancer cell death [18]. These findings collectively suggest that AURKA can act as a target of psoralen for its anticancer activities. Therefore, our research aims to ascertain psoralen's effects on BC cell growth and apoptosis, as well as the relationship between its antitumor effects in BC development and AURKA expression.

Materials and Methods

Cell culture

Human normal bladder epithelial cells SV-HUC-1 (CRL-9520) and BC cells, 5637 (HTB-9), T24 (HTB-4), UM-UC-3 (CRL-1749), and J82 (HTB-1), were acquired from the ATCC (Manassas, VA, USA). BC cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; 11965092, ThermoFisher Scientific, Waltham, MA, USA), whereas SV-HUC-1 cells were cultured in F-12K Medium (30-2004, ATCC Manassas, VA, USA). Cells were maintained in basal growth media supplemented with 10% fetal bovine serum (FBS, F2442, Sigma-Aldrich, St. Louis, MO, USA) at 37 °C in a humidified atmosphere containing 5% CO_2. All cell lines were authenticated by short tandem repeat analysis and confirmed to be free of mycoplasma contamination.

Cell Counting Kit (CCK)-8 Assay

To investigate how psoralen influenced the viability of BC and SV-HUC-1 cells, the CCK-8 test was conducted. Psoralen (N1332, APExBIO, Houston, TX, USA) was dissolved in dimethyl sulfoxide (DMSO, HY-Y0320C, MCE, Monmouth Junction, NJ, USA). Cells were plated in 96-well plates at a density of 5×10^3 cells/well and cultured in complete medium (8 h), followed by replacement with fresh complete medium containing various doses of psoralen (0, 4, 6, 8, 10, and 20 µg/mL) [20]. CCK-8 reagent (C0037, Beyotime, Shanghai, China) was added to cells at 24 and 48 h (1 h, 37 °C), and absorbance was recorded with a microplate reader (450 nm, BMG LABTECH, Offenburg, Germany).

Bioinformatics Analyses

AURKA expression in BC was analyzed based on Gene Expression Profiling Interactive Analysis 2 (GEPIA 2, http://gepia2.cancer-pku.cn/#index, web version updated in 2019, Peking University, Beijing, China). SwissTargetPrediction (http://swisstargetprediction.ch/, based on ChEMBL 23, Swiss Institute of Bioinformatics, Lausanne, Switzerland) was utilized to predict potential targets of psoralen. GeneCards (https://www.genecards.org/, version 5.21, Weizmann Institute of Science, Rehovot, Israel) was employed to retrieve BC-related genes. The genes with a relevant score of more than 50 were chosen to generate equivalent sets. Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/, version 2.1.0, Centro Nacional de Biotecnología–CSIC, Madrid, Spain) was used to create the intersection of the sets to identify BC-related genes that psoralen could target.

Cell Transfection

AURKA overexpression plasmids were constructed using the pcDNA3.1 vector (V79520, ThermoFisher Scientific, Waltham, MA, USA), with an empty vector as a negative control (NC). Transfection (24 h) was performed in T24 and 5637 cells using AURKA overexpression plasmids (2.5 µg per well in 6-well plates) with Lipofectamine 3000 transfection reagents (L3000015, ThermoFisher Scientific, Waltham, MA, USA) at a DNA-to-Lipofectamine 300 ratio of 1:3 (µg:µL) and 5 µL P3000 reagent according to the manufacturer's instructions. The efficiency of transfection was evaluated by Western blot analysis and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Cell Treatment

The 5637 and T24 cells were divided into the following groups: Control, Psoralen 10, NC, AURKA, and Psoralen + AURKA. In the Psoralen 10 group, cells underwent exposure to 10 µg/mL psoralen for 24 h. Cells in the Control groups were treated with an equivalent volume of DMSO as vehicle control. For the NC and AURKA groups, cells were transfected with corresponding plasmids (24 h). After being transfected with the AURKA overexpression plasmid (24 h), cells in the Psoralen + AURKA group were treated with 10 µg/mL psoralen (24 h). All drug preparation and cell treatment procedures were performed under subdued light, and cells were cultured in a standard incubator maintained in the dark to prevent psoralen photoactivation.

Colony Formation Assay

The 5637 and T24 cells were trypsinized (9002-07-7, Sigma-Aldrich, St. Louis, MO, USA) and seeded into six-well plates at a density of 1×10^2 cells/well. After 24 h of inoculation, the culture medium was replaced with fresh medium containing 10 µg/mL Psoralen-10, and the cells were continuously exposed to Psoralen-10 for 14 days, with

Table 1. Primary and secondary antibodies used in Western blotting.

Antibody	Catalog No.	Molecular weight (kDa)	Dilution	Supplier
AURKA	ab52973	46	1:50,000	Abcam, Cambridge, UK
PCNA	ab92552	29	1:1000	Abcam, Cambridge, UK
Cleaved caspase-3	ab2302	17	1:500	Abcam, Cambridge, UK
Bcl-2	ab32124	26	1:1000	Abcam, Cambridge, UK
Bax	ab32503	21	1:1000	Abcam, Cambridge, UK
GAPDH	ab181602	36	1:10,000	Abcam, Cambridge, UK
Goat anti-rabbit IgG	A32731	—	1:10,000	Thermo Fisher Scientific, USA

AURKA, aurora kinase A; PCNA, proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma-2; Bax, Bcl-2-associated X protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IgG, immunoglobulin G.

the drug-containing medium refreshed every 3 days. Visible colonies were fixed in paraformaldehyde (4%, R22038, Yuanye Biotechnology, Shanghai, China) for 15 min and stained with crystal violet (0.1%, 60505ES25, YEASEN, Shanghai, China) for 5 min. The total number of colonies containing more than 50 cells in each well was counted manually under an inverted microscope (IXplore Standard, Japan). Colony formation efficiency was normalized to that of the control group, which was set as 100%.

