Ligand-independent Activation of the Androgen Receptor by Interleukin-6 and the Role of Steroid Receptor Coactivator-1 in Prostate Cancer Cells*

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The androgen receptor (AR) can be activated in the absence of androgens by interleukin-6 (IL-6) in human prostate cancer cells. The events involved in ligand-independent activation of the AR are unknown, but have been suggested to involve phosphorylation of the AR itself or a receptor-associated protein. Steroid receptor coactivator-1 (SRC-1) has been shown to interact with the human AR and to modulate ligand-dependent AR transactivation and is regulated by phosphorylation by MAPK. To date, no one has examined the role of SRC-1 in ligand-independent activation of the AR by IL-6 or other signaling pathways known to activate the full-length receptor. This study addressed this and has revealed the following. 1) SRC-1 similarly enhanced ligand-independent activation of the AR by IL-6 to the same magnitude as that obtained via ligand-dependent activation. 2) Androgen and IL-6 stimulated the MAPK pathway. 3) MAPK was required for both ligand-dependent and ligand-independent activation of the AR. 4) Phosphorylation of SRC-1 by MAPK was required for optimal ligand-independent activation of the AR by IL-6. 5) Protein-protein interaction between endogenous AR and SRC-1 was dependent upon treatment of LNCaP cells with IL-6 or R1881. 6) Protein-protein interaction between the AR N-terminal domain and SRC-1 was independent of MAPK. 7) Ligand-independent activation of the AR did not occur by a mechanism of overexpression of either solely wild-type SRC-1 or mutant SRC-1 that mimics its phosphorylated form.

The androgen receptor (AR)† is a ligand-mediated transcription factor that belongs to the superfamily of steroid receptors (1). These receptors have similar protein structures that are composed of a N-terminal domain (NTD) that contains AF-1 (activation function-1), a DNA-binding domain (DBD), a hinge region, and a ligand-binding domain that contains AF-2. After the ligand binds to the AR, the heat-shock proteins are dissociated from the AR, and the ligand-receptor complex translates into the nucleus, binds specific androgen response elements (AREs) on the chromosome, interacts with coactivators, and modulates the expression of androgen-regulated genes (2). In the absence of androgen, the AR can be activated by growth factors and interleukin-6 (IL-6) and elevation of intracellular cyclic AMP (3–8). One possible mechanism underlying ligand-independent activation of the AR by these alternative pathways may involve phosphorylation of either the AR itself or receptor-associated proteins such as coactivators (7, 8).

Coactivators are proteins that generally do not bind DNA, but are recruited to the promoter through protein–protein interactions with transcription factors such as the AR usually in a ligand-dependent manner. Interactions between the receptor and coactivator enhance receptor-dependent transcription. The first identified member of the coactivator family that regulates steroid receptor action was steroid receptor coactivator-1 (SRC-1) (9). Phosphorylation of SRC-1 by mitogen-activated protein kinase (MAPK) is required for optimal progesterone receptor-dependent transcription and for functional cooperation with cAMP response element-binding protein-binding protein (10). SRC-1 interacts with both AF-1 and AF-2 of the AR and enhances ligand-dependent transactivation to increase transcription of androgen-regulated genes (11, 12).

