Induction of the SUMO-specific Protease 1 Transcription by the Androgen Receptor in Prostate Cancer Cells*

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Tasneem Bawa-Khalfe†, Jinke Cheng‡, Zhengxin Wang§, and Edward T. H. Yeh†‡§

From the †Research Center for Cardiovascular Diseases, Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases, The University of Texas Health Science Center, Houston, Texas 77030 and the Departments of ‡Cardiology and §Cancer Biology, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030

Prostate cancer, the most frequently diagnosed carcinoma in males, is readily modulated via the transcriptional activity of androgen receptors. Our recent publication reported that androgen receptor-dependent transcription is significantly elevated with expression of the human sentrin/SUMO-specific protease (SENP1) in the androgen-sensitive human prostate cancer cell line (LNCaP). In situ hybridization studies indicated an elevation of SENP1 message in prostatic intraepithelial neoplasia and prostate cancer lesions as compared with normal prostate epithelia. This study aimed to delineate the mechanism for the regulation of SENP1 message and to determine the pathophysiological consequence of SENP1 induction with respect to prostate cancer. Real-time PCR confirmed the elevation of SENP1 mRNA in prostate cancer cells as compared with normal prostate epithelial cells. Chronic androgen exposure of LNCaP cells prompted an enhancement in the SENP1 transcript selectively. This androgen-mediated augmentation of SENP1 was absent with co-administration of the androgen receptor antagonist bicalutamide and in androgen receptor-negative prostate cancer PC-3 cells, indicating an androgen receptor-dependent event. Activation of the androgen receptor was required for binding an identified androgen response element and positively regulating SENP1 promoter activity. Abrogation of elevated SENP1 mRNA in prostate cancer cells significantly decreased androgen-mediated cell growth. Because increased SENP1 expression directly modulated androgen receptor-dependent cell proliferation and transcription, SENP1 could play an important role in prostate carcinogenesis.

Small ubiquitin-like modifier (SUMO)3 mediates a diverse array of cellular events by conjugating to numerous protein substrates. Recent reports have focused on the ability of SUMOylation to regulate the transcriptional activity of nuclear receptors, transcription factors, and co-regulatory proteins (1–6). The three mammalian SUMO proteins, SUMO1, SUMO2, and SUMO3, form covalent bonds with cellular targets in a sequential manner analogous to ubiquitin (7, 8). A family of human SUMO-specific proteases (SENP) (9) can selectively deconjugate SUMOylated proteins and hence dictate SUMO dynamics. Our laboratory cloned the first human SUMO protease, SENP1 (9), and since its discovery, SENP1 has become the best characterized of the SENP family. It is localized exclusively in the nucleus and deconjugates many SUMO substrates (9–11). SENP1 shares the greatest homology with SENP2 (9, 12, 13). In contrast, SENP1 differs significantly from SENP3 and SENP5, which constitute an independent subfamily based on their nucleolar localization and preference for deSUMOylation of SUMO2 and SUMO3 conjugates (14).

Recently, we reported that SENP1 plays a prominent role in the regulation of the androgen receptor (AR)-dependent transcription (10). Under physiological conditions, the AR dictates the development and normal growth of the prostate gland. The androgen 5α-dihydrotestosterone binds AR to prompt translocation of this active receptor from the cytosol to the nucleus. The androgen-activated ARs bind specific DNA sequences or androgen response element (ARE) located on the promoter region of a gene. The AR then prompts the recruitment of various co-regulatory proteins to directly modulate the transcription of these genes.

Changes in the transcriptional activation of the AR promote prostate cancer (PCa) cell proliferation and survival (15–17). We observed that AR activity is modulated by SENP1 in PCa cells; overexpressing SENP1 increases AR-transcriptional activity via de-SUMOylation of the co-regulatory protein, HDAC1 (10). Reduction of endogenous SENP1 in these cells, using interfering RNA directed specifically against SENP1, significantly decreases expression of the AR-regulated-prostate-specific antigen gene (10). Similarly, SENP1 moderates the expression of the cell cycle regulator, cyclin D1; diminishing SENP1 in PCa cells decreases cyclin D1 levels (18). Previous studies indicate that enhanced expression of cyclin D1 is readily observed in advanced PCa (19, 20) and contributes to PCa pro-

PCa, prostate cancer; QRT-PCR, quantitative reverse transcription-PCR; SENP, sentrin/SUMO-specific protease; SP1P, SENP1 promoter plasmid; EMSA, electrophoretic mobility shift assay; ANOVA, analysis of variance.
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gression (21, 22). Therefore, the expression of SENP1 in PCa cells modulates major factors in PCa progression.

