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Original Articles

Activation of PSGR with β-ionone suppresses prostate cancer progression by blocking androgen receptor nuclear translocation

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ABSTRACT

The prostate-specific G protein-coupled receptor (PSGR) is a class A G protein-coupled receptor (GPCR) that is specifically expressed in prostate epithelial cells, and its expression has been linked to prostate cancer (PCa) progression. Here, we show that activation of PSGR with its ligand β-ionone, an end-ring analog of β-carotenoid, can suppress PCa cell growth both *in vitro* and *in vivo* model. Dissection of the mechanism underlying this relationship reveals that activation of PSGR by β-ionone suppresses AR nuclear translocation via phosphorylation of AR at residue Ser650 by p38 and JNK, which leads to the suppression of AR transactivation, further suppressing PCa cell growth. Overall, we link a cancer cell-specific GPCR with the nuclear AR and show that targeting PSGR can provide us a new target to combat PCa better.

1. Introduction

Prostate cancer (PCa) is the most commonly diagnosed cancer in males worldwide [1], and its progression is linked to androgen receptor (AR) signaling [2,3]. Targeting androgens with androgen deprivation therapy (ADT) is a standard treatment for advanced PCa that can successfully prolong survival rates [4]. However, several side effects, including decreased libido and fertility, osteoporosis and metabolic disorders, can be induced by ADT and remain a concern for many patients [5–9]. Furthermore, most patients fail to respond to ADT after an average of 12–18 months, and their tumors progress into the castration-resistant stage [10–12]. The development of new therapies that can suppress AR signaling with few side effects and that can overcome castration resistance is therefore needed to better suppress PCa at late castration-resistant stages.

Early studies have indicated that GPCRs can play tumor promoting or suppressive roles in the presence of excess ligand [13–15]. Most GPCRs expressed in prostate cancer cells can stimulate ERK [16]. ERK, in turn, can phosphorylate the AR at several sites, thereby increasing the transcription of AR target genes. GPCRs that signal through Gαs, such as the prostaglandin E2 (PGE2) receptors EP2 and EP4 and the β-adrenergic receptor, can also stimulate the AR through the accumulation of cAMP and protein kinase A (PKA) activation, therefore synergizing with low levels of androgens to activate AR [17]. Thrombin, angiotensin II, bradykinin, endothelin and LPA receptors are overexpressed in prostate cancer and share the ability to stimulate RHOA through Gαq and/or $G\alpha_{13}$. In turn, Rho GTPases seem to have a common role in their ability to promote prostate cancer cell growth [18].

Yet, despite the association between GPCRs and cancer progression and the fact that GPCRs are one of the most 'druggable' classes of molecules, with therapeutics targeting these proteins representing approximately 25% of the market, there are relatively few cancer treatments targeting GPCRs [19].

The prostate-specific G protein-coupled receptor (PSGR) is a class A GPCR that was initially identified as a prostate-specific tumor biomarker [20–22]. It is specifically expressed in prostate epithelial cells, and its expression increases significantly in human prostate intraepithelial neoplasias and prostate tumors; however, the expression level of PSGR shows an inverse relationship with Gleason score, suggesting that PSGR may play a suppressive role in prostate cancer progression [23]. Activation of PSGR signaling can reduce the growth of prostate cancer cells [24].

β-Ionone, an end-ring analog of β-carotenoid, forms a subclass of cyclic isoprenoids and is present mainly in grapes and wine aromatizers [25]. β-Ionone is also a specific ligand for PSGR [24].

Here, we found that PSGR, after activation via its ligand β-ionone, can suppress AR-positive PCa cell growth. The mechanism underlying

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this function may be related to the phosphorylation of AR at residue Ser650 by p38 and JNK, resulting in the suppression of AR translocation and transactivation. Our results provide a novel approach towards suppressing PCa progression by targeting PSGR that incurs very few side effects compared to classic ADT.

2. Materials and methods

2.1. Human specimens

Normal renal, bladder, and prostate tumor specimens and adjacent normal prostate tissues were collected from patients at the First Affiliated Hospital, Xi'an Jiaotong University, China. All specimens were obtained on the basis of their availability for research purposes and under a protocol approved by the local Medical Ethics Committee of the First Affiliated Hospital, Xi'an Jiaotong University, China.

2.2. Cell culture

LNCaP, DU145, PC-3 and CWR22Rv1 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS). The C4-2 cell line was a gift from Dr. Jer-Tsong Hsieh (Southwestern Medical Center) and was grown in RPMI-1640 media containing 1% penicillin and streptomycin, and 10% FBS.

2.3. Reagents and materials

GAPDH (6c5), PSGR, PSA and AR (N-20) antibodies were purchased from Santa Cruz Biotechnology (Paso Robles, CA). FKBP5, Histone H3, p-P38 and p-JNK antibodies were purchased from Abcam (San Diego, CA). Androgen Receptor (phospho Ser650) antibody was purchased from GeneTex (Radnor, PA). The $Ca2^+$ indicator fluo-3/AM was purchased from Life Technologies (Grand Island, NY). β-Ionone (I12603) and the Nuclei EZ Prep Nuclei Isolation Kit (NUC101) was purchased from Sigma (St. Louis, MO). The p38 inhibitor SB203580 and the JNK inhibitor SP600125 were purchased from Cell Signaling Technology (Boston, MA).

2.4. Cell $Ca2^+$ imaging

Cells were incubated (40 min/37 °C) in PBS containing 5 mM Fura-3 AM. After removal of extracellular Fura-3 AM, the cells were washed with PBS. For $Ca2^+$-detection experiments, the cells were treated with 100 μM β-ionone and then observed under a confocal fluorescence microscope at 0 min, 30 min and 60 min post-treatment.

2.5. MTT assay

PCa cells were counted and plated at a density of 5000 per well in 24-well plates. The cells were then treated with β-ionone at different concentrations. On days 0, 2, 4, and 6, the cells were submitted to an MTT assay.

2.6. RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated using TRIzol reagent (Invitrogen, Grand Island, NY). One microgram of total RNA was subjected to reverse transcription using Superscript III transcriptase (Invitrogen, Grand Island, NY). Quantitative real-time PCR (qRT-PCR) was performed using a Bio-Rad CFX96 system with SYBR green to determine the mRNA expression levels of genes of interest. The sequences of the primers for AR, PSR, PSA and FKBP5 PCR amplification were 5′- CCAGGGACCAT GTTTTGCC-3' (forward) and 5′- CGAAGACGACAAGATGGACAA-3' (reverse) (AR), 5′- ACTTCACACATGCCACCTTTG-3' (forward) and 5′-GAAGACCACGATGCAGTTTCC-3' (reverse) (PSGR), 5′- GGCAGCATTG AACCAGAGG-3' (forward) and 5′-GAAGCACACCATTACAGACAAG -3' (reverse) (PSA), 5′- CTCCCTAAAATTCCCTCGAATGC − 3' (forward) and 5′- CCCTCTCCTTTCCGTTTGGTT-3' (reverse) (FKBP5), respectively. All the experiments were performed in triplicate. Expression levels were normalized to the expression of GAPDH RNA.

2.7. Nuclear and cytoplasmic protein extraction

After treatment, the cells were washed with PBS and collected using Nuclei EZ Prep Nuclei Isolation Kit according to the manufacturer's instructions. The protein concentration was normalized using the BCA assay. The collected cytoplasmic and nuclear extracts were subjected to western blotting.