Flow Cytometry

Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) apoptosis detection kits (40302ES20, Yeasen, Shanghai, China) were used to detect apoptosis. 1× Binding Buffer was utilized to resuspend the cells (1×10^6 cells/mL), followed by the addition of 5 µL of Annexin V-FITC solution and 10 µL of PI solution in the dark for 10 min. Apoptotic cells were analyzed using CytExpert software after flow cytometric detection (Cytoflex, version 2.0, Beckman Coulter, Brea, CA, USA). The gating strategy excluded debris and doublets based on forward scatter area (FSC-A)/side scatter area (SSC-A) and FSC-A/forward scatter width (FSC-W)/SSC-A/side scatter width (SSC-W), respectively. Apoptotic populations were identified on Annexin V-FITC versus PI biparametric plots, with quadrants set as: Q4 (Annexin V^-/PI^-, viable), Q3 (Annexin V^+/PI^-, early apoptotic), Q2 (Annexin V^+/PI^+, late apoptotic), and Q1 (Annexin V^-/PI^+, necrotic). Total apoptosis was defined as Q2 + Q3.

qRT-PCR

Trizol reagents (MF0403, Maokangbio, Shanghai, China) were used to extract total RNA from cells. SuperScript IV reverse transcriptase (18-090-010, Thermo Fisher Scientific, Waltham, MA, USA) was employed to reverse-transcribe the isolated total RNA into cDNA. cDNAs were amplified via qPCR reaction performed on a detection device (CFX Connect, Bio-Rad, Philadelphia, PA, USA) with PowerUp SYBR Green Master Mix (A25742, ThermoFisher Scientific, Waltham, MA, USA) at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 60 s. The primers used were as follows: AURKA (forward: 5′-GGAAGACTTGGGTCCTTGGG-3′ and reverse: 5′-GAACCGACAGGGGACTTGAC-3′) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: 5′-AATGGGCAGCCGTTAGGAAA-3′; reverse: 5′-GCGCCCAATACGACCAAATC-3′). AURKA expression was normalized to GAPDH and expressed by the $2^{-\Delta\Delta Ct}$ method.

Western Blot Analysis

Radioimmunoprecipitation assay Buffer (C1053, Applygen, Beijing, China) was applied to extract the total protein from the cells. Bicinchoninic acid kits (BL521A, Biosharp, Hefei, China) were used to determine the extracted protein concentration. The protein (40 µg) was loaded separately and electrophoresed using a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (P0672, Beyotime, Shanghai, China). The protein was then transferred onto polyvinylidene fluoride membranes (WJ002, Epizyme, Shanghai, China). After blocking with 5% non-fat milk at room temperature for 1 h, the membranes were incubated overnight at 4 °C with primary antibodies (Table 1), washed with Tris-buffered saline with Tween 20 (TBST, MA0091, Meilunbio, Dalian, China), and then probed with goat anti-rabbit IgG (Table 1) at room temperature for 1 h. Using an imaging apparatus (iBright CL750, ThermoFisher Scientific, Waltham, MA, USA) and an enhanced chemiluminescence reagent kit (SQ201L, Epizyme, Shanghai, China), immunoreactive band visualization was performed. ImageJ software (version 1.54r, NIH, Bethesda, MD, USA) was adopted for quantitative analysis. GAPDH was normalized as the internal control.

Statistical Analysis

SPSS software (Version 26.0, SPSS Inc., Chicago, IL, USA) was used to conduct statistical analyses. The normality of the data distribution was assessed using the Shapiro-Wilk test. The data were found to follow a normal distribution and are presented as mean ± standard deviation (SD) of triplicate independent experiments. One-way analysis of variance (ANOVA) followed by Dunnett's or Tukey's post-hoc test was used for comparisons among multiple groups. Two-way ANOVA followed by Tukey's post-hoc test was

