**Discovery and Mechanistic Characterization of a Novel Selective Nuclear Androgen Receptor Exporter for the Treatment of Prostate Cancer**

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**Abstract**

Despite the success of medical strategies to reduce androgen levels in the treatment of prostate cancer, this disease invariably relapses to a castrate-resistant state that is generally fatal. Although it had been thought that androgen-insensitive cancers no longer relied on the androgen receptor (AR) for growth and survival, it is now clear that this is not the case. Because relapses are known to occur by many mechanisms that keep the AR functionally active, strategies to block AR accumulation in the nucleus may be therapeutically useful. Here, we report the discovery of a selective nuclear androgen receptor exporter (SNARE) that functions to exclude AR from the nucleus. SNARE-1 binds wild-type and mutant ARs and efficiently inhibits their transactivation activity and ability to induce PSA gene expression. SNARE-1 inhibits the androgen-sensitive growth of LNCaP cells and tumor xenografts. Quantitative subcellular localization studies suggest that SNARE-1 inhibits nuclear translocation of AR, but also facilitates export of nuclear AR that has been translocated by an agonist. Mechanistic studies indicate that SNARE-1 rapidly phosphorylates p38 mitogen-activated protein kinase (MAPK) and Ser⁶⁵⁰ of the AR. Additionally, SNARE-1 was found to promote ubiquitination of AR in LNCaP cells. Lastly, SNARE-1 functions as a tissue-selective AR inhibitor, as it fails to phosphorylate p38 MAPK in U2OS bone cells that are stably transfected with AR. In summary, SNARE-1 inhibits AR function by a mechanism that is distinct from clinically available antiandrogens, such that it might inform novel methods to block AR function in androgen-independent prostate cancer. Cancer Res; 70(2); 842–51. ©2010 AACR.

**Introduction**

Prostate cancer is the most frequently diagnosed cancer in males and the second leading cause of cancer deaths in the Western hemisphere. The American Cancer Society estimated that 28,600 prostate cancer–related deaths and 186,000 new cases of prostate cancer occurred in 2008 (American Cancer Society. Cancer Facts and Figures). Risk factors for prostate cancer span a spectrum including, but not limited to, age, race, geographic residence, and gene rearrangements (1). However, androgens (e.g., testosterone) and the androgen receptor (AR) are necessary for the development of prostate cancer (2). Chemical and surgical castration of men with prostate cancer leads to tumor regression, suggesting the importance of androgens in the growth and maintenance of prostate cancer (3, 4). However, after 12 to 18 months, prostate cancer invariably recurs as a castrate-resistant tumor due to a variety of reasons (4, 5).

Androgens act in cells by binding to the AR, a member of the steroid receptor superfamily of transcription factors (6). In the absence of ligand, AR is maintained in an inactive conformation in the cytoplasm by heat shock proteins. Binding of ligand dissociates heat shock proteins, leading to translocation of AR and binding to androgen response elements. AR recruits various coactivators/corepressors and general transcription factors, leading to the transcription or repression of target genes (7).

Because the growth and maintenance of prostate cancer is largely controlled by circulating androgens, treatment of prostate cancer heavily relies on therapies that target AR. Treatment with AR antagonists such as bicalutamide or hydroxyflutamide to disrupt receptor activation has been successfully used in the past to reduce prostate cancer growth (8, 9). All currently available AR antagonists competitively bind AR and recruit corepressors such as NCoR and SMRT to repress transcription of target genes (10). However, altered intracellular signaling, AR mutations, and increased expression of coactivators lead to functional impairment of antagonists or even transformation of antagonists into agonists (10, 11). Studies have shown that mutation of W741 and T877 within AR converts bicalutamide and...
hydroxyflutamide, respectively, to agonists (12, 13). Similarly, increases in intracellular levels of cytokines recruit coactivators instead of corepressors to AR-responsive promoters, subsequently converting bicalutamide to an agonist (10).