We recently reported that SENP1 expression appears enhanced in prostate carcinoma and precursor prostatic intraepithelial neoplasia lesions relative to adjacent normal prostate tissue via in situ hybridization (18). The present study demonstrates that elevation in SENP1 mRNA levels in PCa cells is selective and requires the activation of the AR. Upon activation, AR binds a specific ARE on the SENP1 promoter and enhances SENP1 transcription. Hence, this study is the first to delineate a mechanism for the regulation of expression of a SUMO protease.

EXPERIMENTAL PROCEDURES

Cell Culture Maintenance and Treatment—LNCaP and PC-3 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 1% penicillin/streptomycin, whereas RWPE1 and RWPE2 cells were maintained in keratinocyte serum-free medium supplemented with 5 ng/ml human recombinant epidermal growth factor and 0.05 mg/ml bovine pituitary extract. For hormone treatment, the maintenance medium was replaced with phenol red-free RPMI 1640 medium containing 10% charcoal-stripped bovine serum.

RNA Interference and Cell Proliferation—SENP1 small interfering RNA (siRNA) and nonspecific siRNA (NS-siRNA) were purchased from Dharmacon (Chicago, IL), and LNCaP cells were transfected with either of the two siRNAs using the DharmaFECT transfection reagent (Dharmacon). The LNCaP cells were allowed to grow for 48 h after initial transfection. Then, the cells were repleted, and after 4 h, a subset of cells was treated with 10 nM R1881. Five days later, the LNCaP cells were harvested and subjected to determine cell growth and SENP1 levels. To assess changes in cell number, LNCaP cells were stained with trypan blue and counted using a hemocytometer. QRT-PCR and real-time PCR were conducted to evaluate endogenous SENP1 mRNA levels following the indicated siRNA treatment.

RNA Isolation, Real-time PCR, and QRT-PCR—Cells were prepared for RNA isolation using the RNA-Bee reagent (Tel-Test Inc., Friendswood, TX) according to the manufacturer's instructions.

The TaqMan Master Mix reagents (Applied Biosystems, Branchburg, NJ) were utilized for the real-time PCR. Primers for either SENP1 (400 nM; forward, 5'-TTG GCC AGA GTG CAA ATG G-3' and reverse, 5'-TCG GCT GCT TCT TGA TTT TTG TAA-3') or the 18S rRNA (40 nM; forward, 5'-TAA CGA ACG AGA CTC TCG CAT-3' and reverse, 5'-CGG ACA TCT AAG GGC ATC ACA G-3') were utilized. SENP1 mRNA levels or relative quantities values (RQ values) were calculated using the TaqMan ABI PRISM 7900 sequence detector system (Applied Biosystems).

Alternatively, QRT-PCR was conducted with the One Step RT-PCR kit from Qiagen (Valencia, CA) to illustrate changes in SENP1 levels with respect to the housekeeping gene actin or glyceraldehyde-3-phosphate dehydrogenase. The following primers (40 nM) were used for amplification of SENP1, actin, or glyceraldehyde-3-phosphate dehydrogenase mRNA: SENP1, 5'-ATA GGA TCC ATG CAG GCA GTG AAG AAT TGG A-3' (forward) and 5'-GCG GTG CGA TCG ACA AGA GTT TTC GGT GGA G-3' (reverse); actin, 5'-TCT TGG GTA TGG AAT CCT GTG GCA-3' (forward) and 5'-ACT CCT GCT TGC TGA TCC ACA TCT-3' (reverse); and glyceraldehyde-3-phosphate dehydrogenase, 5'-GGT ACT TTA TTG ATG GTA GCA-3' (forward) and 5'-GGT ACT TTA TTG ATG GTA CAT GAC-3' (reverse).