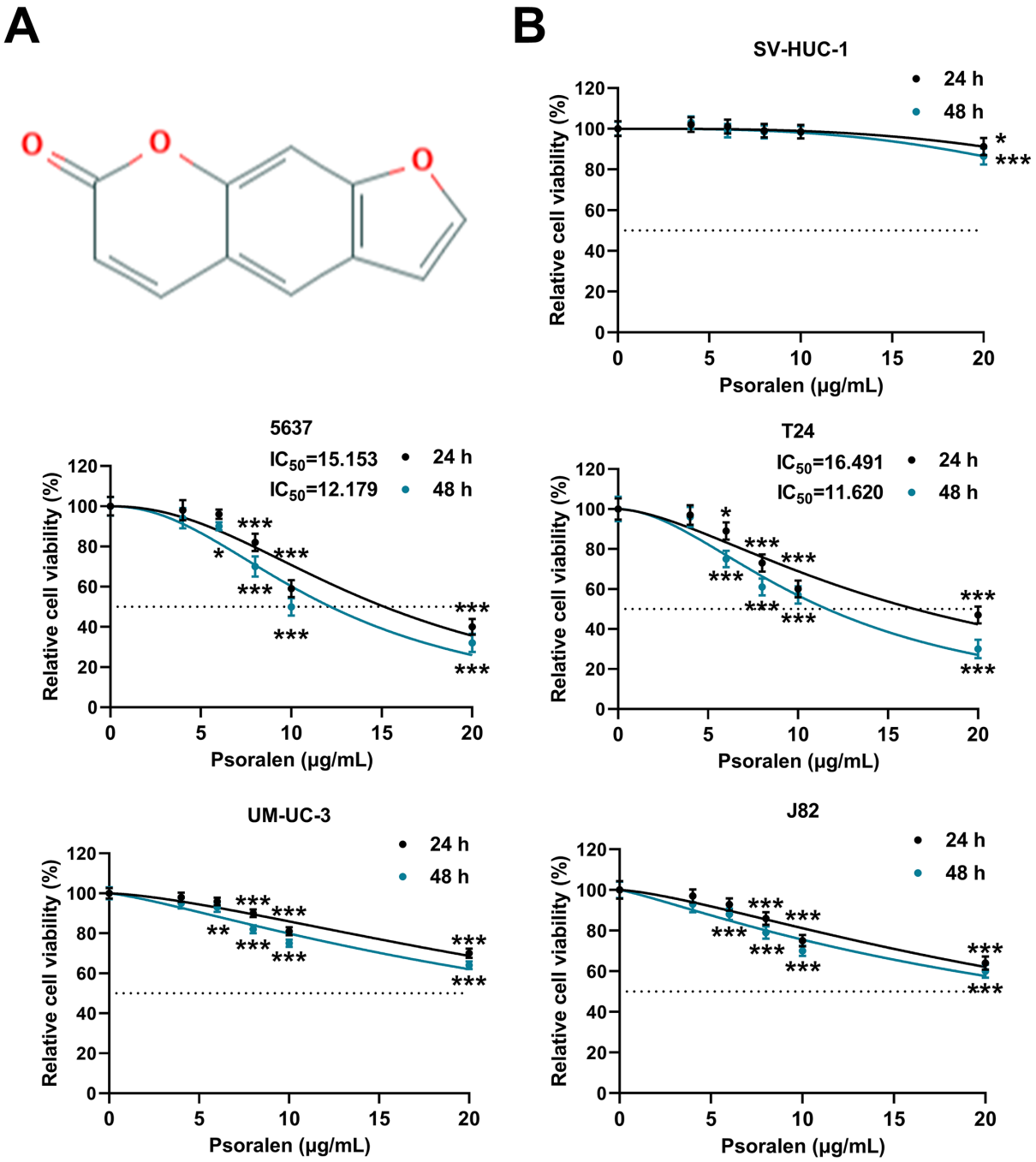

Fig. 1. Psoralen decreased BC cell viability in a concentration-dependent manner. (A) Chemical structure of psoralen. (B) Viability of human normal bladder epithelial SV-HUC-1 cells and human BC (5637, T24, UM-UC-3 and J82) cells treated with different concentrations (0, 4, 6, 8, 10 and 20 μg/mL) of psoralen (CCK-8 assay). Data are mean ± standard deviation (n = 3 independent biological replicates). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; * vs Psoralen 0 μg/mL. BC, bladder cancer; CCK-8, cell counting kit-8.

used for comparisons involving two factors (concentration and time). An independent *t*-test was used to compare differences between two groups. A Venn diagram was generated to evaluate BC-related genes that psoralen targets. A $p < 0.05$ was considered statistically significant.

Results

Psoralen Decreased BC Cell Viability in a Concentration-Dependent Manner

Fig. 1A depicts the chemical structure of psoralen. To assess how psoralen impacts BC cell viability, SV-HUC-1 cells and 4 BC cell lines were treated with different con-