2.8. Western blot analysis

Cells were lysed in RIPA buffer, and proteins (20 μg) were separated on 8–10% SDS/PAGE gels and then transferred onto PVDF membranes (Millipore, Billerica, MA). After blocking the membranes, they were incubated with the appropriate dilutions (1:1000) of specific primary antibodies. Then, the blots were incubated with HRP-conjugated secondary antibodies and visualized using an ECL system (Thermo Fisher Scientific, Rochester, NY).

2.9. Immunofluorescence staining

Cells were plated in an 8-well Lab-Tek II Chamber Slide. After treatment, the cells were fixed in 4% paraformaldehyde. The cells were incubated with AR antibody and Alexa Fluor 594-conjugated goat anti-rabbit IgG. Permafluor mountant (Thermo) was added and coverslips were applied and sealed. Finally, the cells were observed under a Nikon confocal microscope, and images were captured.

2.10. Luciferase reporter assays

Cells were plated in 24-well plates and transfected with androgen response element (ARE)-luc pGL3 and pRL-TK-luciferase plasmids using lipofectamine (Invitrogen) according to the manufacturer's instructions. The cells were lysed, and luciferase activity was detected using a dual luciferase assay with pRL-TK-luciferase as an internal control. Each sample was normalized to the activity of pRL-TK-luciferase, and data are presented as the mean ± SE from at least three independent experiments.

2.11. Construction of an androgen receptor Ser650-mutant plasmid and shRNA

A PWPI vector was submitted to a blunt-end digestion with Pac I and Pme I. We replaced the AGC codon encoding serine 650 with a GCC codon to mutate the serine to alanine. To accomplish this, we designed the following primers to clone two overlapping fragments: the inner mutant primer F: GCTTCCAGCACCACCGCCCCCACTGAGGAG, the full-length AR primer R: TCCTGCAGCCCGTAGTTTTCACTGGGTGTGGAAA TAGA, the inner mutant primer R: CTCCTCAGTGGGGGCGGTGGTGCT GGAAGC and the full-length AR primer F: GTGAGGAATTTCGACATTT AAATTTAATGGAAGTGCAGTTAGGGCTGGGAAGGGTC. We inserted these two fragment into a PWPI vector using the Gibson assembly method. PSGR shRNA (shPSGR) contructed with target sequence 5′-GCAGTGCTCAACAATACAGTA-3′ according to Addgene's pLKO.1 protocol.

2.12. In vivo studies

Male 6- to 8-week-old nude mice were used. Twelve mice were subcutaneously injected with C4-2 cells (1×10^6 cells in a 1:1 mixture with Matrigel) and then divided into two groups: one group was treated

with β-ionone in the solvent of corn oil and the other group was treated with corn oil only. Two weeks after the injection of C4-2 cells, the mice in the treatment group were intraperitoneally injected with β-ionone (75 mg/kg) every other day. Tumor growth in live mice was monitored, and the mice were later sacrificed after another 4 weeks, and their tissues were harvested for further examination by IHC staining. All animal studies were performed under the supervision and guidelines of the Xi'an Jiaotong University Animal Care and Use Committee.

2.13. IHC staining

Mouse prostate tissues were fixed in 10% (v/v) formaldehyde in PBS, embedded in paraffin, and cut into 5-μm sections. The prostate sections were deparaffinized in xylene solution and rehydrated using a gradient of ethanol concentrations, after which immunostaining was performed. A semiquantitative scoring system was used to evaluate the expression level as follows. The proportion of epithelial cells staining positively was termed category A and was assigned scores from 0 to 4 (A = 0 (0%); 1 (< 25%); 2 (25–50%); 3(50–75%); 4(> 75%). Intensity of staining was termed category B and was scored as 0 (negative); 1 (weak); 2 (moderate); 3 (strong). A final score was calculated by A plus B [26].

2.14. Statistics

All statistical analyses were carried out with SPSS 19.0 (SPSS Inc, Chicago, IL). The data were presented as the mean ± SEM. Differences in the mean values between two groups were analyzed using a two-tailed Student's *t*-test, and the means of more than two groups were compared with one-way ANOVA. $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Differential expression of PSGR and AR in various PCa cell lines

Using a qPCR assay and western blotting to examine the expression of PSGR and AR in various PCa cell lines, including the LNCaP, C4-2, PC-3, DU145 and CWR22Rv1 (22Rv1) cell lines, we found that PSGR had high expression in the LNCaP, C4-2 and DU145 cells and very low expression in the PC-3 and 22Rv1 cells (Fig. 1A). In contrast, high AR expression was detected in the LNCaP, C4-2 and 22Rv1 cells, and very low AR expression was detected in the DU145 and PC-3 cells (Fig. 1B).

Since PSGR is a class A G protein-coupled receptor (GPCR). Stimulation of GPCRs could form a second messenger, inositol 1,4,5-triphosphate (IP3), which could result in an efflux of Ca2+ from the endoplasmic reticulum (ER) to the cytoplasm and an elevation of intracellular Ca2+, so activation of PSGR with its ligand β-ionone could also induce influx of Ca2+ in PSGR-positive cells [27]. To further verify the differential expression pattern of PSGR in the various PCa cell lines and its activation by the ligand β-ionone, we used immunofluorescence staining to visualize free $Ca2^{+}$ in the cells with the calcium-sensitive dye fluo-3-AM in the presence of the ligand. The results revealed that activation of PSGR by β-ionone could induce Ca^{2+} influx, as evidenced by the staining that was present only in the PSGR-positive cell lines, namely, the LNCaP, C4-2 and DU145 cell lines (Fig. 1C). These results agree well with the qPCR and Western blotting results presented in Fig. 1A.

In addition, IHC staining with a PSGR antibody was used to assay PSGR expression in PCa samples and normal prostate tissues. The results revealed a higher expression of PSGR in well-differentiated PCa compared with normal prostate tissues, and the expression of PSGR decreased in poorly differentiated PCa (Fig. 1D–G). We also examined PSGR expression in human normal bladder and renal tissues, but no expression of PSGR was found in these tissues (Supplementary Fig. 1).

3.2. Activation of PSGR by β-ionone suppresses AR-positive PCa cell growth

We next assayed how β-ionone-mediated activation of PSGR influences PCa cell growth using an MTT assay. The results revealed that the addition of β-ionone could suppress PCa cell growth only in cells with positive expression of both AR and PSGR, including the LNCaP and C4-2 cell lines (Fig. 2A). In contrast, the addition of β-ionone showed little effect on AR-negative/PSGR-positive PCa cells (DU145 cells), PSGR-negative/AR-positive PCa cells (22Rv1 cells) or PSGR-negative/AR-negative PCa cells (PC-3 cells) (Fig. 2A). We also found that β-ionone could suppress colony formation in LNCaP and C4-2 cells but not in DU145, 22Rv1 or PC-3 cells (Supplementary Fig. 2A). These results suggest that β-ionone may function first through activation of PSGR and then through AR signaling to suppress PCa cell growth.

To further confirm this conclusion, we stably transfected DU145 cells (AR-negative and PSGR-positive) with AR (Fig. 2B) and demonstrated that β-ionone was able to suppress AR-mediated increases in DU145 cell growth (Fig. 2C). We also stably transfected 22Rv1 cells (PSGR-negative and AR-positive) with PSGR (Fig. 2D) and demonstrated that β-ionone was able to suppress the growth of 22Rv1 cell with PSGR overexpression (Fig. 2E). In addition, LNCaP and C4-2 cells submitted to knockdown of PSGR expression showed less sensitivity to β-ionone compared with the corresponding parental cells (Supplementary Figs. 2B–C).