Identification of the SENP1 Promoter Region—The human SENP1 gene, located on chromosome 12, contains a large 5'-untranslated region with the translation start site on exon 2. A series of computational analyses were conducted to identify a candidate SENP1 promoter region. First, to establish the transcriptional start site, the expressed sequence tag data base was searched to find a sequence that extends farther upstream than the first nucleotide of the SENP1 gene (GenBankTM accession number NM_014554); none were found. Therefore, the first base pair of the untranslated region was taken as the putative transcriptional start site. Then, SENP1 was blasted against the completed human genome available on the National Center for Biotechnology Information data base to acquire the sequence upstream of this putative transcriptional start site. Next, the National Institutes of Health-developed Proscan Version 1.7 Bioinformatics Program was used to detect a core promoter region within a 3-kb upstream sequence; the program recognizes transcriptional elements required for binding of RNA polymerase II (23). A 250-bp region located 25 bp upstream of the putative transcriptional start site was identified as the core pro-
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FIGURE 2. AR activation regulates SENP1 mRNA expression in PCa cells. RNA was isolated from the specified prostate epithelial cell lines, and real-time PCR was performed using the appropriate primers and probes; 18 S rRNA served as the endogenous control in all experiments. A, representative real-time PCR exhibits the increase in SENP1 mRNA levels in transformed RWPE2 and PCa LNCaP cells as compared with normal prostate epithelial RWPE1 cells. Inset, QRT-PCR produced results similar to the real-time PCR with the following cell lines: lane 1, RPWE1; lane 2, RWPE2; and lane 3, LNCaP. B, SENP1, but not SENP2, RNA levels increased following 24-h R1881 (20 nM) treatment as compared with untreated control LNCaP cells. C, SENP1 expression was enhanced in LNCaP cells, which endogenously express AR, but not the AR-independent PC-3 cells (Student’s t test; *, p < 0.05). D, LNCaP cells were treated with 20 nM R1881 in the presence or absence of the AR antagonist BIC (5 μM). In the presence of BIC, R1881-mediated up-regulation of SENP1 is completely abolished (ANOVA; *, p < 0.05). All data presented are the mean ± S.E. values of two to five independent experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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moter with a score of 64.23. This program and the TRANSFAC data base confirmed the absence of a TATA box and the presence of multiple Sp1-binding sites within this core promoter region. It is well established that Sp1 binds to GC-rich regions and is involved in recruiting RNA polymerase II and thereby inducing gene transcription (24). The putative SENP1 promoter included a GC-rich region or CpG island as verified by the European Bioinformatics Institute-supported CpGPlot/CpGReport tool.

When aligned with an analogous 3-kb 5′ region of the mouse SENP1/SuPr-2 gene (GenBank™ accession number NM_144851) using tbl2seq, the putative human SENP1 promoter contained two highly conserved regions. Yamaguchi et al. (25) identified the first region, which was located within intron 1 and shared 80% homology between the two species. The mouse ortholog included an enhancer domain within this region; disruption of this area with proviral integration reduced the expression of the SENP1/SuPr-2 gene. The second homologous domain was located in the 5′ region in both species and included 474 nucleotides that share 75% identity.

Identification of Potential AREs—Potential AREs were identified on the 4.1-kb sequence based on 1) alignment with the accepted palindromic ARE sequence 5′-AGAACA nnn TGT-TCT-3′ and 2) conservation of the 2 G and 2 C nucleotides within the ARE that are essential for AR binding (26–28). Based on these two criteria, six potential AREs were recognized; three AREs were located proximal to the CpG island, whereas the other three AREs were at least 1 kb away. One potential ARE was identified in intron 1 within close proximity to the enhancer region identified by Yamaguchi et al. (25). Because the ARE can be located in enhancer regions downstream of the promoter, this downstream ARE was also assessed for regulation of SENP1 promoter activity. According to the location of these putative AREs, we designed additional fragments that excluded one or more of these AREs.