Prostate-specific antigen (PSA) is an androgen-regulated gene that is routinely used by oncologists and urologists to monitor treatment responses, prognosis, and progression in patients with prostate cancer (13). Patients with advanced prostate cancer treated with hormone ablation therapy inevitably succumb to their disease as the tumor progresses to an androgen-independent condition, an early sign of which is a rising titer of serum PSA. There is mounting evidence that ligand-independent activation of the AR may play a role in hormone-refractory disease. This is because the AR is expressed in hormone-refractory prostate cancer (14), and many of the same genes that are increased by androgens in androgen-dependent prostate cancer xenografts become elevated in androgen-independent prostate cancer xenografts in castrated hosts (15). PSA gene expression can be induced in prostate cancer cells by IL-6 via ligand-independent activation of the AR (8). Currently, the underlying molecular mechanism of ligand-independent activation of the AR is not known. A possible mechanism may include increased expression of coactivators of the AR such as SRC-1, which has been reported to be increased in a large number of recurrent prostate cancer tissue (16, 17).
IL-6 has gained considerable clinical interest for a number of reasons, including the following. 1) Prostate cancer predominately metastasizes to bones that express IL-6 (4, 18). 2) Epithelial cells from normal, hyperplasia, and carcinoma prostate tissue also secrete IL-6 in culture media (19). 3) The IL-6 receptor is expressed in normal prostate, high-grade prostatic intraepithelial neoplasia, and cancer (20, 21). 4) IL-6 is elevated in the sera of patients with metastatic prostate cancer (19, 22, 23) and hormone-refractory disease (24). 5) An increase in proliferation of prostate cancer cells has been shown in response to IL-6 (8, 25–27) with neutralizing antibody inhibiting the proliferation of PC-3 and DU145 prostate cancer cells (28–30).

In this study, the mechanism of ligand-independent activation of the AR by IL-6 in prostate cancer cells was further investigated. Although much has been reported about the mechanism of ligand-dependent activation of steroid hormone receptors and their interaction with SRC-1, nothing has been reported about the mechanism of ligand-independent activation of the AR and the role of SRC-1. Here we report for the first time that SRC-1 enhances ligand-independent activation of the AR NTD by IL-6 via a pathway that is dependent upon MAPK in LNCaP human prostate cancer cells.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Materials—**LNCaP human prostate cancer cells were maintained in RPMI 1640 medium supplemented with 5% (v/v) fetal bovine serum (Invitrogen), 100 units/ml penicillin, and 100 mg/ml streptomycin at 37 °C in an atmosphere of 5% CO2. All chemicals were purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise. Bovine serum albumin (BSA) was purchased from Sigma, unless stated otherwise.

**Plasmids—**The human AR cDNA was a gift from Dr. A. O. Brinkmann (Erasmus University, Rotterdam, The Netherlands). AR-Rg-tk-luciferase (where AR is the androgen response region and tk is thymidine kinase) (31) and AR-(1–558)-Gal4 DBD, the Gal4 DBD, p558×Gal4UAS-TATA-luciferase, and AR-(1–233)-Gal4 DBD (7, 8) were described previously. PSA (6.1 kb)-luciferase was provided by Dr. J.-T. Hsieh (Center for Genetic Medicine, University of Texas Southwestern Medical Center, Dallas, TX). The expression vectors for SRC-1 were supplied by Drs. Bert W. O'Malley and Nancy L. Weigel (Baylor College of Medicine, Houston, TX) (10).

**Transfection and Luciferase Assay—**LNCaP cells (3 × 105/well) were seeded in six-well plates and incubated with RPMI 1640 medium containing 5% fetal bovine serum for 24 h. Transfection was performed using Lipofectamine® reagent (5 μl/well; Invitrogen) according to previous published methods (7). The total amount of plasmid DNA was normalized to 3 μg/well by the addition of a control plasmid that encoded the luciferase gene, but lacked the promoter insert. After 24 h, the medium was replaced with serum-free RPMI 1640 medium containing 1 mg/ml bovine serum albumin with R1881 or IL-6. Cells were collected after a 24- or 48-h incubation using the lysis buffer provided with the Promega luciferase kit. Luciferase activities were measured using the Promega dual-luciferase assay system with the aid of a multiplate luminometer (EG&G Berthold). Luciferase activities were normalized by the protein concentration of the samples (8, 10) as measured by the method of Bradford (32). The results presented as -fold induction give the relative luciferase activity of the treated cells divided by that of the control cells.

All transfection experiments were carried out in triplicate wells and repeated three to seven times using at least two sets of plasmids that were prepared separately.