Together, the results shown in Fig. 2A–E and Supplementary Figs. 2A–C demonstrate that PCa cells positive for both AR and PSGR expression can be targeted by β-ionone, a ligand of PSGR.

3.3. Activation of PSGR by β-ionone decreases AR transactivation without influencing AR expression

To dissect why activation of PSGR by β-ionone requires the presence of AR, we first examined whether β-ionone-mediated activation of PSGR influences AR protein expression. Interestingly, we found that activation of PSGR in this context has little influence on AR expression at both the protein and mRNA levels in LNCaP and C4-2 cells (Fig. 3A–D). However, activation of PSGR by β-Ionone could suppress expression of the AR target genes FKBP5 and PSA at both the protein and mRNA levels in LNCaP and C4-2 cells (Fig. 3A–D).

We further employed an ARE-Luciferase assay to examine whether activation of PSGR by β-ionone could influence AR transactivation activity. The results revealed that activation of PSGR by β-ionone suppressed DHT-induced AR transactivation in LNCaP and C4-2 cells (Fig. 3E and F).

Together, the results shown in Fig. 3A–F suggest that activation of PSGR by the ligand β-ionone can decrease AR transactivation without influencing AR expression.

3.4. Dissection of the mechanism underlying how β-ionone-mediated PSGR activation suppresses AR transactivation

We first examined whether activation of PSGR by the ligand β-ionone could influence AR nuclear translocation. As shown in Fig. 4A, the addition of DHT led to AR nuclear translocation in LNCaP cells. Importantly, adding β-ionone to activate PSGR suppressed this DHT-induced AR nuclear translocation in LNCaP cells (Fig. 4A). Similar results were obtained using C4-2 cells (Fig. 4A). We also applied the Western blotting assay to observe the AR distribution in cytosol and nuclei in these two cell lines with the treatment of DHT and β-ionone to activate PSGR, and we obtained similar results (Fig. 4B).

Early studies have suggested that phosphorylation of AR at residue Ser650 by p38 and JNK could suppress AR nuclear translocation [24,28]. We were interested to determine whether activation of PSGR by the ligand β-ionone could influence AR nuclear translocation through the activation of the p38 and JNK signaling pathways. The results revealed that activation of PSGR by β-ionone increased the phosphorylation (Fig. 4C) of residue Ser650 in AR in both LNCaP and C4-2 cells based on the detection of antibodies specific for Ser650

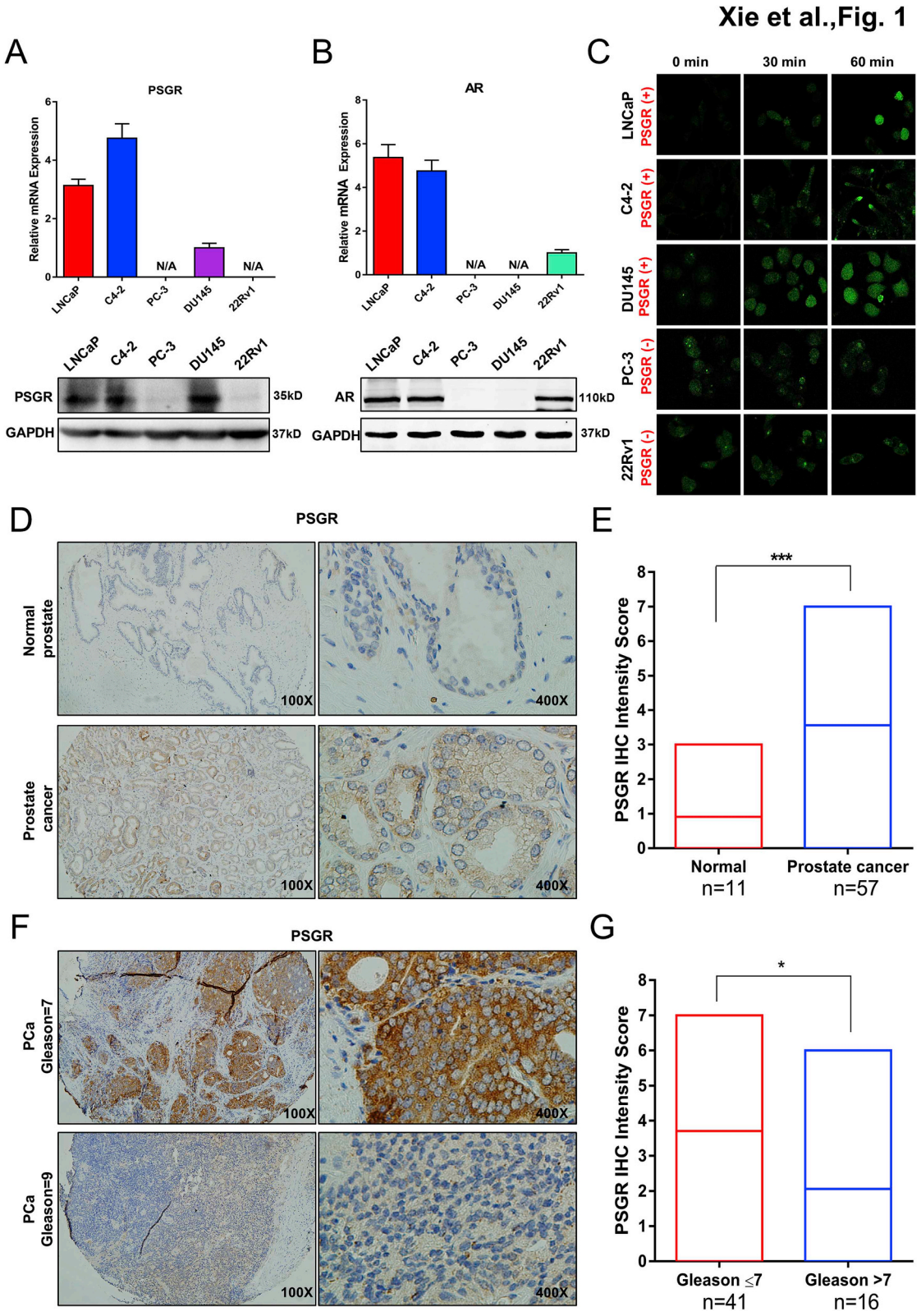

Fig. 1. Differential expression of PSGR and AR in various PCa cell lines. (**A**) Q-PCR and Western blotting results showing the expression levels of PSGR in the PCa cell lines LNCaP, C4-2, PC-3, DU145 and CWR22Rv1 (22Rv1). (**B**) Q-PCR and Western blotting results showing the expression levels of AR in the PCa cell lines LNCaP, C4-2, PC-3, DU145 and 22Rv1. (**C**) Immunofluorescence staining of free intracellular Ca^{2+} using the calcium-sensitive dye fluo-3-AM. (**D, E**) IHC staining with a PSGR antibody to show PSGR expression in human normal prostate tissues and PCa tissues. ***P $<$ 0.001. (**F, G**) IHC staining with a PSGR antibody to show PSGR expression in human PCa tissues with different Gleasons. *P $<$ 0.05.