Promoter–Luciferase Construct—The potential SENP1 promoter described above was amplified from the genomic DNA of LNCaP cells using specific primers (supplemental Table S1). The primers for the initial SENP1 promoter plasmid (SP1P) and five deletion constructs (supplemental Table S1) included a cleavage recognition site for either NheI on the forward primers or XhoI on the reverse primers to facilitate subcloning into the multiple cloning regions of the pGL3-basic vector. The plasmids, which fused the respective promoter regions with a firefly luciferase reporter gene, were co-transfected with the control Renilla luciferase plasmid. Following appropriate treatment, LNCaP and/or PC-3 cells were lysed using the cell lysis buffer provided with the Dual-Luciferase Reporter 1000 assay system (Promega), and relative luciferase activity was assessed with a luminometer; the relative luciferase activity is used to represent the ratio of the firefly luciferase count to the Renilla luciferase count.

Chromatin Immunoprecipitation (ChiP)—PC-3 cells were treated as indicated, and following treatment, the protein-DNA complexes were cross-linked with 1% formaldehyde for 10 min. The cells were lysed, and subsequently the cell lysate was sonicated to shear the DNA. Then, 600 μg of DNA from each treatment group was collected. To reduce nonspecific background, the cell lysate was first incubated with agarose beads and salmon sperm DNA. The supernatant was collected, and 20 μl of it was saved to analyze as the initial input for the reaction. The remaining lysate was incubated overnight with 2 μg of the anti-AR antibody. The protocol provided with the ChiP kit (Upstate Biotechnology) was followed for immunoprecipitation, elution, and reverse cross-linking of the protein-DNA complex. The eluted DNA was purified with the PCR purification kit (Qiagen). Both the eluted and input products were then subjected to PCR analysis using the indicated primers (supplemental Table S1).

Electrophoretic Mobility Shift Assay (EMSA)—Specific oligonucleotides labeled with biotin on the 5′ end (supplemental
Table S2) were purchased from Integrated DNA Technologies. A non-radioactive LightShift chemiluminescent EMSA kit (Pierce) was used to detect interaction between the respective oligonucleotides and the 107-amino acid DNA-binding domain of the AR (AR-DBD); it was previously illustrated that the AR-DBD can successfully bind AREs in the absence of AR (29). Briefly, each oligonucleotide (20 fmol) was incubated with the AR-DBD (50 ng) and 50 ng/μl of the nonspecific competitor DNA, poly(dI-dC), for 20 min in binding buffer (Pierce), 0.1% Nonidet P-40, 20 mM KCl, and 100 μg/ml bovine serum albumin. The reaction was terminated with the addition of the loading buffer, and subsequently each sample was loaded onto a pre-run polyacrylamide gel. The samples were then transferred to a nylon membrane, and the biotin-labeled oligonucleotides were detected via chemiluminescence. When present, the relative density of the slower migrating band (representative of AR-DBD-bound oligonucleotide) was evaluated using the FluorChem-8900 (Alpha Innotech Corp., San Leandro, CA).

Data Analysis—Nonlinear regression analysis was conducted with GraphPad Prism Version 4.0 (GraphPad Software). Either Student’s t test or ANOVA (concurrently with Tukey’s post hoc test) was employed to calculate p values and evaluate differences between groups.

RESULTS

Induction of SENP1 by Androgen in Prostate Cancer Cells Modulates Cell Proliferation—In situ hybridization studies illustrated more intense staining for SENP1 mRNA in prostatic intraepithelial neoplasia and PCa cells as compared with normal adjacent prostate epithelium (18). The consequence of SENP1 overexpression in PCa cells remained undefined. Overexpression of SENP1 in PCa cells enhanced AR-dependent transcription (10, 18). Activated AR induces the transcription of numerous genes that are required to modulate cell proliferation (30–34); therefore, we postulated that androgen-activated AR could enhance SENP1 levels to regulate PCa cell growth. LNCaP cells were transfected with SENP1-targeting or non-targeting siRNA, and after 2 days they were treated with 10 nM R1881 for an additional 5 days. Transfection of the SENP1-siRNA successfully ablated endogenous SENP1 mRNA (Fig. 1A, second lane) as compared with the NS-siRNA (first lane). This ablation was confirmed by real-time PCR (data not shown). The androgen treatment significantly elevated SENP1 mRNA (Fig. 1A, third and fourth lanes); however, SENP1 message was greater in cells transfected with
NS-siRNA (third lane) than SENP1-siRNA (fourth lane). Consequently, the lower SENP1 mRNA following SENP1-siRNA treatment prompted a significant decrease in the ability of R1881 to enhance LNCaP cell proliferation (Fig. 1B) as compared with treatment with the NS-siRNA ($p < 0.05$). In the absence of R1881, 7-day SENP-siRNA treatment has little effect on cell growth (data not shown). Therefore, androgen-induced SENP1 mRNA contributed to AR-mediated PCa cell proliferation.