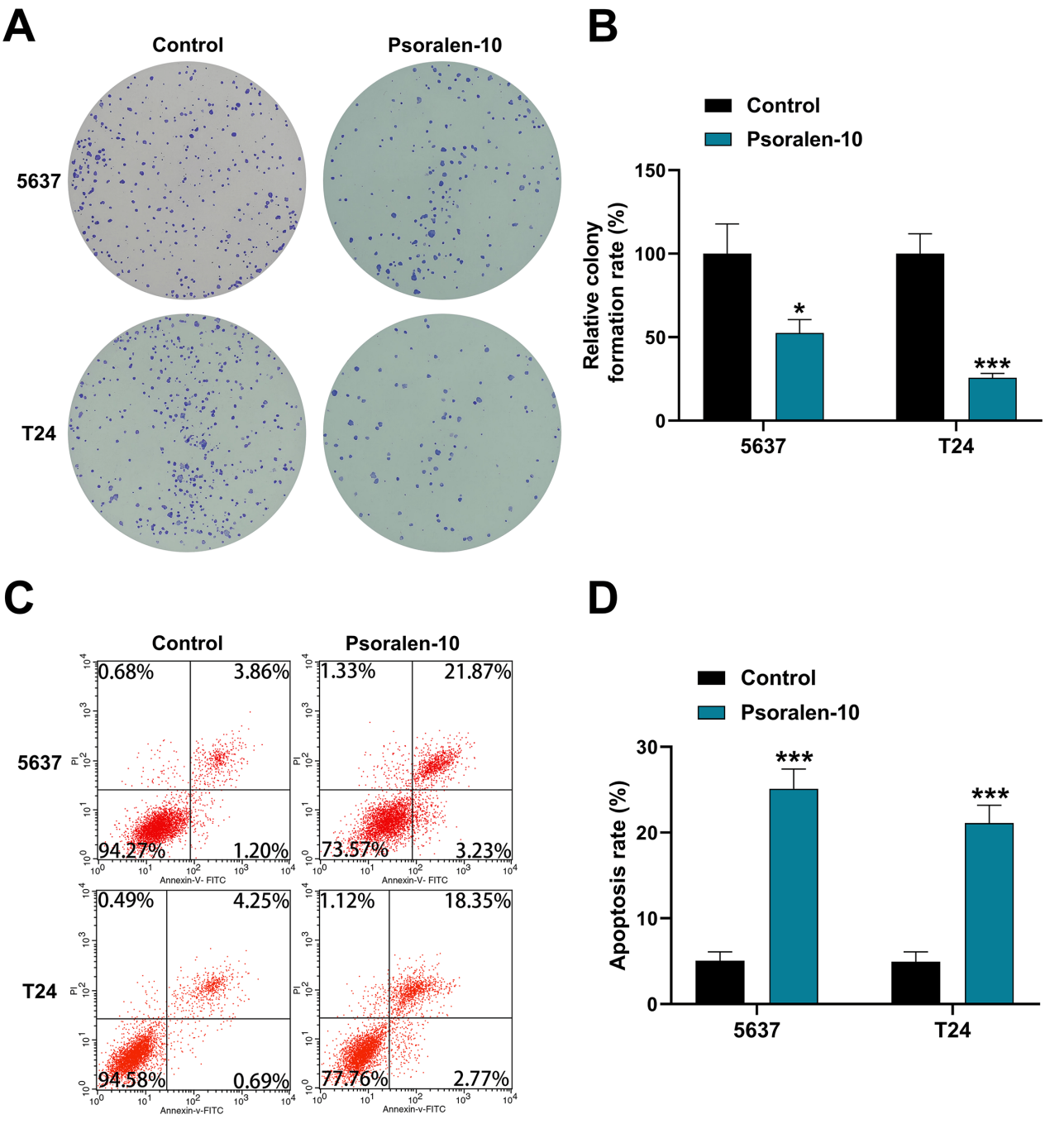

Fig. 2. Psoralen inhibited BC cell proliferation and enhanced apoptosis. (A,B) The colony-forming ability (colony formation assay) (C,D) and apoptosis (flow cytometry) of psoralen (10 µg/mL)-induced 5637 and T24 cells. Data are mean ± standard deviation (n = 3 independent biological replicates). $^{*}p < 0.05$, $^{***}p < 0.001$; * vs Control. BC, bladder cancer.

centrations of psoralen (0, 4, 6, 8, 10, and 20 µg/mL). As reflected in CCK-8 assay, psoralen decreased SV-HUC-1 cell viability at 24 and 48 h at a concentration of 20 µg/mL ($p < 0.05$; Fig. 1B). Psoralen at concentrations ≥8 µg/mL reduced the viability of 5637, UM-UC-3 and J82 cells after 24 h ($p < 0.001$; Fig. 1B). At 48 h, concentrations ≥6 µg/mL exerted a dose-dependent inhibitory effect on the viability of 5637, UM-UC-3 and J82 cell ($p < 0.05$; Fig. 1B). For T24 cells, psoralen at concentrations ≥6 µg/mL diminished cell viability at both 24 and 48 h ($p < 0.05$; Fig. 1B). Based on the CCK-8 assay results, the 24 h half-maximal inhibitory concentration (IC50) values of psoralen for 5637 and T24 cells were determined by four-parameter logistic regression as 15.153 µg/mL and 16.491 µg/mL, respectively, with corresponding 48 h IC50 values of 12.179 µg/mL and 11.620 µg/mL. For UM-UC-3, J82, and SV-

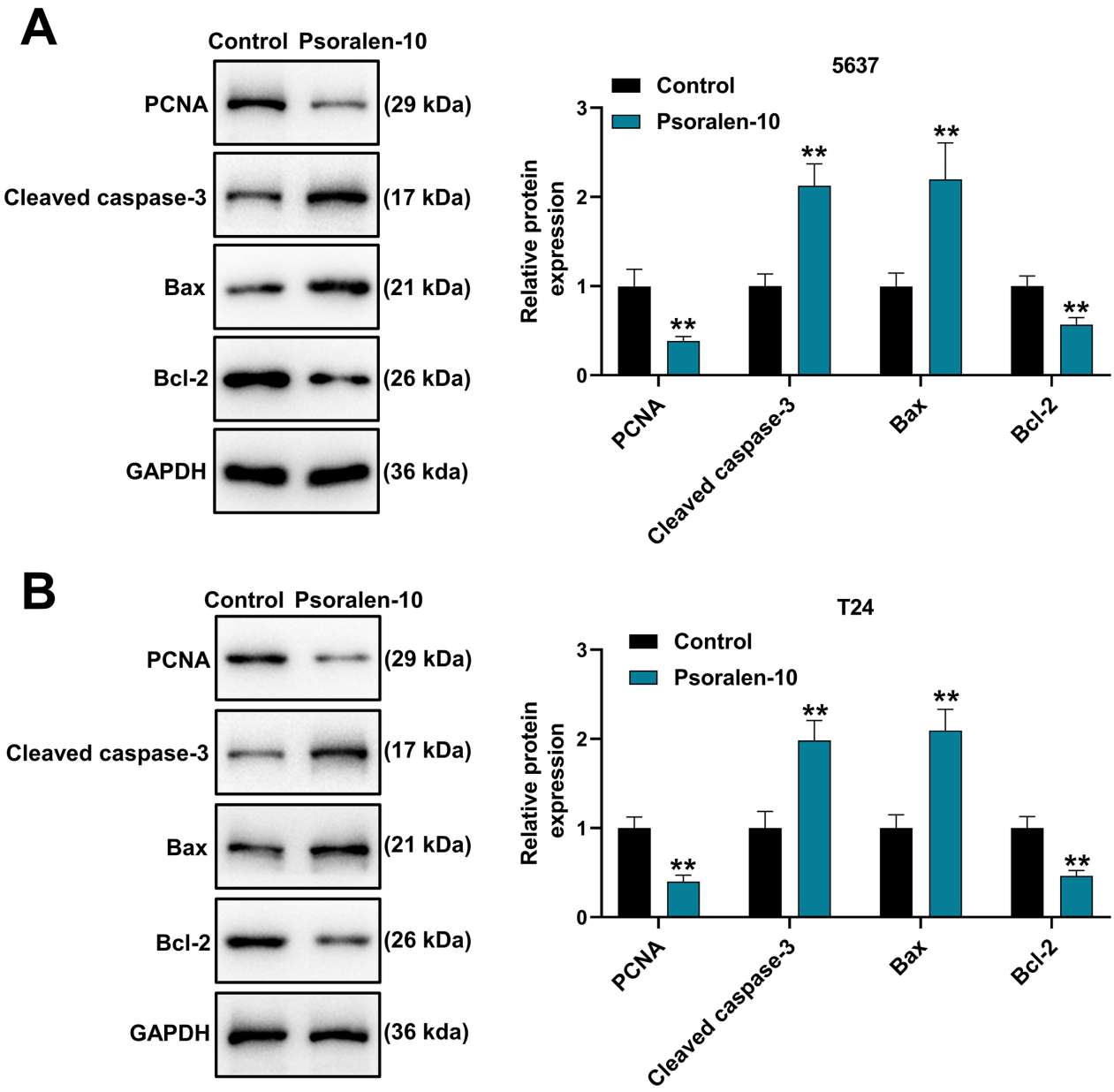

Fig. 3. Psoralen regulated proliferation-/apoptosis-related molecule expression levels in BC cells. (A,B) PCNA, Cleaved caspase-3, Bax and Bcl-2 expression levels in psoralen (10 µg/mL)-induced 5637 (A) and T24 (B) (Western blotting, GAPDH as a reference gene). Data are mean ± standard deviation (n = 3 independent biological replicates). $^{**}p < 0.01$; * vs Control. BC, bladder cancer; PCNA, proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; GEPIA 2, Gene Expression Profiling Interactive Analysis 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