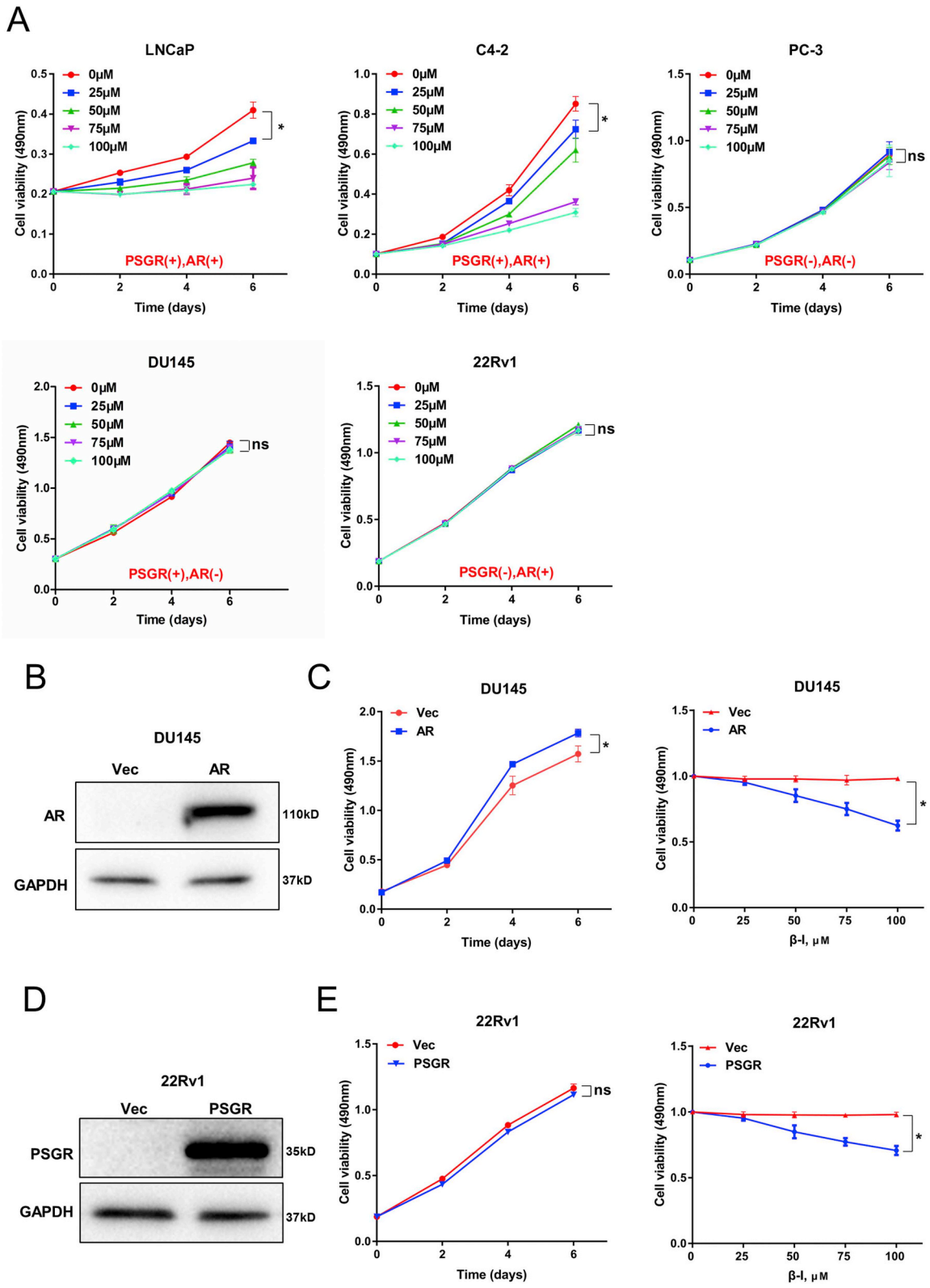

Fig. 2. Activation of PSGR by β-ionone suppresses the growth of AR-positive PCa cell lines. (**A**) An MTT assay was used to determine the effects of different concentrations of β-ionone on PCa cell growth. *$p < 0.05$. ns, no statistical differences. (**B**) Western blotting results showing AR expression after the overexpression of AR in the DU145 cell line. (**C**) MTT assay results showing the effect of AR overexpression on DU145 cell growth and activation of PSGR could inhibit DU145 ($PSGR^+$) cell growth after overexpression of AR. *$p < 0.05$. (**D**) Western blotting results showing PSGR expression after the overexpression of PSGR in the 22Rv1 cell line. (**E**) MTT assay results showing the effect of AR overexpression on 22Rv1 cell growth and activation of PSGR could inhibit 22Rv1 (AR^+) cell growth after overexpression of PSGR. *$p < 0.05$. ns, no statistical differences.