This positive correlation between AR and prostate cancer and the lack of a fail-safe AR antagonist emphasize the need for molecules that inhibit AR function through a novel mechanism that can elicit antagonistic activities within an altered cellular environment. We initiated a mechanism-based discovery program to identify novel AR antagonists that repress AR function not only through competitive binding but also through utilization of intracellular signaling pathways to alter AR nuclear accumulation and degradation by the ubiquitin proteasome pathway. Here, we present evidence of a novel AR antagonist, (R)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-(4-cyanophenoxy)-2-hydroxy-2-methylpropanamide (SNARE-1), which represses AR function by multiple mechanisms ensuring that utilization of alternative mechanisms will not lead to the functional impairment of the antagonist.

Materials and Methods

**Reagents.** Phospho-Ser$^{650}$ antibody was obtained from Abcam, Inc., and actin antibody was procured from Chemicon International.

**Cell culture.** LNCaP cells were grown in RPMI 1640 + 10% fetal bovine serum (FBS). U2OS stably transfected with AR cells were grown in McCoy's 5A medium + 1.5 mmol/L L-glutamine + 10% FBS, and COS-1 or HEK-293 cells were grown in DMEM + 10% FBS. For the chromatin immunoprecipitation and mitogen-activated protein kinase (MAPK) array assays, cells were plated in 150-mm dishes at 10$^7$ per dish in medium supplemented with 1% charcoal-stripped FBS (csFBS). The cells were maintained in 1% csFBS for 6 d to reduce basal occupancy of promoters, with medium changed on days 1 and 3 and before treatment on day 6.

**Chromatin immunoprecipitation assay.** Chromatin immunoprecipitation assays with AR N-20 antibody (Santa Cruz Biotechnology) were done as described previously (14). Real-time PCR was done on an ABI 7300 (Applied Biosystems) using TaqMan PCR master mix at universal condition. The following PCR primers and probe for PSA enhancer region were synthesized by Biosource International: PSA enhancer forward primer, 5′-GCTGGATCTGAGAGATATC-3′; reverse primer, 5′-ACACCTTTTTTTCCTGATTTG-3′; probe FAM, 5′-TGCAAGGATGCTGCTTACTACACACC-3′ TAMRA.

**Bone marrow culture.** Cell culture materials were obtained from Invitrogen. All animal studies were conducted using animal protocols approved by the Institutional Animal Care and Use Committee at the University of Tennessee. Bone marrow cultures were done as described earlier (15). After 12 d, the osteoclasts were stained for tartrate-resistant acid phosphatase activity (TRAP), and cells having 2 or more nuclei were counted under a microscope (15).

**RNA analysis and reverse transcriptase-PCR.** RNA was isolated using Trizol (Invitrogen), and the expression of various genes measured using TaqMan-validated primer probe mix. The expression of individual genes was normalized to 18S RNA levels.

**Growth assay.** LNCaP or PC3 cells were plated at 100,000 per well of a 24-well plate in RPMI supplemented with 1% csFBS. The cells were treated as indicated in the figures. Cell viability was measured using sulforhodamine blue reagent.

**Plasmid constructs and transient transfection.** Rat AR, glucocorticoid receptor, mineralocorticoid receptor, and AR ligand binding domain (LBD) were cloned from rat cDNA into pCR3.1 vector backbone. Mutant AR plasmids were developed internally at Gtx. GRE-LUC and SRC coactivator plasmids were kindly provided by Drs. Nancy L. Weigel and Bert W O'Malley (Baylor College of Medicine, Houston, TX). For transfection, cells were plated at 90,000 per well of a 24-well plate in DMEM + 5% csFBS. The cells were transfected using Lipofectamine (Invitrogen) with 0.25 μg of GRE-LUC, 0.02 μg of CMV-LUC (renilla luciferase), and 25 ng of receptor. The cells were treated 24 h after transfection as indicated in the figures, and luciferase assay was done 48 h after transfection. Transfections of p53 or control siRNA (Accell siRNAs, Dharmaco, Inc.) in LNCaP cells were done in Accell siRNA transfection medium.