HUC-1 cells, cell viability remained above 50% across all tested concentrations (up to 20 µg/mL). A concentration of 10 µg/mL was selected as a sub-IC50 dose that produced measurable inhibitory effects in bladder cancer cells while causing limited short-term reduction in SV-HUC-1 viability. Additionally, T24 and 5637 cells were selected as models for subsequent investigations because they showed a more pronounced sensitivity to psoralen than the other BC cell lines.

Psoralen Inhibited the Function of BC Cells

The results demonstrated that psoralen (10 µg/mL) inhibited clonogenic growth and increased apoptosis of BC cells ($p < 0.05$; Fig. 2A–D). To determine how psoralen affects the expression of proteins linked to apoptosis or proliferation, Western blotting was performed. As demonstrated in Fig. 3A,B, psoralen (10 µg/mL) reduced proliferating cell nuclear antigen (PCNA) and B-cell lymphoma 2 (Bcl-2) in BC cells, while psoralen (10 µg/mL) increased

Cleaved caspase-3 and Bcl-2-associated X protein (Bax) ($p < 0.01$).

AURKA was Highly Expressed in BC and Downregulated by Psoralen in BC Cells

Furthermore, we explored the mechanism underlying psoralen-induced impacts on BC cells. Potential targets of psoralen were predicted using the SwissTargetPrediction tool. Meanwhile, the GeneCards database was searched for BC-associated genes, and genes with a relevance score greater than 50 were chosen. The intersection between the predicted psoralen targets and BC-associated targets identified 11 common genes, considered as potential therapeutic targets of psoralen in BC: NAD(P)H quinone dehydrogenase 1 (NQO1), epidermal growth factor receptor (EGFR), prostaglandin-endoperoxide synthase 2 (PTGS2, also known as cyclooxygenase-2, COX-2), erb-b2 receptor tyrosine kinase 2 (ERBB2), SRC proto-oncogene, non-receptor tyrosine kinase (SRC), MET proto-oncogene, receptor tyrosine kinase (MET), AKT serine/threonine kinase 1 (AKT1), estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2), B-Raf proto-oncogene, serine/threonine kinase (BRAF), and AURKA (Fig. 4A). Through GEPIA 2-based analysis, AURKA was shown to be upregulated in BC samples relative to normal samples ($p < 0.05$; Fig. 4B). Similarly, AURKA were significantly upregulated in BC cells compared to that in SV-HUC-1 cells ($p < 0.001$; Fig. 4C). Psoralen (10 µg/mL) markedly decreased AURKA expression in BC cells compared to control ($p < 0.05$; Fig. 4D,E).

AURKA Overexpression Antagonized Psoralen-Induced Effects on BC Cells

Next, we explored the psoralen-induced effects on cell proliferation and apoptosis in response to AURKA overexpression to determine the role of AURKA in mediating these effects. Psoralen treatment considerably diminished AURKA in BC cells, whereas AURKA overexpression reversed this outcome ($p < 0.05$; Fig. 5A,B). In BC cells, transfection with the AURKA overexpression plasmid significantly increased AURKA expression, whereas psoralen treatment partially reversed this upregulation ($p < 0.01$; Fig. 5A,B). Besides, the data revealed that AURKA overexpression enhanced clonogenic growth and reduced apoptosis in BC cells, whereas Psoralen treatment reversed these effects ($p < 0.05$; Fig. 5C–F). Interestingly, psoralen's inhibitory effects on cell proliferation were lessened by AURKA overexpression, which also inhibited psoralen-induced cell death ($p < 0.01$; Fig. 5C–F). AURKA overexpression continually lowered Cleaved caspase-3 and Bax while enhancing PCNA and Bcl-2; however, these effects were markedly attenuated following Psoralen treatment ($p < 0.05$; Fig. 6A,B). Additionally, psoralen's effects on key proteins linked to apoptosis and proliferation were reversed by AURKA overexpression ($p < 0.05$; Fig. 6A,B).

Discussion

The main challenges in BC treatment include disease recurrence, metastasis, and treatment-related toxicity. Traditional Chinese medicines have been considered potent agents for cancer therapy [15]. For many years, psoralen has been reported as an antitumor agent via induction of cancer cell phenotype changes in many types of cancers [23]. Nevertheless, its potential antitumor effects in BC development remain undefined, prompting the present investigation.

A previous study that established the safe dosage of psoralen for breast cancer treatment suggested that psoralen treatment at 8 µg/mL is a non-cytotoxic treatment regimen that can suppress the growth of breast cancer cells [24]. Psoralen exhibits various safe concentrations across different cancers, as supported by studies on the effect of psoralen on hepatoma and lung cancer cells [17,18]. Therefore, the optimal concentration of psoralen for suppressing BC cell growth without inducing cytotoxicity in noncancerous cells was investigated. In our study, the CCK-8 assay showed that psoralen (10 µg/mL) was non-cytotoxic to normal bladder epithelial cells but reduced BC cell viability; therefore, 10 µg/mL was selected as the working concentration for subsequent psoralen treatment of BC cells. Additionally, psoralen has been shown to inhibit cancer growth through apoptosis or cell cycle inhibition [16,23]. Based on our findings that psoralen inhibited proliferation and promoted apoptosis in BC cells, we propose that BC should be added to the spectrum of cancers subjected to antitumor treatment with psoralen.