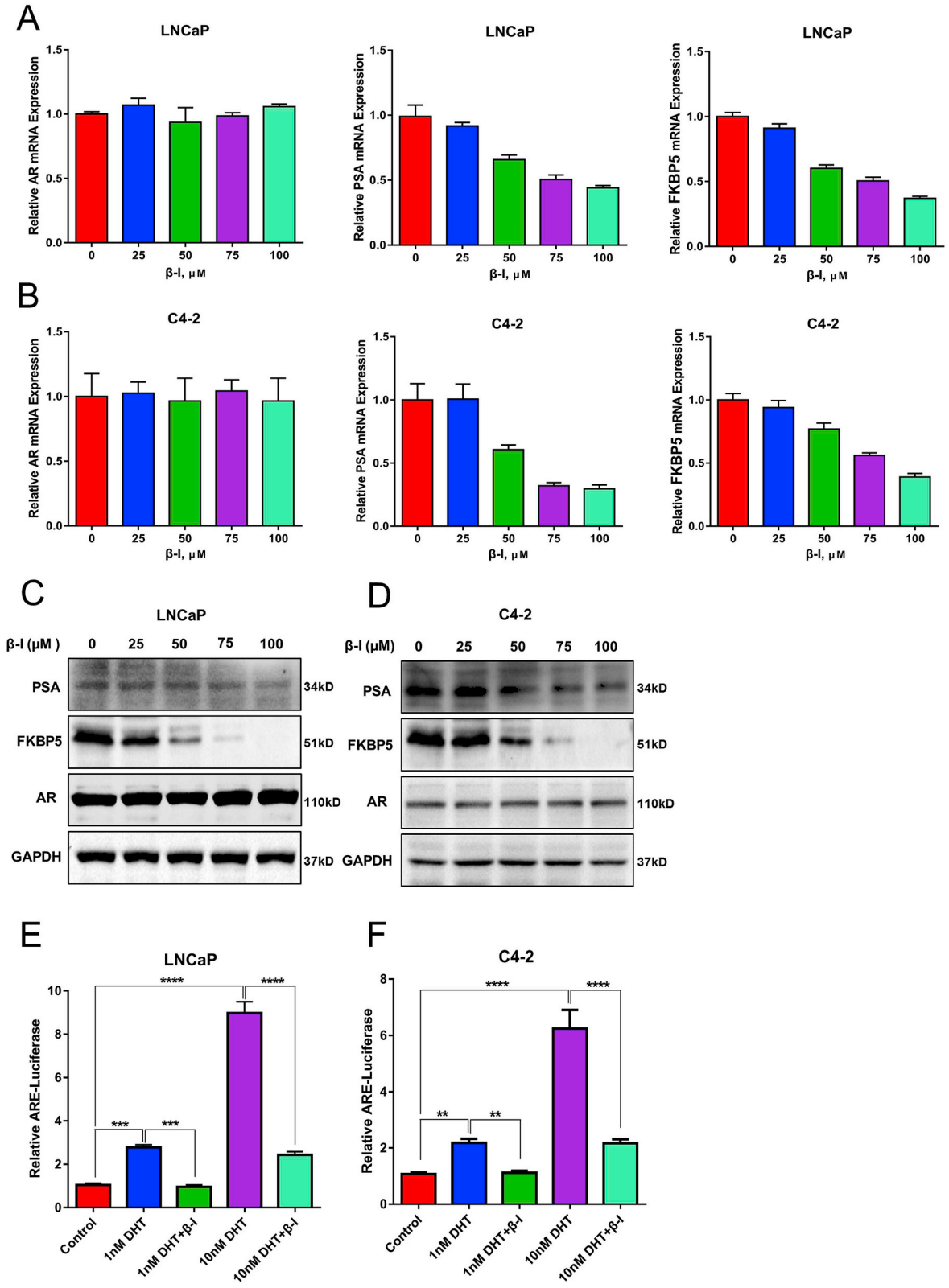

Fig. 3. Activation of PSGR by β-ionone decreases AR transactivation without influencing AR expression. (**A, B**) Real-time PCR to show the mRNA levels of FKBP5, PSA and AR in the LNCaP and C4-2 cell lines with treatment of β-ionone for 48 h. (**C, D**) Western blotting to show the protein levels of FKBP5, PSA and AR in the LNCaP and C4-2 cell lines with treatment of β-ionone for 48 h. (**E, F**) ARE-Luciferase reporter assay results showing AR transactivation in the LNCaP and C4-2 cell lines. **P < 0.01, ***P < 0.001, ****P < 0.0001.

phosphorylation.

Importantly, activation of PSGR by β-ionone also led to activation of the p38 and JNK signaling pathways in both LNCaP and C4-2 cells (Fig. 4D). Similar results were obtained in AR-negative/PSGR-positive PCa cells (DU145 cells) (Supplementary Fig. 3A). Furthermore, addition of the p38 inhibitor SB203580 or the JNK inhibitor SP600125

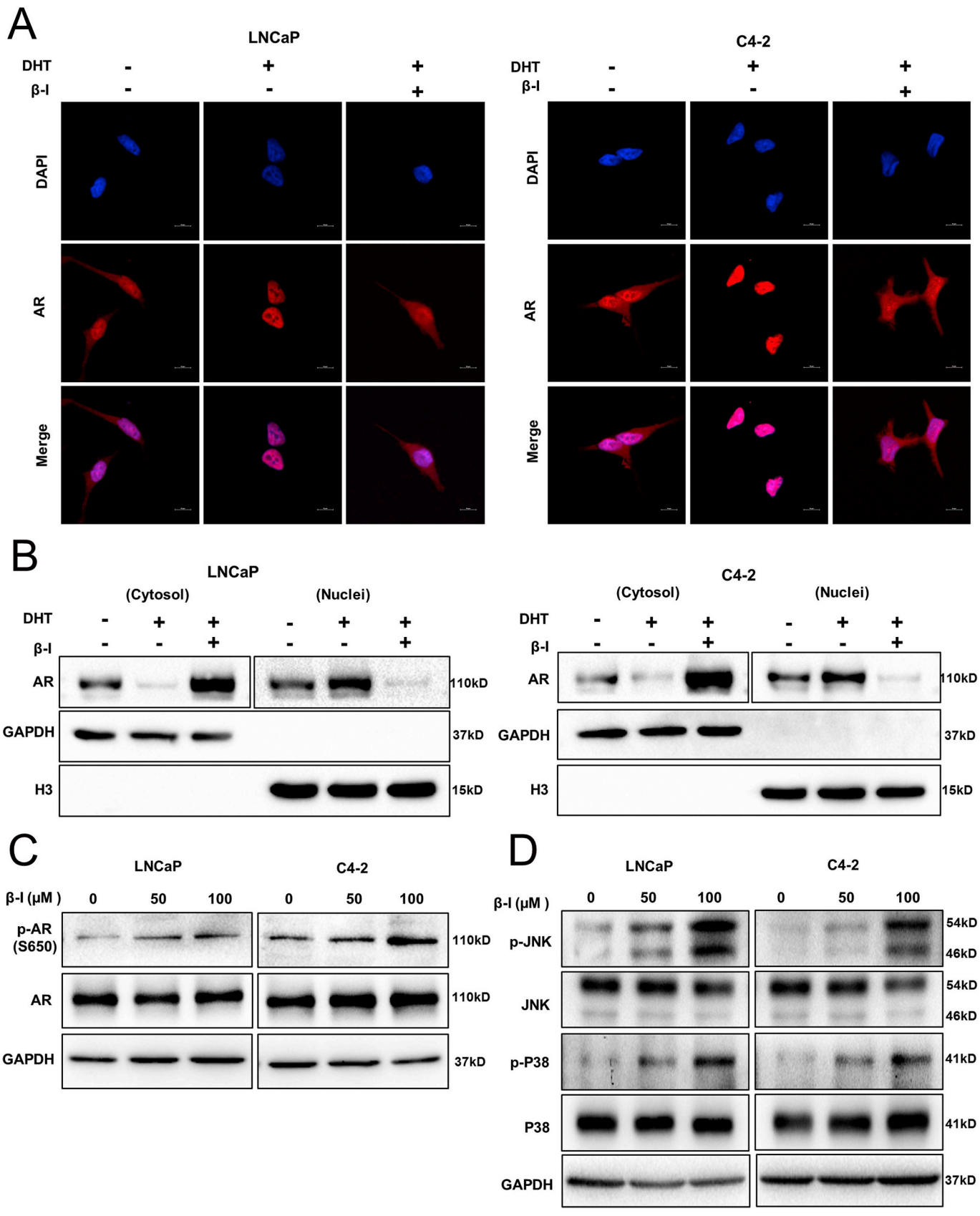

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Fig. 4. Dissection of the mechanism underlying how activation of PSGR by β-ionone leads to suppression of AR transactivation. (**A**) Immunofluorescence staining (200x) showing AR nuclear translocation in the LNCaP and C4-2 cell lines. (**B**) Western blot analysis of nuclear and cytoplasmic fractions from LNCaP and C4-2 cells with the treatment of DHT (10 nM) or β-ionone (100μM) to show AR distribution in nuclei and cytosol. GAPDH and Histone H3 (H3) served as the fractionation controls. (**C**) Western blotting results showing phosphorylation of residue Ser650 in AR with treatment of β-ionone. (**D**) Western blotting results to show p38 and JNK phosphorylation in LNCaP and C4-2 cell lines with treatment of β-ionone. (**E, F**) Western blotting to show AR Ser650 phosphorylation and PSA expression in the LNCaP and C4-2 cell lines treated with DHT (10 nM), β-ionone (100μM), the p38 inhibitor SB203580 (p38i) or the JNK inhibitor SP600125 (JNKi). (**G, H**) Immunofluorescence staining (200x) to show AR distribution in the LNCaP and C4-2 cell lines treated with DHT (10 nM), β-ionone (100μM), the p38 inhibitor SB203580 (p38i) or the JNK inhibitor SP600125 (JNKi). (**I**) The effects of DHT (10 nM) and activation of PSGR by β-ionone (100μM) on AR distribution in the DU145/AR (wild type AR) and DU145/AR-Mutant (mutation of AR Ser650 to alanine) cell lines.