**Immunoblotting—**LNCaP cells (2 × 105/dish) were plated in dishes (10-cm diameter) in RPMI 1640 medium containing 5% fetal bovine serum for 24 h. Heparin transfected with expression vectors encoding SRC-1 (1 μg/dish) and AR-(1–233)-Gal4 DBD, AR-(1–558)-Gal4 DBD, or AR-(1–308)-Gal4 DBD (10 ng/dish) with 60 μg/l of protein were treated with vehicle, R1881 (10 nm), or IL-6 (50 ng/ml) for 6 h. Cells were harvested; nuclear lysates were prepared and precleared with anti-mouse IgG and protein A/G-agarose (Santa Cruz Biotechnology, Inc.); antibody AR441 (4 μg/ml) was added; and the mixture was incubated overnight at 4 °C. The immunoprecipitates were washed four times with phosphate-buffered saline and resuspended in sample buffer. The immunocomplexes were separated by SDS-PAGE (7%). Western blot analysis was carried out with anti-SRC-1 antibody. AR NTD-Gal4 DBD fusion proteins were immunoprecipitated from LNCaP cells (2 × 105/dish) that were plated in dishes (10-cm diameter) in RPMI 1640 medium containing 5% fetal bovine serum for 24 h. Cells transfected with expression vectors encoding SRC-1 (1 μg/dish) and AR-(1–233)-Gal4 DBD, AR-(1–558)-Gal4 DBD, or AR-(1–308)-Gal4 DBD (10 ng/dish) with 60 μg/l of protein were treated with vehicle, R1881 (10 nm), or IL-6 (50 ng/ml) for 6 h. Cells were harvested, and whole cell and nuclear lysates were prepared as described previously (33). After the supernatants of the cells were pre-cleared by the addition of 0.25 μg of rabbit IgG and 20 μl of protein A/G-agarose, anti-Gal4 DBD antibody (2 μg; RK5C1, Santa Cruz Biotechnology, Inc.) was added, and the mixture was incubated for 1 h at 4 °C. The immunoprecipitates were washed four times with ice-cold lysis buffer and resuspended in sample buffer. Immunocomplexes were separated by SDS-PAGE (7%). Western blot analyses were carried out with anti-SRC-1 antibody (1:200; 20-h incubation).

**RESULTS**

**Overexpression of SRC-1 Enhances Androgen-dependent and Androgen-independent Induction of ARE-driven Reporter Gene Constructs—**The role of SRC-1 as a coactivator of the unliganded AR that has been activated by alternative pathways (ligand-independent activation) has not been described. Therefore, we sought to determine whether SRC-1 plays a role in ligand-independent activation of the AR by IL-6. To do this, LNCaP human prostate cancer cells expressing endogenous AR were transiently cotransfected with ARE-driven reporter plasmids and the expression vector for SRC-1 prior to treatment with synthetic androgen (R1881) or IL-6. The first ARE-driven reporter used was the PSA (6.1 kb)-luciferase reporter gene construct. This luciferase construct contains both the enhancer and promoter regions of the clinically relevant PSA gene and is induced by androgens (8, 34). As shown in Fig. 1A, R1881 (10 nm) induced the PSA (6.1 kb)-luciferase reporter construct by ~70-fold. Transfection of the SRC-1 expression plasmid (0.2 μg) increased this activity to ~140-fold, or by 2-fold over that without SRC-1 (compare the second and sixth bars). IL-6 (50 ng/ml) induced the PSA (6.1 kb)-luciferase construct by ~17-fold in the absence of androgen (Fig. 1B). Cotransfection of the SRC-1 expression plasmid (0.2 μg) increased this activity to ~38-fold, or again by ~2-fold over that without SRC-1 (compare the second and sixth bars). Basal activity was not altered by enhanced levels of SRC-1 (first, third, and fifth bars).