We investigated associated molecular alterations to confirm the effects of psoralen on BC cells. PCNA, essential for DNA replication and repair, is a factor that anchors DNA polymerases and other DNA editing enzymes, and can interact with a large number of proteins to modulate several DNA-related cellular events, including DNA repair, cell proliferation and apoptosis [25]. Targeting PCNA protein has been established as a therapeutic strategy in chemotherapy against highly proliferating malignancies. Cleaved caspase-3, the executioner of the apoptotic pathway, is induced to initiate apoptosis. In line with our findings regarding psoralen's effects on BC cell proliferation and apoptosis, psoralen-treated BC cells showed higher levels of Cleaved caspase-3 and Bax, along with diminished levels of PCNA and Bcl-2.

Next-generation sequencing has facilitated the identification of genetic changes in BC development, which has led to the identification of several potential therapeutic targets. AURKA has been found to be aberrantly expressed in and capable of regulating the onset and progression of various cancer types [26]. In BC patients, highly expressed AURKA is reported to be associated with advanced tumor stage, high tumor grade, invasiveness, and a shorter overall survival time [21]. Consistently, our study showed that AU-

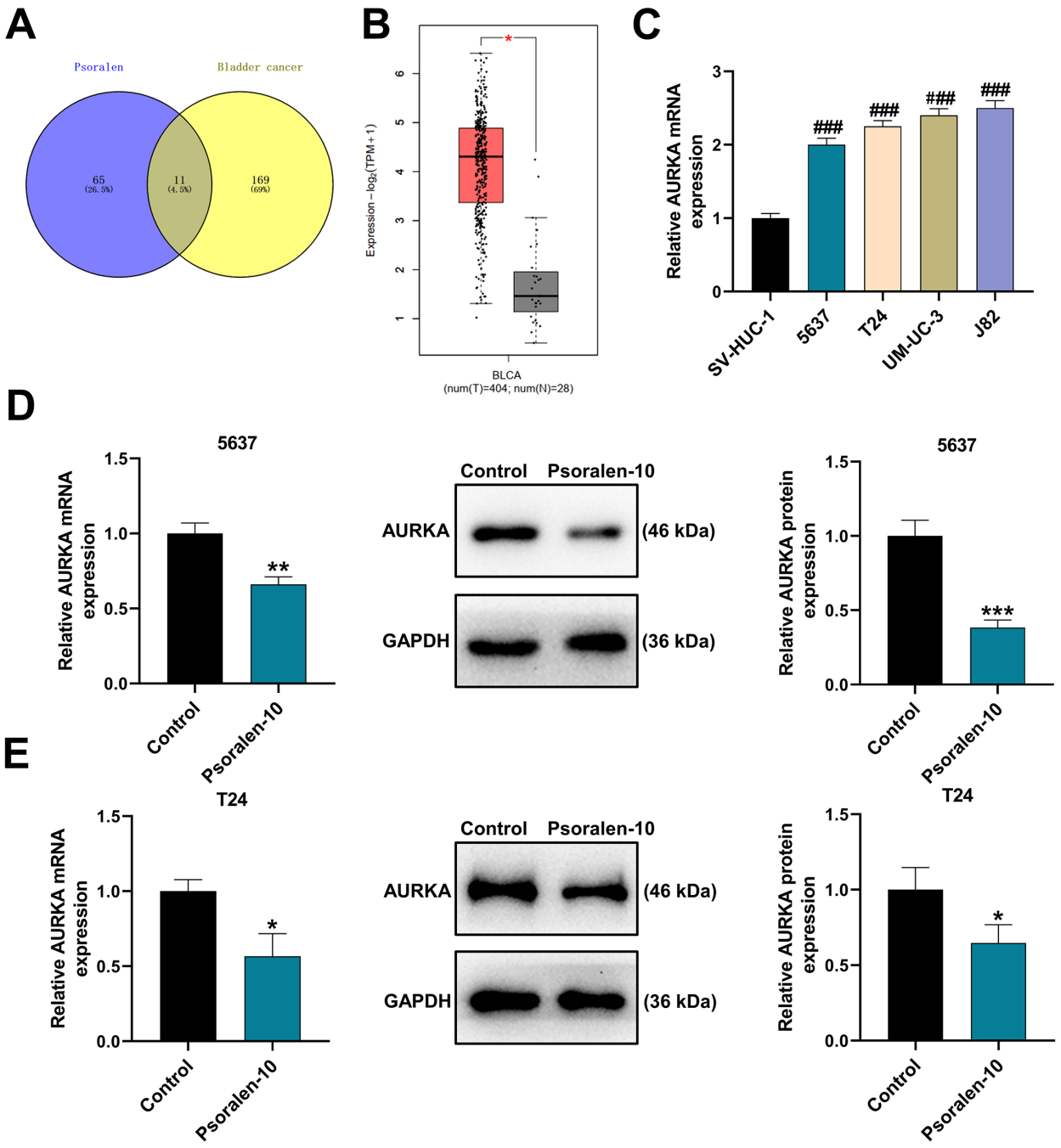

Fig. 4. AURKA was significantly upregulated in BC samples and cells, yet its expression was markedly diminished following psoralen exposure in BC cells. (A) Venn diagram illustrating the overlap between BC-associated genes from GeneCards and psoralen-related targets predicted by SwissTargetPrediction. (B) AURKA expression in BC and normal tissues analyzed using GEPIA 2. (C) AURKA mRNA expression in SV-HUC-1 and BC cell lines (5637, T24, UM-UC-3, and J82) determined by qRT-PCR. (D,E) Effects of psoralen (10 μg/mL) treatment on AURKA expression in 5637 (D) and T24 (E) cells. AURKA mRNA and protein expression were evaluated by qRT-PCR and Western blotting. Data are mean ± standard deviation (n = 3 independent biological replicates). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{\#\#\#}p$ or $^{***}p < 0.001$; * vs Normal; $^{\#}$ vs SV-HUC-1. BC, bladder cancer; AURKA, aurora A kinase; qRT-PCR, quantitative reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