**Elevation of SENP1 Levels in PCa Cells Requires Activation of AR**—The expression of SENP1 mRNA was analyzed by both real-time PCR and QRT-PCR in the normal prostate epithelial cell line RWPE1, the transformed cell line RWPE2, and the PCa cell line LNCaP. RWPE2 cells, which are derived from RWPE1 cells, expressed a 2-fold greater level of SENP1 mRNA than their parental counterparts (Fig. 2A), suggesting that transformation of normal prostate epithelial cells induced SENP1 expression. Similarly, LNCaP cells also revealed higher SENP1 levels than RWPE1 cells (Fig. 2A). These results supported our observation in human prostate tissue that SENP1 levels are altered within PCa cells compared with normal prostate cells.

We recently reported that 24-h exposure of human AR-positive LNCaP cells to the synthetic androgen R1881 (20 nM) was sufficient to enhance SENP1 expression by 5-fold (18). Previous studies have established that the normal prostate epithelial cell line RWPE1 expresses functional AR (35). However, treatment of RWPE1 cells with R1881 (20 nM) for 24 h did not alter SENP1 mRNA levels as compared with untreated control cells ($n = 3$, $p = 0.48$; Student’s $t$ test). Therefore, activated AR induces SENP1 mRNA levels specifically in prostate carcinoma cells.

To determine whether the induction was selective for SENP1 in PCa cells, we evaluated the expression of a closely related SENP family member, SENP2, using specifically designed primers and probes (Fig. 2B). LNCaP cells exposed to the same concentration of R1881 exhibited a significantly greater level of SENP1 than SENP2 mRNA. Further real-time PCR analysis indicated that SENP1 levels paralleled the R1881 concentration in LNCaP cells; increasing R1881 concentration elevated SENP1 levels (Fig. 2C). This concentration-dependent induction of SENP1 mRNA was absent in the AR-deficient PCa cells, PC-3 (Fig. 2C). Concomitant administration of the AR antagonist bicalutamide (BIC, 5 µM) (Fig. 2D) with R1881 (20 nM) ablated the induction of SENP1 in LNCaP cells. The unaltered SENP1 levels in AR-negative PC-3 cells (Fig. 2C) and in the presence of the AR antagonist (Fig. 2D) indicated that activation of the AR is required for androgen regulation of SENP1 expression.

**Activation of AR Regulates the SENP1 Promoter Activity**—Activation of the AR prompts transcription of numerous genes; hence we investigated whether the active AR modulates SENP1 gene transcription to augment SENP1 mRNA expression in PCa cells. Specific primers (supplemental Table S1) were used to clone the 4.1-kb SP1P fragment from the genomic DNA of the LNCaP cells. The fragment was subsequently fused to a firefly luciferase reporter gene in the promoter-less vector, pGL3-basic. In LNCaP cells, transfection of the SP1P plasmid (250 ng) produced a 11-fold greater luciferase response than transfection of an equivalent amount of the empty pGL3-basic vector, suggesting that the 4.1-kb clone included a functional promoter (mean relative luciferase activity values: pGL3-basic vector, 0.007, $n = 4$; SP1P plasmid, 0.082, $n = 4$; Student’s $t$ test, $p < 0.05$). Although functional in both cell lines, the SP1P plasmid exhibited five times less promoter activity in PC-3 than in LNCaP cells (Fig. 3A). To evaluate the contribution of the AR to SENP1 promoter activity, both cell lines were transfected with SP1P, and a subset of the population was co-transfected with AR cDNA (500 ng). Titrating concentrations of R1881 for 24 h produced a concentration-dependent induction of luciferase activity in both the presence and absence of exogenous AR in LNCaP cells (Fig. 3B); however, in the presence of AR, R1881 was more effective at prompting the response. In contrast, the addition of AR was required for promoter activity in AR-deficient PC-3 cells, and with the inclusion of AR, a more efficacious and potent R1881-induced luciferase response was observed (Fig. 3C). Hence, an activated AR mediated SENP1 promoter activity in PCa cells.