The second ARE-driven reporter used was AR-Rg-tk-luciferase, which is an artificial reporter construct that contains three tandem repeats of the rat probasin ARE1 and ARE2 regions upstream of the thymidine kinase promoter (S. Schwartz, unpublished observations). Overexpression of SRC-1 (0.2 μg), R1881 induced this reporter by ~8000-fold (sixth bars). Consistent with previous reports, IL-6 induction of this reporter was poor in comparison with that achieved with...
R1881, and only a 6-fold induction was achieved in the absence of ectopic SRC-1 (Fig. 1D, second bar), whereas an ~11-fold induction was measured in the presence of ectopic SRC-1 (0.2 μg) (sixth bar). Thus, ectopic SRC-1 enhances both androgen-dependent and androgen-independent induction of ARE-driven reporters to a similar extent (2-fold), and these effects are not specific to one particular ARE-driven reporter gene construct.

**Effect of Inhibiting MAPK Activity on IL-6 Induction of the PSA (6.1 kb)-Luciferase Reporter Construct in the Presence of Ectopic SRC-1**—MAPK has been suggested to play an important role in androgen-independent prostate cancer (35, 36). MAPK also directly phosphorylates SRC-1 at Thr\(^{1179}\) and Ser\(^{1185}\) (10). To provide insight into the role of MAPK in the enhanced induction of ARE-driven reporters by overexpression of SRC-1, we examined the PSA (6.1 kb)-luciferase reporter construct in LNCaP cells exposed to an inhibitor of MAPK (U0126). Inhibition of MAPK reduced the induction of PSA-luciferase by R1881 both in the presence and absence of ectopic SRC-1 (Fig. 2A). Similarly, inhibition of MAPK reduced the induction of PSA-luciferase by IL-6 both in the presence and absence of ectopic SRC-1 (Fig. 2B). Inhibition of MAPK decreased both androgen-dependent and androgen-independent induction of PSA-luciferase activity in both the presence and absence of ectopic SRC-1. AR protein levels were not altered by treatment of cells with either IL-6 or U0126 (Fig. 2C). AR protein levels were slightly increased in cells treated with R1881, which is consistent with previous reports that ligand stabilizes the AR protein (37).

**IL-6 and Androgen Activate the MAPK Pathway**—Treatment of LNCaP cells with dihydrotestosterone leads to a rapid and reversible activation of MAPK (38). To test whether the MAPK pathway was being activated in our system with IL-6 and R1881, we performed Western blot analyses using antibodies that detect the phosphorylated MAPK isoforms p44 and p42. Western blot analyses showed that phosphorylation of isoforms p44 and p42 was maximum after 15 min of exposure of LNCaP cells to R1881 and IL-6 (Fig. 3, lanes 3 and 5). U0126 completely blocked the phosphorylation of MAPK in cells exposed to R1881 and IL-6 (compare lanes 3 and 4 and lanes 5 and 6). Thus, phosphorylation of MAPK was increased in LNCaP cells treated with R1881 or IL-6.

**Phosphorylation at Threonine 1179 and Serine 1185 of SRC-1 Is Required for Optimal Stimulation of Androgen-independent Increases in PSA-Luciferase Activity by IL-6**—Recently, it was shown that MAPK phosphorylates SRC-1 at Thr\(^{1179}\) and Ser\(^{1185}\), which is required for optimal stimulation of both ligand-dependent and ligand-independent activation of the ectopic progesterone receptor in COS cells cotransfected with the GRE\(_{2}\)-E1bCAT reporter construct (10). To date, this has not been explored with the AR in the context of ligand-dependent or ligand-independent activation. Therefore, to determine whether phosphorylation of SRC-1 by MAPK is required for androgen-independent induction of PSA by IL-6 via ligand-independent activation of the AR, mutant SRC-1 was compared with wild-type SRC-1 in LNCaP cells. The results presented in Fig. 4 show that mutation of Thr\(^{1179}\) and Ser\(^{1185}\) of SRC-1 to alanines (T1179A/S1185A) reduced IL-6 induction of PSA-luciferase activity to a level comparable to that achieved in the absence of ectopic expression of wild-type SRC-1 (compare bars 4 and 2). Mutation of Thr\(^{1179}\) and Ser\(^{1185}\) of SRC-1 to glutamic acids (T1179E/S1185E) restored SRC-1-enhanced induction of PSA-luciferase activity by IL-6 to a level comparable to that achieved with wild-type SRC-1 (compare bars 5 and 3). Alanine mutations mimic a loss of phosphorylation, whereas glutamic acid mutations maintain a negative charge to mimic phosphorylation (10). Thus, optimal ligand-independent activation of the AR and induction of PSA-luciferase activity by IL-6 require phosphorylation of SRC-1 at Thr\(^{1179}\) and Ser\(^{1185}\).