RKA was increased in BC and that AURKA overexpression enhanced BC cell expansion and inhibited apoptosis. According to a previous study, plant extracts can initiate antitumor actions by directly targeting AURKA [27]. In our study, SwissTargetPrediction predicted AURKA as a possible target of psoralen. Subsequently, we found that in BC

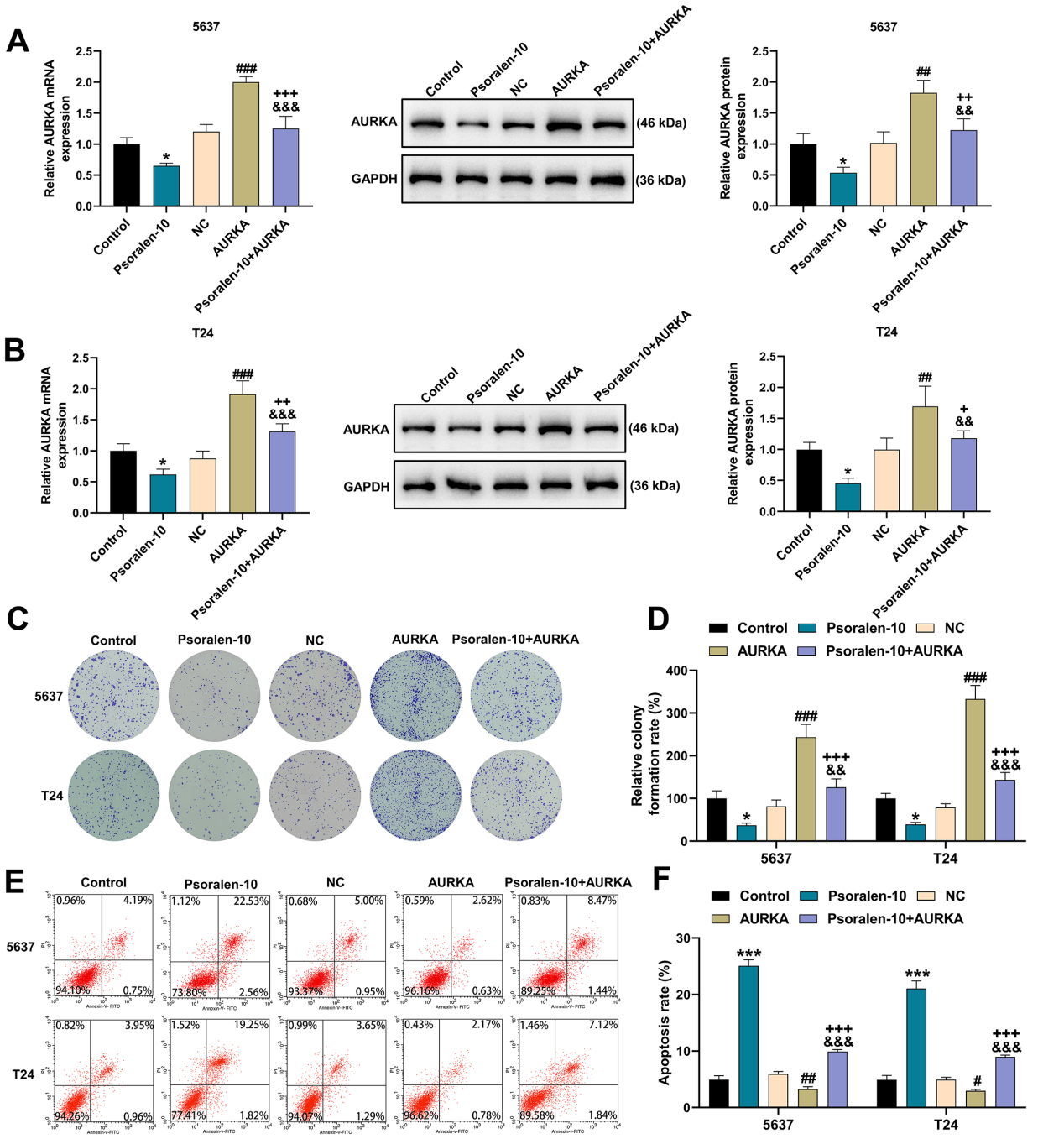

Fig. 5. AURKA overexpression antagonized psoralen-induced effects on BC cell proliferation and apoptosis. (A,B) AURKA expression (qRT-PCR, GAPDH as a reference gene) (the leftmost one) and Western blotting (the rightmost two, GAPDH as a reference gene), (C,D) colony-forming abilities (colony formation assay), and (E,F) apoptosis (flow cytometry) of AURKA overexpression plasmid-transfected, psoralen (10 μg/mL)-induced BC (5637 and T24) cells. Data are mean ± standard deviation (n = 3 independent biological replicates). $^{*}p$ or $^{\#}p$ or $^{+}p < 0.05$; $^{\&\&}p$ or $^{\#\#}p$ or $^{++}p < 0.01$; $^{***}p$ or $^{\#\#\#}p$ or $^{\&\&\&}p$ or $^{+++}p < 0.001$; * vs Control; $^{\#}$ vs NC; $^{\&}$ vs Psoralen; $^{+}$ vs AURKA. BC, bladder cancer; AURKA, aurora A kinase; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

cells, psoralen decreased AURKA protein and mRNA expression and antagonized AURKA overexpression-induced effects; meanwhile, the effects of psoralen were antagonized by AURKA overexpression, which suggests that the regulation of AURKA may contribute to the psoralen-induced inhibition of BC cell proliferation and promotion of apoptosis.