**Human phospho-MAPK array.** Cells were plated and maintained in 1% csFBS as indicated above and were treated with vehicle or SNARE-1 for 45 min. The cells were washed, harvested, and blotted on human pMAPK array (ARY002, R&D Biosystems) as instructed by the manufacturer.

**Western blotting and immunoprecipitation.** Immunoprecipitation and Western blots were done as described earlier (14).

**Microscopic experiments.** Microscopic experiments were done at Baylor microscopic core facility in HeLa cells stably transfected with AR, as described in detail earlier (16).

**Xenograft experiment.** Nude mice obtained from Harlan were housed at five animals per cage and were allowed free access to water and commercial rodent chow (Harlan Teklad 22/5 rodent diet 8640). During the course of the study, the animals were maintained on a 12-h light:12-h dark cycle. Xenograft tumors were established in nude mice as previously published (17). Briefly, a mixture of 4:1 LNCaP/stromal cells (1 × 10$^6$ LNCaP cells and 0.25 × 10$^6$ stromal cells per animal) were suspended in 0.0375 mL RPMI + 10% FBS and 0.0625 mL Matrigel per animal and were injected s.c. Once the tumor size reached 100 mm$^3$, the animals were randomized and treated with vehicle (polyethylene glycol/ethanol/DMSO at 1:1:2 ratio) or SNARE-1 for 4 wk ($n = 9$). Tumor volume and body weight were measured. Tumor volume was calculated using the following formula: length × width × width × 0.5236. One animal in the vehicle-treated group and three animals in the SNARE-1–treated group died due to non–tumor-related incidents.

**Cell cycle analysis.** Cell cycle analysis was done using propidium iodide staining as previously described (18). Terminal deoxyribo-nucleotidyl transferase–mediated dUTP nick end labeling...
(TUNEL) staining and quantification were done by Paragon Biosciences.

Statistics for animal experiments were done using Wilcoxon rank sum test.

Results

**SNARE-1 inhibits AR function specifically.** The AR antagonistic effect of SNARE-1 was tested and compared with that of bicalutamide. Figure 1A shows that SNARE-1 inhibited R1881-stimulated AR transactivation with inhibition comparable to that of bicalutamide. SNARE-1 not only inhibited reporter gene activity but also inhibited R1881-induced transcription of AR target genes, PSA and NKx3.1, in LNCaP cells (Fig. 1B).

To determine if the AR LBD is necessary for the antagonism, an AR construct lacking LBD was created and tested in a transactivation assay. SNARE-1 inhibited full-length AR transactivation but failed to inhibit the constitutive activity of the AR lacking its LBD (Fig. 1C).

Although the LBDs of glucocorticoid receptor and mineralocorticoid receptor share low homology with AR, similarity in folding of the 12 α-helices makes cross-reactivity between the ligands a potential problem (19). Hence, the ability of SNARE-1 to inhibit glucocorticoid receptor and mineralocorticoid receptor transactivation was tested. SNARE-1 inhibited only ligand-induced AR transactivation but had no effect on glucocorticoid receptor or mineralocorticoid receptor transactivation (Fig. 1D).

**SNARE-1 inhibits coactivator-augmented wild-type and mutant AR transactivation.** Earlier studies showed that antagonists fail to inhibit AR and prostate cancer cell growth when coactivators are overexpressed (20). In addition, overexpression of AR or coactivators led to castrate-resistant prostate cancer and the conversion of the AR antagonist bicalutamide into an agonist (21, 22). AR transactivation studies with SNARE-1 and bicalutamide were done in cells overexpressing the AR coactivator SRC-2. As shown in Fig. 2A, overexpression of SRC-2 converted bicalutamide, but not SNARE-1, into an agonist.