**IL-6 Activation of the Human AR NTD Is Enhanced by SRC-1 and Blocked by Inhibition of MAPK**—IL-6 activates the...
AR NTD (8), and SRC-1 interacts directly with the AR NTD in a ligand-independent manner (11, 12). Therefore, we sought to determine whether SRC-1a would enhance activation of the AR NTD by IL-6. To do this, the yeast Gal4 system was employed using a chimeric construct of the AR NTD (amino acids 1–558) fused to the Gal4 DBD (AR(1–558)-Gal4 DBD) (7, 8). LNCaP cells were cotransfected with the expression vector encoding the Gal4 DBD, AR-(1–558)-Gal4 DBD, SRC-1, and a reporter gene containing the Gal4-binding site (p5-luciferase (1 kb)-TATA-luciferase). As shown in Fig. 5A, IL-6 had a slight effect on the induction of the Gal4-luciferase activity in cells expressing only the Gal4 DBD (compare bars 1 and 2). A ligand-dependent and ligand-independent activation, respectively, of the AR in LNCaP cells. LNCaP cells were transiently cotransfected with PSA (6.1 kb)-luciferase (1 μg/well) with or without SRC-1a (0.2 μg/well) for 24 h, pretreated with U0126 (10 μM) or vehicle for 2 h before the addition of IL-6 (50 ng/ml) or R1881 (10 nM), and then incubated for an additional 48 h under serum-free conditions. The bars represent the means ± S.E. of three separate experiments, each performed in triplicate. C, levels of AR protein in LNCaP cells treated with R1881, IL-6, or U0126 for 24 h. Forty micrograms of protein in whole cell lysates was loaded in each lane, separated by SDS-PAGE, blotted, analyzed for the AR protein, and normalized to β-actin.

To test the effects of MAPK on the activation of the AR NTD by IL-6 in the presence of ectopic SRC-1, the MEK inhibitor U0126 was employed (Fig. 5B). U0126 had negligible effects upon basal transactivation of AR(1–558)-Gal4 DBD (compare bars 9 and 10). In the presence of IL-6, U0126 reduced the Gal4 reporter activity both in the presence (compare bars 15 and 16) and absence (compare bars 13 and 14) of cotransfected SRC-1. The levels of AR-(1–558)-Gal4 DBD fusion protein were consistent in cells treated with R1881, IL-6, and U0126 (Fig. 5C). These results suggest that activation of the AR NTD by IL-6 in the absence and presence of ectopic SRC-1 is dependent upon the MAPK pathway.

Phosphorylation at Threonine 1179 and Serine 1185 of SRC-1 Is Required for Optimal Activation of the AR NTD by IL-6—To determine the role of MAPK phosphorylation of SRC-1 in ligand-independent transactivation of the AR NTD by IL-6, we compared wild-type SRC-1 with the mutant forms (T1179A/S1185A and T1179E/S1185E) using the Gal4 system. The results presented in Fig. 6 show that when Thr1179 and Ser1185 of SRC-1 were mutated to alanines (T1179A/S1185A, T1179E/S1185E), the levels achieved with wild-type SRC-1 (SRC-1 wt) restored the coactivation to levels consistent in cells treated with R1881, IL-6, and U0126 (Fig. 5C). These mutations in SRC-1 reduced activation of the AR NTD by IL-6. To determine whether SRC-1 wt would enhance activation of the AR NTD by IL-6, we compared wild-type SRC-1 with the mutant forms (T1179A/S1185A and T1179E/S1185E) using the Gal4 system. The results presented in Fig. 6 show that when Thr1179 and Ser1185 of SRC-1 were mutated to alanines (T1179A/S1185A, T1179E/S1185E), the levels achieved with wild-type SRC-1 (SRC-1 wt) restored the coactivation to levels consistent in cells treated with R1881, IL-6, and U0126 (Fig. 5C). These mutations in SRC-1 reduced activation of the AR NTD by IL-6.
To date, all studies examining SRC-1 interaction with AR have failed to examine the endogenous proteins in whole cells. Thus, interaction between SRC-1 and AR was investigated using endogenous complexes isolated from LNCaP prostate cancer cells that were exposed to R1881 and IL-6. These studies revealed that SRC-1 was co-immunoprecipitated with the AR only in cells exposed to R1881 or IL-6 (Fig. 7, lanes 7 and 8). No interaction between the AR and SRC-1 was observed in the absence of these compounds (lane 6).