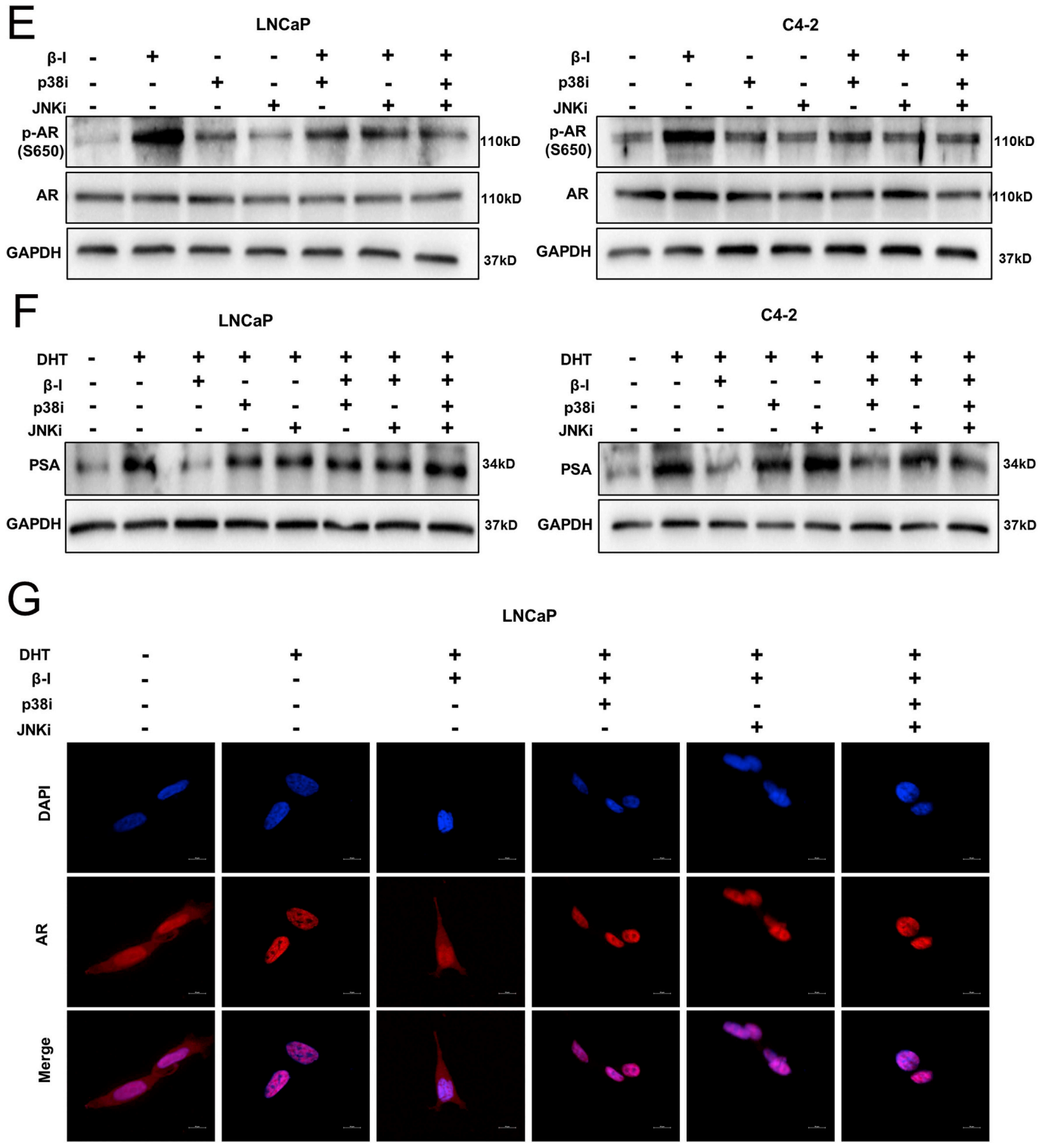

Fig. 4. (*continued*)

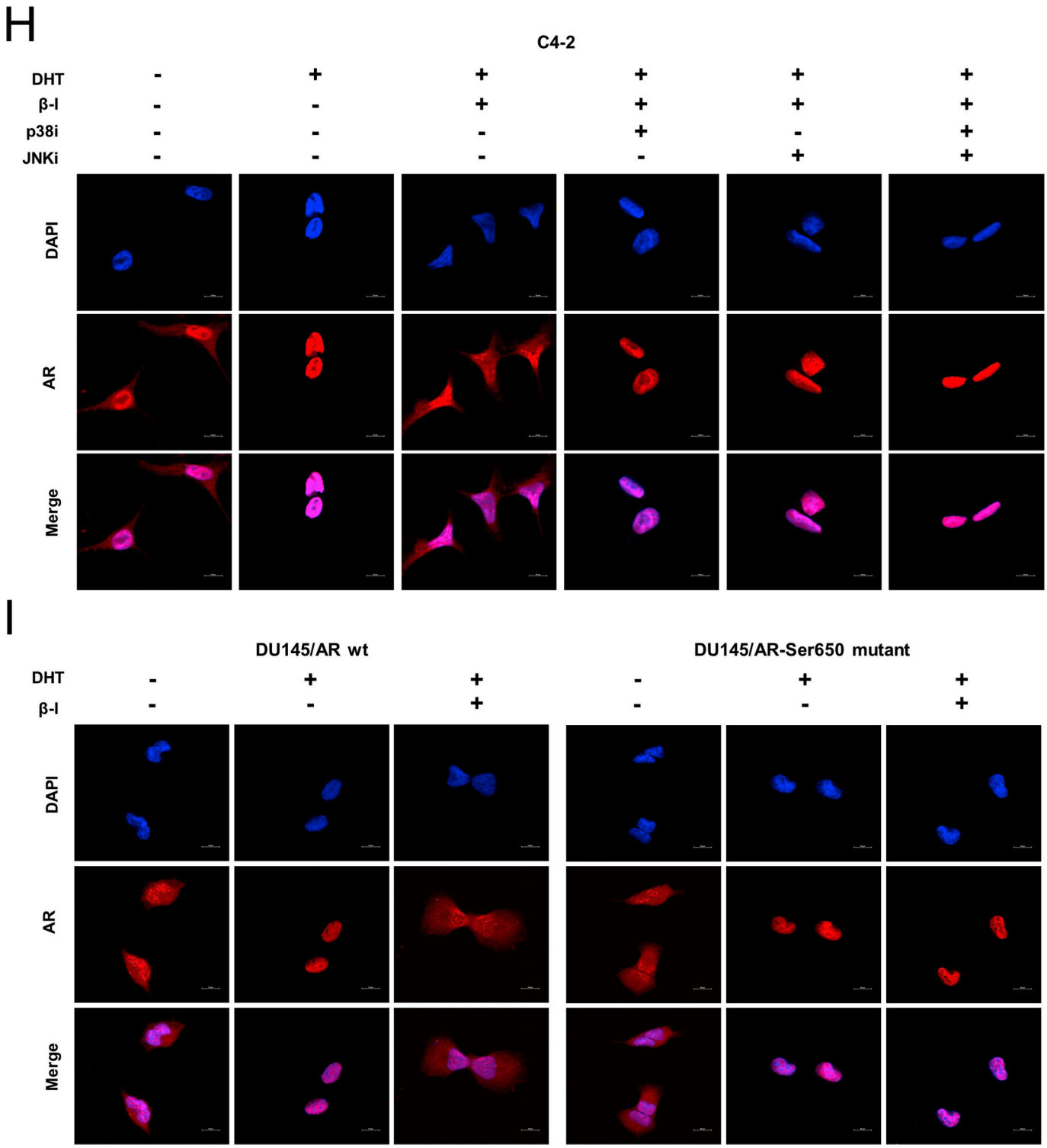

Fig. 4. (*continued*)

suppressed the phosphorylation of residue Ser650 in AR and PSA expression decrease induced by PSGR activation (Fig. 4E and F). More importantly, these inhibitors also reversed β-ionone-induced cytosolic translocation of AR (Fig. 4G and H).