Various deletion fragments were constructed to determine the minimal region required for AR regulation of SENP1 promoter activity (Fig. 3D). The native activity of each deletion construct was compared with the empty pGL3-basic vector (Fig. 3D). All deletion constructs, which included a large portion of the 5’ region, maintained promoter activity, whereas Del-4 did not (Fig. 3D). The smallest deletion construct with intact endogenous activity was Del-3; hence the core promoter required for activity was within a 1.4-kb region on Del-3. Interestingly, the Del-3 construct produced a 31-fold greater luciferase response as compared with the empty pGL3-basic vector and exhibited significantly greater native activity than all other constructs (ANOVA, $p < 0.05$). It is probable that a repressor region existed upstream of the Del-3 region, and therefore,

**FIGURE 3.** AR modulates the activity of the proposed SENP1 promoter. A, LNCaP and PC-3 cells were transfected with the SP1P plasmid (250 ng) and pRL vector (20 ng). The cells were grown for 24 h in normal maintenance medium, and then relative luciferase activity was calculated. The SP1P plasmid produced greater native activity in LNCaP than PC-3 cells (Student’s $t$ test; $p < 0.05$). B, and C, LNCaP and PC-3 cells, respectively, were exposed to titrating concentration of R1881 following transfection with 250 ng of SP1P in the presence or absence of 500 ng of AR. Concurrently, both PCa cells were transfected with the Renilla vector, which served as the control for transfection efficiency. The relative luciferase activity for each R1881 concentration was compared with that of SP1P alone, and hence the y axis represents the -fold induction over the relative luciferase activity of SP1P alone. t test analysis ($p < 0.05$; GraphPad Prism) indicated an increase in efficacy of R1881 in the presence of AR in both cell lines. D, shown is a schematic illustration of the various deletion fragments is generated to identify the minimal promoter region required for activity. Six potential ARESs (black boxes) were detected 2.7 kb upstream and 1.5 kb downstream of the first nucleotide within the large 5’-untranslated region. A CpG island (striped box) and two conserved regions (cross-hatched boxes) were also identified. LNCaP cells were treated in a manner analogous to that described for A. When compared with the empty pGL3-basic vector, all deletion constructs exhibited native activity in LNCaP cells with the exception of Del-4. ANOVA analysis indicated that the Del-3 construct had significantly greater promoter activity than other constructs ($p < 0.05$). E, the ability of increasing concentrations of R1881 to prompt promoter activity in the presence AR (500 ng) was assessed in LNCaP cells. -Fold induction of relative luciferase activity was calculated for each deletion fragment as described for B and C. Four deletion constructs, specifically Del-1, -2, -3, and -6, displayed comparable log EC$_{50}$ values whereas Del-4 did not ($p < 0.05$).
A R1881 concentration-response curve comparable with Del-1
"Experimental Procedures." The Del-6 plasmid helped assess
three potential AREs located proximal to the core
postulated that the AR could bind either one or more of
the constructs nor two upstream AREs (Del-3 construct) lessened
fore, the elimination of neither the one downstream ARE (Del-6
R1881 promoter response in both LNCaP and PC-3 cells (Fig. 3
luciferase response for Del-3 was assessed in the presence of the
was mediated via activation of the AR, the R1881-mediated
moter and within the highly homologous upstream region pres-
ion and regulation.

As anticipated, the loss of the promoter prevented Del-4
from eliciting a luciferase response in the presence of increasing
R1881 (Fig. 3E). However, the Del-4 fragment expressed a
potential ARE as identified by the criteria described under "Experimental Procedures." The Del-6 plasmid helped assess
the contribution of this ARE, which was in close proximity to
the conserved region previously identified (25). Del-6 exhibited
a R1881 concentration-response curve comparable with Del-1
and -2; Del-6 expressed a log EC_{50} value not significantly
greater or less than the other two constructs (Fig. 3E). There-
fore, the elimination of neither the one downstream ARE (Del-6
construct) nor two upstream AREs (Del-3 construct) lessened
the ability of R1881 to prompt luciferase activity. Hence we
postulated that the AR could bind either one or more of the
three potential AREs located proximal to the core SENP1 pro-
moter and within the highly homologous upstream region pres-
ent on Del-3.