**Functional and Physical Interaction between SRC-1 and the AR NTD**—The region of the AR NTD required for ligand-independent activation and recruitment of SRC-1 by IL-6 has not been reported. Therefore, we mapped the region of the AR NTD required for potentiation of IL-6 activation by SRC-1. LNCaP cells were cotransfected with the expression vector encoding the Gal4 DBD, AR-(1–558)-Gal4 DBD, AR-(1–558)-Gal4 DBD, SRC-1, and a reporter gene containing the Gal4-binding site (p5×Gal4UAS-TATA-luciferase). The results shown in Fig. 8A show that IL-6 failed to significantly activate the AR-(1–233)-Gal4 DBD protein either in the presence or absence of ectopic SRC-1 (bars 5–8) as compared with the AR-(1–558)-Gal4 DBD protein (bars 10 and 12). This suggests that either amino acids 234–558 of the AR or, alternatively, that the entire NTD (amino acids 1–558) of the AR may be required for biological activity by IL-6 regardless of the levels of SRC-1, which is in agreement with previous studies mapping transactivation of the AR by IL-6 to amino acids 234–558 in the absence of ectopic SRC-1 (8).

To test whether amino acids 1–233 physically interact with SRC-1, co-immunoprecipitation assays were carried out in LNCaP cells exposed to IL-6. As expected, IL-6 induced protein-protein interactions between SRC-1 and AR-(1–558)-Gal4 DBD in LNCaP cells (Fig. 8B, compare lanes 5 and 6). However, inconsistent with the transactivation studies (Fig. 8A), IL-6 also induced protein-protein interactions in LNCaP cells expressing AR-(1–233)-Gal4 DBD (Fig. 8B, compare lanes 3 and 4). No interactions were observed in the absence of IL-6 (lanes 3 and 5) or between SRC-1 and the Gal4 DBD (compare lanes 1 and 2). Inhibition of MAPK did not prevent interaction be-

**Fig. 5. Effect of SRC-1 on the activity of the human AR NTD. A,** transactivation assays were performed in LNCaP cells cotransfected with p5×Gal4UAS-TATA-luciferase (1 µg/well) and AR-(1–558)-Gal4 DBD (50 ng/well) or the Gal4 DBD (50 ng/well) with or without SRC-1a (0, 0.1, and 0.2 µg/well) for 24 h prior to incubation with IL-6 (50 ng/ml) or vehicle for an additional 24 h before harvesting and measuring the relative luciferase activities. **B,** the effects of inhibition of MAPK on transactivation of the AR were examined in LNCaP cells cotransfected with p5×Gal4UAS-TATA-luciferase (1 µg/well) and AR-(1–558)-Gal4 DBD (50 ng/well) or the Gal4 DBD (50 ng/well) with or without SRC-1a (0.2 µg/well) for 24 h prior to incubation with IL-6 (50 ng/ml) or vehicle for an additional 24 h. LNCaP cells were pretreated with the MEK inhibitor U0126 (10 µM) or vehicle for 2 h prior to the addition of IL-6 (50 ng/ml) or vehicle and then incubated for an additional 24 h before harvesting and measuring the relative luciferase activities. The bars represent the means ± S.E. of three separate experiments, each performed in triplicate. **C,** shown are the levels of AR-(1–558)-Gal4 DBD protein fusion in LNCaP cells treated with R1881, IL-6, or U0126 for 24 h. Forty micrograms of protein in whole lysate was loaded in each lane, separated by SDS-PAGE, blotted, and analyzed for the AR protein using antibody AR441 (2 µg/ml). The membranes were stripped and normalized to β-actin protein levels using anti-β-actin antibody Ab-cam (1:5000). **RLU,** relative luminescent units.
**DISCUSSION**