To determine if overexpression of coactivators compromises the function of SNARE-1, cells overexpressing coactivators were treated with R1881 alone or in combination with 10 μmol/L SNARE-1. Whereas coactivators increased AR transactivation efficiently, SNARE-1 maintained a dominant antagonistic effect (Fig. 2B) in cells overexpressing coactivators.
Hundreds of AR mutations exist in prostate cancer, with many of these mutated AR exhibiting resistance to antagonist treatments. Two well-characterized point mutants in the AR that have been identified in prostate cancer are the W741L and T877A mutants (23, 24). Because SNARE-1 is an arylpropionamide, we also investigated the role of H874 in its binding to AR (25). Hence, H874 was mutated to phenylalanine (H874F), and transactivation of the wild type and mutants was studied in the presence of SNARE-1 and bicalutamide. SNARE-1 and bicalutamide inhibited wild-type AR and T877A- and H874F-AR (Fig. 2C). However, as published earlier (23), bicalutamide failed to inhibit W741L-AR, and this mutant converted bicalutamide into an agonist. In contrast, SNARE-1 inhibited transactivation by the W741L-AR (Fig. 2C).

**SNARE-1 inhibits prostate cancer growth in vitro and in vivo.** LNCaP cell growth assays were done in the presence or absence of SNARE-1 to examine its AR antagonistic effect on prostate cancer cell growth. SNARE-1 inhibited LNCaP proliferation stimulated by R1881 (Fig. 3A) or by full serum (Fig. 3B). To ensure that the antiproliferative effects of SNARE-1 are not limited to LNCaP cells alone, growth assay was done in another AR-positive cell line, VCaP. Similar to the results obtained in LNCaP, SNARE-1 also inhibited the

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**Figure 2.** A, overexpression of SRC-2 converts bicalutamide to an agonist. HEK-293 cells were transfected as described in the legend to Fig. 1 and with increasing amounts of SRC-2. The amount of transfected plasmid was balanced with vector plasmid. The cells were treated with 10 μmol/L of bicalutamide or SNARE-1 and luciferase activity was measured. B, SNARE-1 inhibits coactivator-induced AR transactivation. COS-1 cells were transfected with 25 ng of AR in combination with 0.25 μg of backbone or expression plasmids for SRC-1, SRC-2, or SRC-3. The cells were treated with the indicated concentrations of R1881 alone (broken lines) or in combination with 10 μmol/L SNARE-1 (solid lines) and luciferase activity was measured. C, SNARE-1 inhibits AR harboring prostate cancer point mutants. COS-1 cells were transfected as indicated in Fig. 1 with wild-type AR or T877A-, H874F-, or W741L-AR and treated with various concentrations of R1881 (circles), SNARE-1 (line), bicalutamide (squares), or a combination of 0.1 nmol/L R1881 and various concentrations of SNARE-1 (diamonds) or bicalutamide (triangles), and luciferase activity was measured. n = 3.
growth of VCaP cells, indicating that it is not limited to only one AR-positive prostate cancer cell line (data not shown). However, SNARE-1 failed to inhibit the growth of AR-null PC3 cells (Fig. 3C) or AR-positive androgen-insensitive C4-2 cells (data not shown), indicating that AR expression or androgen dependence is required for its antagonistic activity.

Cell cycle studies were done in LNCaP cells to evaluate the mechanism of SNARE-1–mediated growth inhibition. SNARE-1 treatment (10 μmol/L) led to loss of cells in S phase and accumulation of cells in the G0-G1 or G2-M phases (Fig. 3D), indicating a near-complete inhibition of replication and DNA synthesis. SNARE-1 also differed from bicalutamide (21) in inducing apoptosis of LNCaP cells (Supplementary Fig. S1), showing that the two antagonists function through different mechanisms to induce prostate cancer cell death.