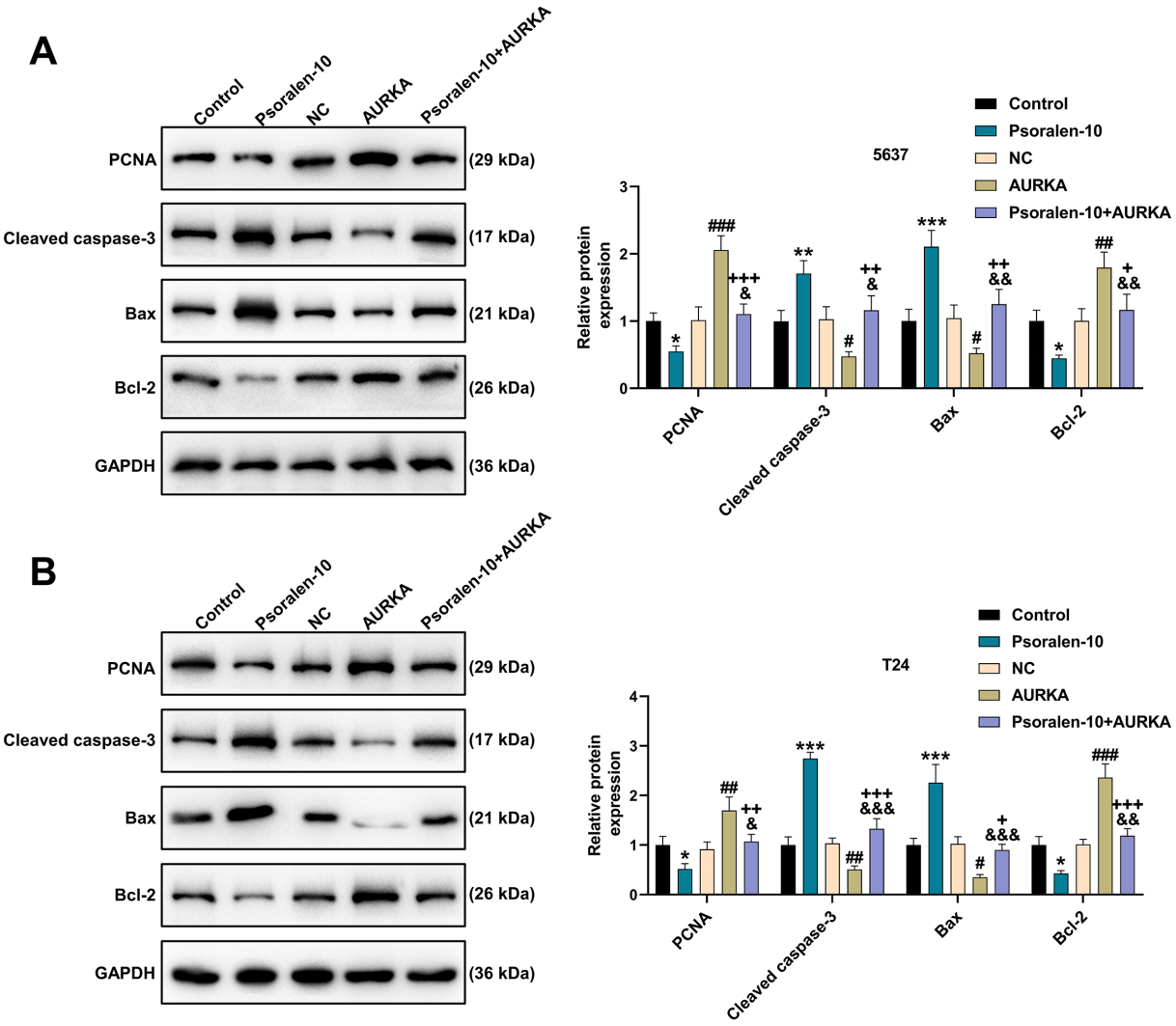

Fig. 6. AURKA overexpression antagonized psoralen-induced effects on proliferation or apoptosis-related molecules in BC cells. (A,B) PCNA, Cleaved caspase-3, Bax and Bcl-2 expression levels in AURKA overexpression plasmid-transfected, psoralen (10 μg/mL)-induced BC (5637 (A) and T24 (B)) cells (Western blotting, GAPDH as a reference gene). Data are mean ± standard deviation (n = 3 independent biological replicates). $^{*}p$ or $^{\#}p$ or $^{\&}p$ or $^{+}p < 0.05$; $^{**}p$ or $^{\#\#}p$ or $^{\&\&}p$ or $^{++}p < 0.01$; $^{***}p$ or $^{\#\#\#}p$ or $^{\&\&\&}p$ or $^{+++}p < 0.001$; * vs Control; $^{\#}$ vs NC; $^{\&}$ vs Psoralen; $^{+}$ vs AURKA. BC, bladder cancer; AURKA, aurora A kinase; qRT-PCR, quantitative reverse transcription polymerase chain reaction; PCNA, proliferating cell nuclear antigen; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Nevertheless, there are still some limitations in this study. AURKA rescue experiment lacks a matching control "Psoralen + NC" group, which means the influence of the transfection vector or the transfection process itself cannot be completely excluded. Additionally, the conclusion that psoralen directly targets AURKA is primarily based on database prediction and overexpression rescue experiments, whereas direct binding evidence (e.g., SPR, CETSA, or molecular docking) has not been obtained. Validation using AURKA-specific inhibitors or siRNA-mediated knockdown, as well as exploration of downstream signaling pathways, are also lacking. Future research should explore its long-term toxicity *in vivo*, the direct interaction between psoralen and AURKA, and the underlying downstream mechanisms.

Conclusion

Our findings demonstrate that psoralen may exert anti-proliferative and pro-apoptotic effects in BC cells *in vitro*, potentially through AURKA inhibition. These results suggest that psoralen warrants further investigation as a promising natural compound for BC therapy, and further *in vivo* studies are required to confirm its therapeutic potential.

Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

ZW is the sole contributor to this manuscript. The author confirms sole responsibility for the conception and design of the study; the acquisition, analysis, and interpretation of data; the preparation of the manuscript; final approval of the version to be published; and for being accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The author declares no conflict of interest.

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