To validate that the androgen-induced luciferase response
was mediated via activation of the AR, the R1881-mediated
luciferase response for Del-3 was assessed in the presence of the
AR antagonist BIC. Addition of BIC significantly lowered the
R1881 promoter response in both LNCaP and PC-3 cells (p < 0.05) (Fig. 4A and B), respectively and confirmed that activa-
tion of the AR modulated SENP1 promoter activity.

**DISCUSSION**

*In situ* hybridization studies and tissue microarray data sug-
gest an augmentation of SENP1 mRNA in PCa samples as com-
pared with normal prostate tissue (18). In the present study, we
observed that the SENP1 mRNA levels were elevated in the
transformed RWPE cells, RWPE2, as well as in the PCa cell line
LNCaP as compared with the normal prostate cells, RWPE1
(Fig. 2A). Collectively, these results confirmed the enhanced
expression of SENP1 in prostate cancer. Up-regulation of
SENP1 mRNA was recently reported in an additional adenocar-
cinoma, specifically in thyroid oncocyctic cancer (36). However,
the mechanism and subsequent consequence of SENP1 induc-
tion were not investigated.

Previous results suggested that 24-h treatment of LNCaP
cells with R1881 promotes elevation in SENP1 mRNA; in con-
cells, we observed that the induction of SENP1 mRNA in PCa cells was selective and mediated via activation of the AR. Chronic exposure of LNCaP cells to androgen prompted the up-regulation of SENP1 but not SENP2; this observation was especially interesting because the two SENPs share 57% homology in the catalytic domain and constitute an independent subfamily. Several results from this study supported that enhancement of SENP1 requires AR activation. First, androgen-mediated SENP1 up-regulation was only observed in the cell line that expressed endogenous AR (LNCaP cells) but not in AR-independent cells (PC-3) (Fig. 2C). Second, blockade of the AR with bicalutamide inhibited R1881-induced SENP1 expression. An analogous series of experiments suggested that the SENP1 promoter was also regulated via activation of AR. First, introduction of equivalent amounts of an AR plasmid was required for comparable androgen-mediated SENP1 promoter activity in both PC-3 (Fig. 3C) and LNCaP (Fig. 3B). Second, inhibition of AR activity with bicalutamide significantly blunted androgen-promoted-SENP1 promoter activity in both cell lines (Fig. 4, A and B). Collectively these results illustrated that activation of AR initiates transcription of the SENP1 gene and subsequently enhances SENP1 mRNA levels in PCa cells.

The active AR directly bound the SENP1 promoter to enhance gene transcription. The ChIP and EMSA indicated that the AR bound a region that is highly conserved upstream of both the human and mouse SENP1 genes. Mutation of 1 C and 1 G nucleotide on this ARE profoundly decreased its ability to interact with the AR-DBD (Fig. 5D). Hence, the identified sequence conforms to previous reports that the C and G nucleotides are essential for contact with the AR-DBD (26–28). Interestingly, recent studies suggest that the AR binds DNA in a head to toe orientation, whereas the other members of the nuclear receptor superfamily bind head to toe. Consequently, androgen-occupied AR binds half-sites that appear as direct repeats, or 5′-AGAACA nnn