Steroid receptors show a realignment of helix 12 in the ligand-binding domain that allows binding to LXXLL motifs of coactivators in the presence of ligand (46, 47). AF-2 has been mapped to helix 12 (48) and is highly conserved between these receptors. AF-1 is generally considered to be ligand-independent and has been mapped to the NTDs of steroid receptors. The relative importance of AF-1 and AF-2 in transcriptional activation by the AR varies depending on the gene and possibly ligand (49). SRC-1 has been shown to interact with both the AF-1 and AF-2 domains of the AR (11, 12). Hence, the contribution of SRC-1 in the two different AF regions was anticipated to vary depending on the presence or absence of hormone. This was not shown to be the case. Rather, we have shown here that SRC-1 similarly enhanced both ligand-dependent and ligand-independent activation of the AR by 2-fold. 2) Androgen and IL-6 stimulated the MAPK pathway. 3) MAPK was required for both ligand-dependent and ligand-independent activation of the AR. 4) Phosphorylation of SRC-1 by MAPK was required for optimal ligand-independent activation of the AR by IL-6. 5) Protein-protein interaction between endogenous AR and SRC-1 was independent of MAPK. 6) Ligand-independent activation of the AR did not occur by a mechanism of solely overexpression of either wild-type SRC-1 or mutant SRC-1 that mimics its phosphorylated form.

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to the potential binding sites determined on the AR NTD, as recently reported (50). Alternatively, the fact that the AR is unique from other nuclear receptors in the way that AF-1 contributes most, if not all, of the activity to the ligand-bound AR rather than AF-2 (51–54) may explain why SRC-1 increased both ligand-dependent and ligand-independent transactivation of the AR to the same degree. However, it should be noted that previous reports (51) that the AF-1 region of the AR is ligand-independent have been based on experiments omitting compounds that are known to activate the full-length receptor in the absence of its cognate ligand. Those studies observed that the AR NTD could activate a reporter gene in the absence of the ligand-binding domain to a level comparable to that achieved with the full-length receptor in the presence of ligand and thus referred to this as ligand-independent activation via the AF-1 region. To avoid confusion, we will refer to this as constitutive activation of the AR NTD because, under the same cell conditions, the full-length receptor is not activated; and we will refer to activation of the AR NTD by IL-6, which activates the full-length AR in the absence of androgens, as ligand-independent activation.

MAPK is elevated in androgen-independent prostate cancer tissue (36) and can activate the AR in prostate cancer cell lines (35). The MAPK pathway is activated by both IL-6 and androgen in prostate cancer cells (Fig. 2C) (38). We have shown here that inhibition of MAPK activity blocked both ligand-dependent and ligand-independent activation of the AR as measured by reduction in the activity of ARE-driven reporter gene constructs, regardless of levels of SRC-1. Similarly, a decrease in IL-6-induced transactivation of the AR NTD was also achieved by inhibition of MAPK. Consistent with these data, mutation of the SRC-1 amino acid residues known to be phosphorylated by MAPK to alanine decreased both the induction of PSA-luciferase and transactivation of the AR NTD by IL-6. Mutation of these residues to glutamic acid, which mimics phosphorylation, restored these activities. Together, these data strongly suggest an important role for MAPK in the mechanism of ligand-dependent and ligand-independent transactivation of the AR. It should be stressed that transactivation of the AR was not achieved in the absence of IL-6 with the mutant form of SRC-1 that mimics its phosphorylation state (T1179E/S1185E), thereby indicating that ligand-independent activation of the AR requires an step additional to phosphorylation of SRC-1 or enhanced levels of SRC-1. This may explain why R1881, which activates MAPK as shown in these studies, does not activate the AR NTD, as previously reported (7). Nevertheless the common requirement of MAPK for both ligand-dependent and ligand-independent activation of the AR indicates that inhibition of MAPK activity may constitute a general mechanism for antagonizing AR function in prostate cancer cells.