Finally, transfection of the PSGR-positive DU145 cells with wild-type AR or a Ser650-mutant AR (from serine to alanine) that could no longer be phosphorylated by JNK and p38 revealed that activation of PSGR by β-ionone no longer suppressed DHT-induced Ser650-mutant AR nuclear translocation (Fig. 4I), and DU145 cells with a Ser650-mutant AR showed less sensitivity to β-ionone compared with the corresponding parental cells with wild-type AR (Supplementary Fig. 3B).

Together, the results in Fig. 4A–I and Supplementary Figs. 3A–B shows that activation of PSGR by β-ionone can suppress AR nuclear translocation by leading to the activation of the p38 and JNK signaling pathways, which results in the phosphorylation of AR at residue Ser650. This suppression of AR nuclear translocation may then lead to suppression of AR transactivation, inhibiting PCa cell growth.

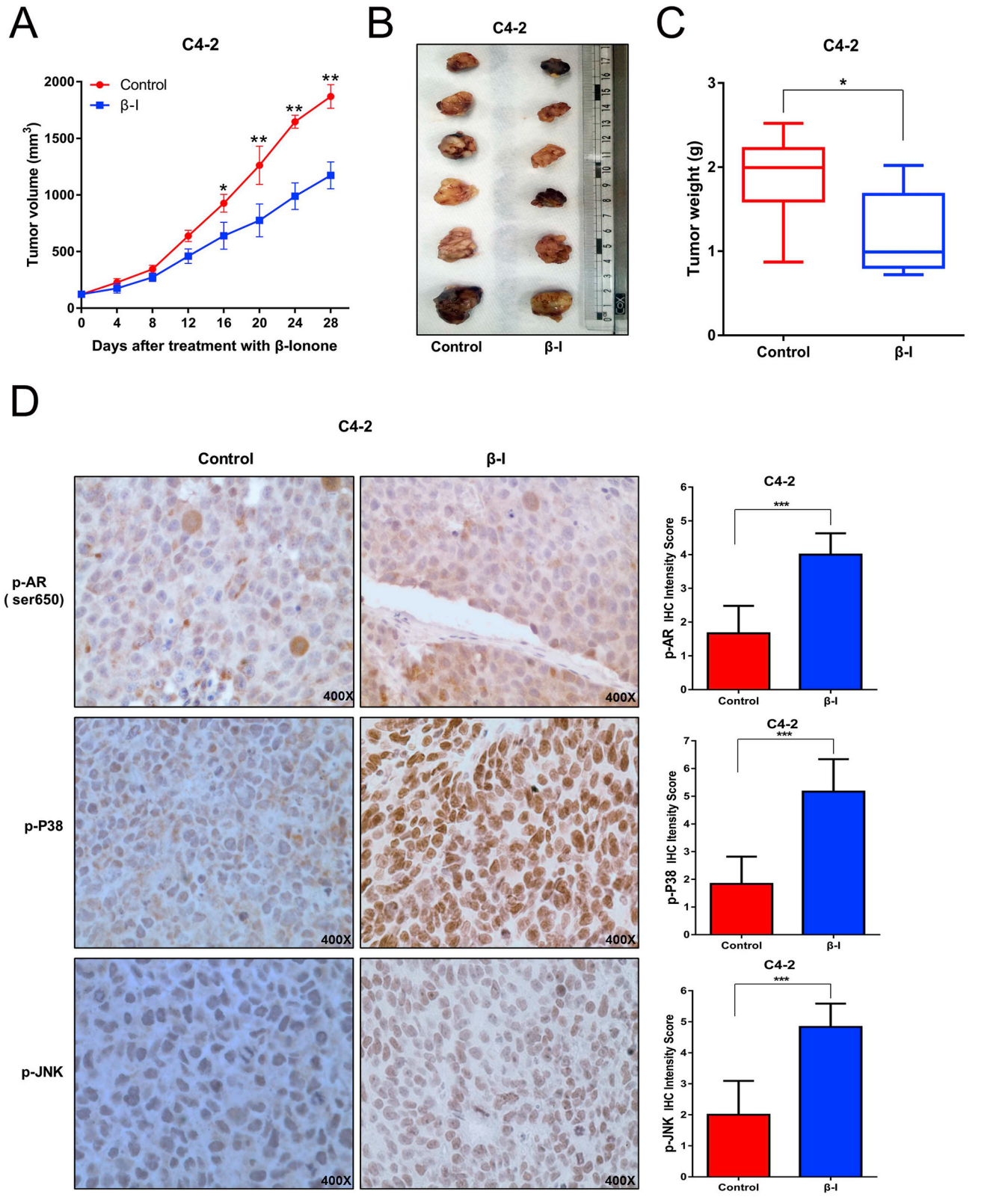

Fig. 5. Activation of PSGR by β-ionone leads to suppression of PCa progression *in vivo*. (**A**) The growth curve of PCa in the control group and the β-ionone treatment group. *$p < 0.05$, **$p < 0.01$. (**B, C**) The volumes and weights of PCa tissues collected from the control group and the β-ionone treatment group. *$p < 0.05$. (**D**) IHC staining of p-AR (Ser650), p-P38 and p-JNK in PCa tissues from the two groups. The right panels show quantitative data. ***$p < 0.001$.

3.5. Activation of PSGR by β-ionone suppresses PCa progression in vivo

To recapitulate the *in vitro* results described above *in vivo* using an animal model, we subcutaneously injected C4-2 cells into 6- to 8-week-old male nude mice. After 2 weeks, the mice were divided into two groups: the mice in the treatment group were intraperitoneally injected with β-ionone (75 mg/kg) in the solvent of corn oil every other day for another 4 weeks [29,30], and the other group was treated with corn oil