We next examined the in vivo pharmacologic effects of SNARE-1 using an in vivo tumor xenograft model using LNCaP epithelial and HPS-19B stromal cells (26). SNARE-1 inhibited stromal-epithelial prostate tumor growth by about 50%, with significant differences within 10 days of dosing and continuing for 4 weeks (Fig. 4A and inset). Furthermore, there was no change in body weight or serum alanine transaminase (data not shown), indicating that the drug did not elicit apparent toxic effects. At 4 weeks, tumor (Fig. 4B) and prostate weight (Fig. 4C), markers of androgen action, were significantly reduced by SNARE-1, indicating efficacious antagonistic actions of SNARE-1 in vivo. Serum and tumor PSA, indicators of the SNARE-1 direct inhibition of AR activity in tumor, were significantly inhibited by SNARE-1 (Supplementary Fig. S2). Serum testosterone was measured and no statistical difference was observed between the two groups. Interestingly, levator ani, an androgen-responsive muscle, did not show any reduction, indicating a possible tissue-selective AR antagonistic effect (data not shown). This effect, however, has to be confirmed in a Hershberger rat model (27).

Histology was done on the tumor sections to better understand the mechanisms of tumor inhibition and apoptosis induced by SNARE-1. SNARE-1 increased the apoptosis of tumor cells by 3-fold (Fig. 4D).

**Figure 3. SNARE-1 inhibits prostate cancer cell growth in vitro.** LNCaP cells maintained in 1% csFBS and treated with 0.1 nmol/L R1881 (A), LNCaP cells maintained in FBS (B), and PC3 cells maintained in FBS (C) were treated with various concentrations of SNARE-1 for 6 d. The cells were fixed and stained with sulforhodamine blue. D, SNARE-1 induces G0-G1, and G2-M arrest. LNCaP cells maintained in FBS were treated with vehicle (open columns) or 10 μmol/L SNARE-1 (filled columns) for 6 d. Cells were fixed and subjected to cell sorting. Columns, mean (n = 3); bars, SE.
AR phosphorylation at Ser<sup>650</sup> by p38 MAPK (32), immunoblot analyses, using phospho-specific antibody, showed that SNARE-1 treatment resulted in phosphorylation of AR Ser<sup>650</sup> (Fig. 5A, right).

Ser<sup>650</sup> phosphorylation by p38 MAPK is known to lead to AR nuclear export (32). Because SNARE-1 phosphorylated p38 MAPK, we examined ligand effects on intracellular AR trafficking, including its subnuclear organization (e.g., hyperspeckling) as an indicator of AR transcriptional competency related to a poorly understood combination of interactions with other proteins and DNA (33–35). As published earlier, R1881 induced robust AR translocation and hyperspeckling (Fig. 5B, top). Bicalutamide induced AR nuclear translocation without hyperspeckling, suggesting that it mechanistically impairs intranuclear AR functions through altering effects upon protein and/or DNA interaction. In marked contrast, SNARE-1 treatment resulted in reduced AR translocation and minimal hyperspeckling. Interestingly, in competition (antagonistic) studies with 0.1 or 1 nmol/L R1881, AR translocation (Fig. 5B, bottom) and hyperspeckling (Fig. 5B, bottom) were also markedly reduced by SNARE-1 with an IC<sub>50</sub> of 100 and 70 nmol/L, respectively. Additionally, brief (~30 minutes) pulse labeling of a HaloTag-AR fusion protein with an irreversible fluorescent ligand followed by imaging studies revealed that SNARE-1 reduced nuclear translocation and facilitated nuclear export (Fig. 5C). These findings corroborate the p38 MAPK and Ser<sup>650</sup> AR phosphorylation data observed when LNCaP cells were treated with SNARE-1.

The effects of SNARE-1 on intracellular AR distribution led us to speculate that R1881-induced recruitment of AR to an androgen-responsive promoter should be inhibited by SNARE-1, but not by bicalutamide. Chromatin immunoprecipitation studies in LNCaP cells indicated that SNARE-1, but not bicalutamide, reduced the AR recruitment by R1881 to the PSA enhancer (Fig. 5D, left), and dose-response studies indicated that SNARE-1 reduced the presence of AR on PSA enhancer at concentration as low as 1 μmol/L (Fig. 5D, right). These results are consistent with reduced levels of AR in the nucleus as well as with reduction of nuclear hyperspeckling.