FIGURE 5. Identification of an ARE on the SENP1 promoter bound by androgen-activated AR. A, the minimal promoter region present on the Del-3 construct, which includes the highly conserved region and three potential AREs, is highlighted. The two PCR amplification sites are also identified and labeled as REGION1 (underlined with the solid line) and REGION2 (underlined with the dashed line). B, PC-3 cells were transfected with empty vector (lanes 1 and 4) or AR (500 ng, lanes 2 and 5) and treated with R1881 (50 nM, lanes 3 and 6) for 24 h; this R1881 concentration prompted a 2-fold induction in SENP1 promoter activity in Fig. 3B. ChIP was performed with anti-AR antibody, and the eluted products (top bands) and initial input (bottom bands) were run on an agarose gel. AR interacts with Region 1 (lanes 1–3), but not Region 2 (lanes 4–6), of the proposed SENP1 promoter only in the presence of androgen (lane 3). C and D, specific 5′-biotin-labeled oligonucleotides were designed to include one potential ARE (supplemental Table S2). The AR-DBD interacted with oligonucleotides but not 2 or 3 as observed in the representative blot and four independent experiments (C). A representative blot and densitometric analyses of three independent experiments illustrated the decrease in ability of the mutated oligonucleotide 1 to interact with AR-DBD; Student’s t test indicated a significant difference between the two groups (∗, p < 0.05) (D).
AGAACA-3’, with greater affinity than the palindromic sequence AGAACA nnn TGTTCT-3’ (37, 38). The identified ARE sequence 5’-GGAGCA nnn GGAACA-3’ on the SENP1 promoter displayed greater similarity to the direct rather than the inverted repeat sequence, 75% versus 50%, respectively; this further suggests that the identified ARE is a high affinity AR-binding site.

Previously, we demonstrated that overexpressing SENP1 in PCa cells enhances AR-dependent transcription (10). AR induces the transcription of numerous genes, which in turn modulate cell proliferation (30–34). SENP1 also positively regulates the expression of the cell cycle regulator cyclin D1 (18). Hence, it is not surprising that reducing endogenous SENP1 levels with siRNA inhibits the G1-S phase transitions.4 The correlation between SENP1, cyclin D1, and cell cycle regulation is currently being investigated in our laboratory. Based on the present results, it is evident that AR-regulated SENP1 also contributes to AR-mediated PCa cell growth (Fig. 1B).

In the current report, we reported a 5-fold induction of SENP1 mRNA expression following chronic exposure to a synthetic androgen. It was difficult to assess changes in SENP1 protein levels because of a lack of good available SENP1 antibodies and low endogenous expression of the SENP1 protein. However, it is clear from our studies that modulation of SENP1 message leads to meaningful biological responses, such as changes in AR-mediated cellular proliferation (current study), AR-dependent transcription (10, 18), and c-Jun-dependent transcription (39). To our knowledge, this is the first report to delineate the mechanism for the regulation of SENP1 in a biological system. The androgen-activated AR binds a specific response element located proximal to the SENP1 promoter. SENP1 promoter activity is enhanced by this activated AR, and thereby, SENP1 mRNA levels are significantly elevated. SENP1 up-regulation completes the positive feedback loop by potentiating AR-dependent transcription and cell proliferation.

The present results, collectively with previous studies (10, 18), suggest that SENP1 could be an important target for future therapeutic treatments of advanced PCa. Currently, androgen ablation therapy is employed to regulate AR activity and cell growth in systemic PCa (15–17). The major drawback to this therapy is that it readily causes the androgen-sensitive PCa to progress to an androgen-independent state or hormone refractory PCa. The exact mechanism responsible for the onset of hormone refractory disease is undefined, but several potential models exist. One model suggests that molecular changes in the PCa cells maintain functional AR signaling in androgen-independent PCa (40, 41). An alternative model suggests that the androgen-deprived environment prompts clonal selection of preexisting androgen-independent over androgen-dependent PCa cells (42, 43). It is intriguing to speculate that androgen ablation therapy is initially effective in treating PCa due to its ability to decrease SENP1 expression because in the current study, we demonstrate that androgen-activated AR regulates SENP1 expression in PCa cells. We anticipate that therapeutic agents designed to selectively lower SENP1 levels would be more effective than androgen ablation therapy in the treatment of advanced PCa. Like androgen ablation, reduction of elevated SENP1 levels lowers AR activity (10, 18) and PCa cell proliferation. However unlike androgen ablation, selective down-regulation of SENP1 could modulate these two events without depleting prostate epithelial cells of androgen. By not altering androgen levels, this SENP1-targeting agent would not prompt the outgrowth of androgen-dependent cancer cells. More extensive studies will be required to define the contribution of SENP1 overexpression to PCa progression.

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