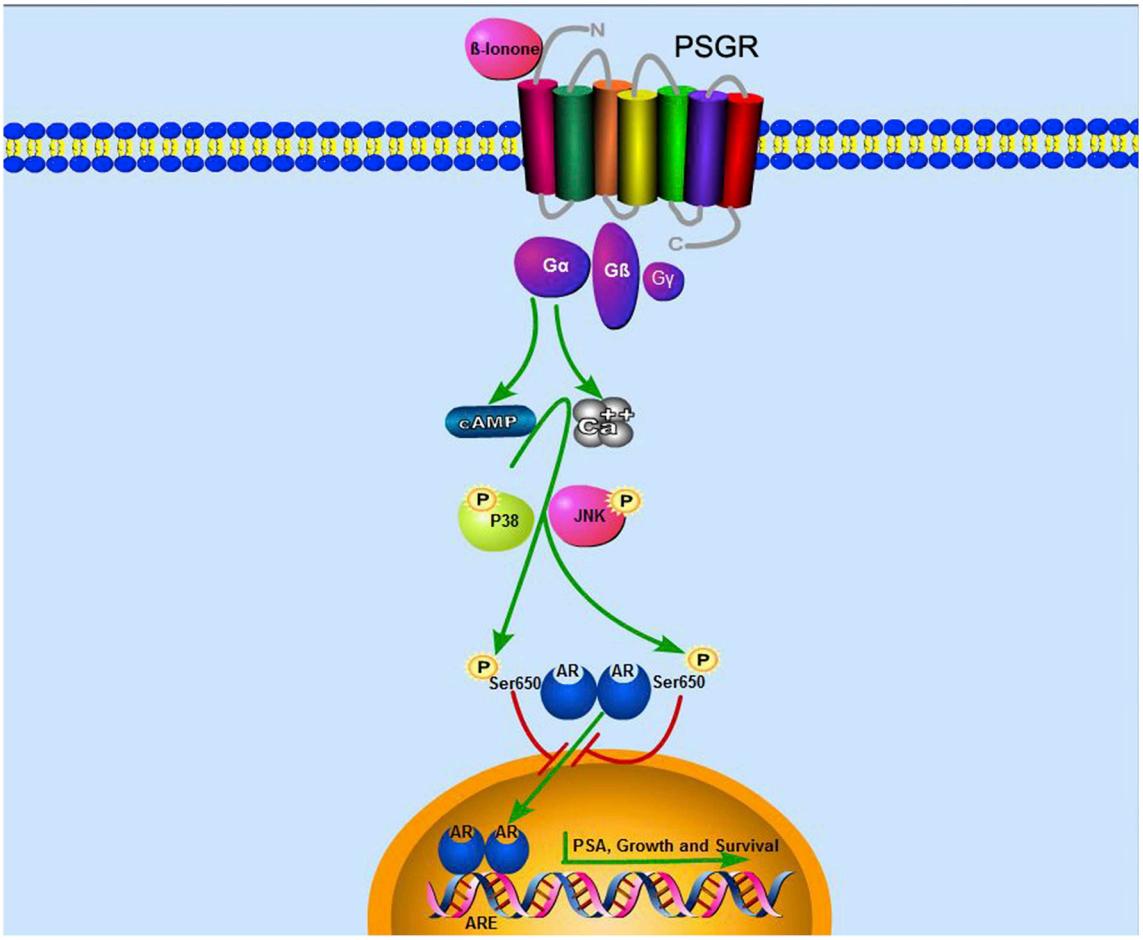

Fig. 6. Mechanism underlying how β-ionone-induced PSGR activation leads to suppression of PCa progression. Activation of PSGR with β-ionone suppresses PCa progression by blocking AR nuclear translocation via activation of the p38 and JNK signaling pathways.

only. After the treatment period, the mice were sacrificed. The results revealed that β-ionone suppressed tumor growth (Fig. 5A), the mice in the control group had larger tumor volumes and weights compared with those in the β-ionone treatment group (Fig. 5B and C). Importantly, IHC staining showed that the expression levels of phosphorylated AR (Ser650), p-P38, and p-JNK were in agreement with the above *in vitro* studies. Overall, these experiments show that activation of PSGR by β-ionone leads to the suppression of AR transactivation through the activation of the p38 and JNK signaling pathways, resulting in the phosphorylation of AR at residue Ser650 (Fig. 5D).

4. Discussion

Although ADT combined with anti-androgen treatment is the current standard for advanced PCa and has shown success in prolonging the survival of patients with PCa, resistance to such therapy can develop, which may be linked to alterations in AR signaling [31]. Furthermore, the systematic suppression of androgen/AR binding causes problematic side effects, including reduced libido and fertility, osteoporosis and metabolic disorders [10–12], because AR signaling is involved in important physiological functions outside of the prostate [32–34]. Thus, great concern remains regarding these treatments.

GPCRs that are excessively activated in PCa have been identified in basic and clinical research. These proteins lead to androgen-independent AR activation, thus sustaining androgen-independent growth of PCa cells [35], either due to the presence of abnormally high levels of ligands for these GPCRs [36] or to overexpression of the GPCRs, such as for endothelin A receptor [37], bradykinin1 receptor [38], and thrombin receptor [39]. However, PSGR is specifically expressed in prostate epithelial cells, and its expression has been linked to PCa development [20–22]. Specifically, the activation of PSGR could suppress prostate cancer due to the resulting inhibition of AR activity. Thus, any potential therapy based on targeting PSGR should produce minimal side effects due to the minimal influence on organs other than the prostate, as only the prostate expresses PSGR.

Recent studies have demonstrated anti-proliferative and apoptosis induction properties of β-ionone in prostate cancer [40–42], which may be via regulating cyclin D1 expression or as an antagonist of AR to suppress PCa [42,43], However, the mechanism underlying it is still unclear. Here we found β-ionone can be as a ligand to activate PSGR to suppress prostate cancer. The anti-tumor effects of β-ionone would be limited to AR+/PSGR + prostate cancer, and may be weakened in prostate cancer with higher Gleason score due to reduced expression of PSGR. However, we still found PSGR expression in prostate cancer with Gleason score higher than 7 in our data, so β-ionone treatment may still elicit anti-tumor effects in prostate cancer with high Gleason score besides low Gleason score. In dissecting the mechanism underlying the above observations, we found that activation of PSGR with β-ionone results in the activation of the p38 and JNK signaling pathways. p38 and JNK are two main members of the MAPK family, which mediates intracellular signaling associated with a variety of cellular activities, including cell proliferation, differentiation, survival, death, and transformation [44,45]. The JNK and p38 signaling pathways are activated not only by proinflammatory (IL-1, IL-6 or TNFα) or anti-inflammatory (EGF or TGF-β) cytokines but also in response to cellular stresses, such as genotoxic, hypoxic, osmotic, or oxidative stress [46,47].

Proapoptotic roles for both p38 and JNK have been established in PCa, and the signaling mediated by these proteins can be induced by 2-methoxyestradiol [48], gamma-tocotrienol [49], melatonin [50] or protoapigenone [51]. It is interesting to note that androgen deprivation, the most common therapy used to treat advanced PCa, may elicit apoptosis via the activation of JNK [52]. In the context of its pro-apoptotic role, JNK has been shown to trigger apoptosis through reactive oxygen species (ROS) production in PCa cells [53,54]. ROS may also induce apoptosis through JNK activation [55]. Here, we showed that PSGR can activate both p38 and JNK, which is consistent with a previous study [24], leading to phosphorylating residue Ser650 in the AR. This phosphorylation inhibits AR-mediated transcriptional activity by promoting its nuclear export, leading to suppression of PCa. Although there are some reports showing that activation of p38 and JNK could affect cell proliferation, differentiation, survival, death by a variety of other signals except AR signal [28,44,45], and here we found that activation of p38 and JNK could suppress AR activity to induce growth inhibition, which is furthermore proved with that mutation of residue Ser650 can reverse PSGR-induced suppression of AR activity, also consistent with a previous report [28].

Here, we show activation of PSGR with β-ionone can suppress PCa cells growth, which is consistent with previous two independent reports [24,56], but seems to contradict with two reports of the same group, in which they show overexpression of PSGR can promote PCa cells growth [57,58]. Regarding to this difference, we consider that PSGR can play a suppressive role in PCa growth with activation by β-ionone, however, It may also play a promoting role in PCa growth with other ligands different from β-ionone.

In summary, our results show that activation of PSGR by β-ionone can lead to the suppression of PCa progression by blocking AR nuclear translocation (Fig. 6), which may provide a new therapeutic approach for combatting PCa through the targeting of this newly identified signaling mechanism.

Conflict of interest

No potential conflicts of interest were disclosed.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2019.03.044.

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