To determine if SNARE-1 induces AR ubiquitination, LNCaP cells were treated with varying concentrations of SNARE-1 or 10 nmol/L R1881. Protein extracts were immunoprecipitated with monoubiquitin or polyubiquitin antibodies and then immunoblotted with AR antibody. Clearly, 10 μmol/L SNARE-1 induced interaction of AR with ubiquitin proteins, suggesting that AR is marked for proteasomal degradation when bound to SNARE-1 (Fig. 6A).

To understand if p38 MAPK is the sole mediator of SNARE-1–dependent AR inhibition and prostate cancer cell growth, LNCaP cells were transfected with p38 siRNA or

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**Figure 4.** A, SNARE-1 inhibits prostate cancer xenograft growth in vivo. LNCaP, HPS-19B (prostate stromal cells), and Matrigel mix were injected s.c. into nude mice, which were than treated with either vehicle (solid line; n = 9) or 30 mg/kg/d SNARE-1 (broken line; n = 7) s.c. for 4 wk. Tumor volumes were measured biweekly. Inset, slopes of the tumor volume graph lines (open columns, vehicle; filled columns, SNARE-1). B and C, tumor (B) and prostate (C) weights were measured at sacrifice (n = 7 for vehicle and n = 5 for SNARE-1). D, SNARE-1 induces apoptosis. Tumor sections obtained from the animals in A (n = 8 for vehicle and n = 5 for SNARE-1) at sacrifice were stained for TUNEL. The TUNEL-positive cells were counted and represented as percent stained cells. B to D, open columns, vehicle-treated animals; filled columns, SNARE-1–treated animals. Columns, mean; bars, SE. *, P < 0.05; **, P < 0.001.
A

Template
Vehicle R1881 Bicalutamide SNARE-1
Phospho-Ser650 AR
Actin

B

Translocation

Hyperspeckling

C

Percent nuclear AR

D

AR/input

SNARE-1 (nmol/L)

SNARE-1 (nmol/L)

SNARE-1 (μmol/L)

+0.1 nmol/L R1881
control siRNA. Cells were then treated with SNARE-1, and PSA gene expression was measured. Figure 6B indicates that inhibition of p38 MAPK partially reversed SNARE-1-mediated inhibition of PSA expression, indicating that p38 MAPK is an important but not the sole contributor to the inhibitory effects of SNARE-1.

The AR also plays critical roles in bone and muscle function. To test the effect of SNARE-1 on p38 MAPK in an anabolic tissue–based cell line, U2OS bone cells were stably transfected with AR and then treated with SNARE-1 under the same conditions as with the LNCaP cells. Phospho-MAPK array blotting results suggested the absence of p38 MAPK phosphorylation by SNARE-1 (Fig. 6C). If there is no p38 MAPK phosphorylation, then SNARE-1 may not induce bone loss and may be a tissue-selective inhibitor of AR function. To confirm this, we cultured rat bone marrow cells in the presence of SNARE-1 and stained for TRAP-positive multinucleated osteoclasts. As expected, the positive control, receptor activator of NF-κB ligand (RANKL) and macrophage colony stimulating factor (MCSF), but not SNARE-1 or R1881, induced the formation of TRAP-positive osteoclasts (Fig. 6D).

Discussion

Although more than 200 molecules targeting several pathways of prostate cancer are in various stages of development, AR is still considered a central player in castrate-resistant prostate cancer. Targeting AR directly with an antagonist efficiently reduces its activity. However, eventually antagonism is lost through several mechanisms. Hence, targeting AR by