There is a consensus in the literature concerning the importance of cell-specific and promoter-specific responses for steroid receptor coactivators, yet the majority of studies examining coactivators and the AR in the context of prostate cancer have used yeast, CV-1, HeLa, and COS cells and physiologically non-relevant promoters. The results presented here are the first to show interaction of endogenous AR and SRC-1 as well as interaction between the AR NTD and SRC-1 in human prostate cancer cells. These studies reveal a number of discrepancies with the results obtained using other cell types or methods. In yeast, it was shown that the AR NTD (residues 1–556) and AR-(360–494) interact with SRC-1e in a ligand-independent manner (11). We have shown here that stimulation of LNCaP cells with IL-6 was required for interaction between SRC-1 and the AR NTD (residues 1–558 and 1–233) and that no interaction was detected in its absence. In COS cells, a decrease in ligand-independent activation of the AR was observed in the presence of increased levels of SRC-1a (11), whereas in CV-1 cells, enhanced SRC-1a increased constitutive AR AF-1 (residues 1–555) activity (12). We have shown here that in LNCaP human prostate cancer cells, SRC-1a slightly enhanced constitutive AF-1 activity, whereas in the presence of IL-6 (which activates the full-length receptor), SRC-1 markedly enhanced transactivation. The fact that SRC-1 enhanced IL-6-induced transactivation of AR-(1–558), but not AR-(1–233), was not due to an inability of SRC-1 to interact with AR-(1–233), as shown in immunoprecipitation studies. Rather, the inability of SRC-1 and IL-6 to activate AR-(1–233) was probably due to this region of the AR lacking the essential AF-1 regions required for transactivation (49, 51). IL-6-induced protein-protein interaction between the AR NTD and SRC-1 was not prevented by an inhibitor of MAPK despite the fact that transactivation was blocked by inhibition of MAPK. This suggests that inhibition of phosphorylation of either SRC-1 or the AR NTD does not prevent protein-protein interactions, but rather transactivation. This implies that protein-protein interaction between SRC-1 and the AR NTD is independent of MAPK activity. Similarly, phosphorylation at Thr1179 and Ser1185 of SRC-1 does not alter the binding of SRC-1 to the progesterone receptor (10).

In conclusion, we have shown that SRC-1 enhanced both ligand-dependent and ligand-independent transactivation of the AR to a similar extent by a mechanism that is dependent upon MAPK and phosphorylation of SRC-1. Phosphorylation by MAPK was not required for IL-6-induced protein-protein interaction between the AR NTD and SRC-1. Therefore, the decrease in transactivation of the AR resulting from inhibition of phosphorylation of SRC-1 may be due to a loss of cooperation between SRC-1 and the MAPK response element-binding protein-protein binding as described for the progesterone receptor (10). However, a mechanism additional to MAPK phosphorylation of SRC-1 is required for ligand-independent activation of the AR because a mutation of SRC-1 that mimics phosphorylated SRC-1 was not sufficient to mediate transactivation in the absence of IL-6. Understanding this additional underlying mechanism(s) may help to elucidate the events involved in the progression of prostate cancer to androgen independence and the biological and clinical significance of ligand-independent activation of the AR by IL-6.

Acknowledgments—We thank N. Mendelev for excellent technical assistance and Dr. R. Snoek for helpful discussions.

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J. Biol. Chem. 2002, 277:38087-38094.
doi: 10.1074/jbc.M203313200 originally published online August 5, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M203313200

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