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Figure 6. A, SNARE-1 induces AR-ubiquitination. LNCaP cells were treated and monoubiquitin and polyubiquitin proteins were immunoprecipitated, run on SDS-PAGE, and immunoblotted with AR antibody. B, p38 siRNA partially reverses SNARE-1–inhibited PSA gene expression. LNCaP cells were transfected with nonspecific or p38 siRNA and were treated for 24 h as indicated in the figure. RNA was extracted and PSA expression was measured and normalized to 18S. Inset, knockdown of p38. Filled columns, p38 siRNA–transfected cells; open columns, nonspecific siRNA–transfected cells. C, SNARE-1 fails to rapidly phosphorylate p38 MAPK in U2OS-AR cells. U2OS-AR cells were maintained in McCoy + 1% csFBS for 5 d and treated for 45 min with vehicle or 100 nmol/L SNARE-1. Protein extracts were subjected to pMAPK array blot. The spots inside the circles indicate p38α and p38γ (B9 and B10 and C9 and C10, respectively). D, SNARE-1 does not increase TRAP-positive multinucleated osteoclasts. Primary bone marrow cells from rats were cultured in the presence of RANKL + MCSF (R+M) to differentiate into osteoclasts. The cultures were treated with 10 μmol/L SNARE-1 or 10 nmol/L R1881. After 10 d, the cells were stained and the number of TRAP-positive multinucleated osteoclasts was counted. Columns, mean; bars, SE.
multiple mechanisms would be an even more efficient method to inhibit its activity and prostate cancer cell growth.

Recent observation from Dr. Sawyers’ group (21) showed that keeping AR out in the cytoplasm is a valid strategy to overcome the problem of resistance to antagonist treatment. SNARE-1 follows this strategy mechanistically, and hence, long-term treatment will not lead to resistance. Both antagonists (SNARE-1 and RD162 or MDV3100) prevent translocation of AR into the nucleus. In addition, SNARE-1 also exports the AR that is already present in the nucleus. Supplementary Table S1 summarizes the similarities between the two ligands and their distinction from bicalutamide.

SNARE-1 is a member of an evolving class of ligands that inhibit AR function and prostate cancer growth by activating antiproliferative p38 MAPK, inhibiting nuclear accumulation by phosphorylating Ser650 of AR and leading to AR ubiquitination. The data presented here suggest that SNARE-1 has the potential to function as a next-generation treatment for prostate cancer.

Recent studies indicated that overexpression of coactivators or depletion of corepressors will lead to a reversal in the pharmacologic profile of bicalutamide, making its function as an agonist (10). However, SNARE-1 functioned as an antagonist even when coactivators were overexpressed (Fig. 2B). SNARE-1 also retained its antagonistic potential when tested with AR harboring commonly occurring prostate cancer mutations (Fig. 2C). These results indicate that SNARE-1 functions at least one level beyond a simple competitive AR inhibitor.

SNARE-1 inhibited nuclear translocation and facilitated nuclear export of AR with an IC50 of 100 nmol/L (Fig. 5B and C), suggesting that altered AR trafficking and subnuclear organization takes place through mechanisms that are distinct from direct AR binding, such as activation of p38 MAPK and Ser650 AR phosphorylation (32).

Several studies have highlighted the anticancer properties of p38 MAPK. Activation of p38 MAPK by external stimuli leads to G2-M arrest and, subsequently, apoptosis (30). It also functions as a competitive inhibitor of the oncogenic kinase ERK (36). Universal activation of p38 MAPK, although useful to inhibit tumorigenesis, could lead to inflammatory or neurodegenerative diseases. Hence, tissue-selective activation of p38 MAPK is critical to limit side effects. We suspect that SNARE-1 might function as a tissue-selective activator of p38 MAPK because it failed to activate this kinase in U2OS-AR bone cells (Fig. 6C). siRNA to p38 MAPK partially reversed androgen-dependent PSA expression, indicating that p38 MAPK is one mechanism through which SNARE-1 mediates its antiproliferative effects.

Future studies with molecules that target these multiple pathways will help to increase the potency and efficacy of their tumor suppressive properties. Studies with androgen-independent prostate cancer cells that express AR are under way to determine the ability of SNARE-1 to inhibit prostate cancer cells that have escaped hormone dependency.

Disclosure of Potential Conflicts of Interest

J.T. Dalton: ownership interest, GTx, Inc. The other authors disclosed no potential conflicts of interest.

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